Title

METAL DRUGS AND THE ANTICANCER IMMUNE RESPONSE

Authors

Bernhard Englinger[1,#], Christine Pirker[1,#], Petra Heffeter[1,2], Alessio Terenzi[2,3+], Christian R Kowol[2,3], Bernhard K Keppler[2,3], Walter Berger[1,2*]

Affiliations

[1] Institute of Cancer Research and Comprehensive Cancer Center, Department of Medicine I, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria.

[2] Research Cluster "Translational Cancer Therapy Research", University of Vienna and Medical University of Vienna, Vienna, Austria.

[3] Institute of Inorganic Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Str. 42, A-1090, Vienna, Austria.

[#] These authors contributed equally to this work.

[+] Current adress: Donostia International Physics Center, Paseo Manuel de Lardizabal 4, Donostia, 20018 Spain

[*]**Corresponding author**

Walter Berger, walter.berger@meduniwien.ac.at

Abstract

The immune system deploys a multitude of innate and adaptive mechanisms not only to ward off pathogens but also to prevent malignant transformation ("immune surveillance"). Hence, a clinically apparent tumor already reflects selection for those malignant cell clones capable of evading immune recognition ("immune evasion"). Metal drugs, besides their well-investigated cytotoxic anticancer effects, massively interact with the cancer-immune interface and can reverse important aspects of immune evasion. This topic has recently gained intense attention based on combination approaches with anticancer immunotherapy (e.g. immune checkpoint inhibitors), a strategy recently delivering first exciting results in clinical settings. This review summarizes the promising but still extremely fragmentary knowledge on the interplay of metal drugs with the fidelity of anticancer immune responses but also their role in adverse effects. It highlights that, at least in some cases, metal drugs can induce long-lasting anticancer immune responses. Important steps in this process comprise altered visibility and susceptibility of cancer cells towards innate and adaptive immunity, as well as direct impacts on immune cell populations and the tumor microenvironment. Based on the gathered information, we suggest initiating joint multidisciplinary programs to implement comprehensive immune analyses into strategies to develop novel and smart anticancer metal compounds.

Table of contents

1. Introduction

1.1. Immunological effects contribute to the activity of anticancer metal drugs

Evidence has accumulated recently that - besides direct immunotherapeutic approaches - also more or less all other forms of local and systemic cancer therapies including surgery and irradiation as well as chemotherapy (e.g. with anticancer metal drugs), antiangiogenic approaches and targeted drugs might exert massive and partly even opposing impacts on systemic and tumor-associated immunological parameters.[3] In earlier days, based on the well-known chemotherapy-induced myelosuppression and lymphocytopenia associated with a massive risk for infections, it was taken for granted that chemotherapy would primarily lead to immune suppression.[4-8] Unexpectedly, several lines of evidence from cell biological, preclinical and clinical observations suggest that -when using appropriate combination schemes - cytotoxic chemotherapy and immunotherapy might even exert a highly synergistic anticancer activity.[9] Hence, chemotherapy might reverse important aspects of "immune evasion" and "immune-subversion" (compare chapter 1.2) at the side of cancer cells, but also impact on several types of immune cells resulting in enhanced anticancer effects.[8,10-13] These potentially detrimental or beneficial interactions between cytotoxic chemotherapy and anticancer immune responses are multifaceted and might strongly depend on the nature of the used treatment scheme including not o.nly the applied compounds/combinations,[14] but also dose and schedule as well as the interconnection with other treatment modalities.[11,12,15] The latter is currently even more in the focus of interest when combining chemo- with immunotherapy approaches like the recent, highly successful application of immune checkpoint inhibitors.[16] Moreover, it has to be considered that, during a therapeutic intervention e.g. by cytotoxic chemotherapy, not only the malignant cell compartment itself, but also the dynamic cancer microenvironment might be remodeled, creating attractive but at the same time often transient constellations for new therapeutic interventions.[3,12,17] Consequently, a detailed know-how on the underlying molecular mechanisms at the side of the cancer cells and the diverse compartments of the microenvironment is essential to fully harness these fascinating but often also fragile novel therapeutic possibilities.

One class of cytotoxic agents that might come into focus of novel combination strategies with immunotherapies are anticancer metal drugs. This anticipation is based especially on recent highly promising chemoimmunotherapy data for combination of platinum (Pt) drugs and programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) checkpoint inhibitor antibodies in non-small cell lung cancer (NSCLC) (compare chapter 8.3.1).[9,18,19] Accordingly, several findings outlined in the following sections of this review implicate a complex interplay between classical metal drugs like cisplatin or oxaliplatin as well as novel anticancer metal complexes and the anticancer immune response, both directly impacting on immune effectors and on cancer cell immune recognition.[20-22] From an eagle's perspective, these findings demonstrate that the widely used categorization of systemic cancer therapy as cytotoxic chemotherapy,

targeted therapy, and immunotherapy is drastically oversimplified. Moreover, with respect to the development of novel anticancer metal complexes, this review uncovers that the current know-how regarding interaction with and activation of immune-related anticancer signals is rudimentary at best. Based on the gathered information and the fragmentary picture emerging, we suggest initiating joint multidisciplinary efforts to implement immune biomarkers and tests into strategies to develop novel and smart anticancer metal compounds. Thereby, the exceptional and powerful aspects of metal complexes, allowing the combined utilization of the unique characteristics of the metal core together with the (e.g. tumor-specific, controlled) release of bio- and probably immuno-active ligands, should be fully exploited.[23-27] These approaches can strongly support the (pre)clinical development of anticancer metal complexes, especially within combination settings with immunotherapeutic strategies, thus re-positioning anticancer metal complexes into the landscape of successful systemic cancer therapy development.

Before the available data concerning anticancer metal drugs and their immune effects are outlined and discussed in chapters 2-8, an overview on the immunological background of malignant transformation and progression as well as of the major players of the immune system is given in the following chapter.

1.2. The immune system and cancer - an overview

1.2.1. Cancer immunoediting and general aspects of the (anticancer) immune response

The body's immune system disposes of an exquisite armamentarium of sensors dedicated to efficiently recognize and eliminate foreign pathogens as well as incipient cancer cells. This protection is the result of a fine-tuned education of the immune compartment, leading to the discrimination of "self" from "non-self" molecular features.[28] As early as in the beginning of the 20th century, Paul Ehrlich postulated the immune system to recognize not only foreign invaders, but also aberrant structures arising as a result of malignant transformation.[29,30] In the 1950s, Burnet and Thomas coined the concept of "immunosurveillance", according to which aberrant cells are constantly warded off by the immune system based on antigenic traits recognized as "foreign"[31-33] or - following a newer concept - as "dangerous".[34] During oncogenesis, cancer antigens are generated from genetic mutations and rearrangements, overexpression of otherwise normal genes, as well as altered post-transcriptional and post-translational modifications.[35,36] Based on these concepts, it has become increasingly clear that outgrowth of a clinically apparent tumor is the result of a rigorous and persistent shaping and selection process of tumor cells by the immune system and vice versa, with a constant selection for those malignant cells that are capable of evading immune recognition.[35,37-39] Three major aspects describing this complex interplay have been formulated, summarized as "immunoediting", that depict a dynamic flux of novel phenotypic traits on cancer cells resulting in a continuous evolutionary pressure towards loss of immune recognition.[40-42] 1) In the elimination phase, the immune system successfully kills newly generated (pre)transformed cells.[2,16,43] 2) In the equilibrium phase,

incipient, not fully eradicated cancer cells are held in check by the immune-mediated destruction of newly formed, antigenic clones.[44] 3) Finally, in the escape phase, cancer cells acquire traits that enable them to evade immune cell killing by flying below the immunological radar and finally to establish a clinically apparent tumor.[37,45,46]

Accordingly, a multitude of tightly interconnected innate and adaptive mechanisms exists, which the immune system deploys to ward off cancer cells and preserve the integrity of a multicellular organism (summarized in Figure 1).[2,47,48] The innate system, comprising the myeloid lineage-derived granulocytes (neutrophils, basophils, eosinophils), mast cells, monocytes, macrophages, and dendritic cells (DC), as well as the lymphoid-derived natural killer (NK) cells, mediating rapid clearance of pathogens, is working in an "unspecific" manner and with only limited development of immunological memory (memory NK cells[49-51]). From vertebrates onwards, innate immunity is interconnected with an adaptive immune response, allowing further exuberant specification and persistent immunological memory. Like NK cells, all cells of the adaptive immune system derive from the lymphoid lineage of the hematopoietic system. The adaptive immune compartment disposes of an enormous capacity to detect "non-self" but also tumor-associated (neo)antigens.[52] These functions are mainly executed by two lymphocytic cell types specialized for cellular and humoral adaptive immune protection, namely T and B lymphocytes, respectively.[47,48] Each T and B lymphocyte expresses a unique surface receptor with specific antigen-binding properties, termed T cell receptor (TCR) and B cell receptor (BCR). The combination of mechanisms like gene rearrangements, splicing, polypeptide chain combination and, in case of B cells, somatic hypermutation generates an almost limitless repertoire of antigen receptors underlying potent T cell-mediated and antibody responses.[47,48,53] The interface between the innate and adaptive immune system is provided by several cell types subsumed as antigen-presenting cells (APC). In humans, APC comprise DC, which represent the most specialized and potent type of APC, macrophages, and B cells. Due to their special function as APC, DC are often depicted in an intermediate position between innate and adaptive arm or are even assigned to the latter one. Only APC are enabled to present a specific antigen to T lymphocytes, and to start an adaptive response. In the end, the specific pathogen clearance is exerted by cells of both adaptive and innate immune system.[48]

Central to all these processes is expression of molecules of the major histocompatibility complex (MHC), in humans termed human leucocyte antigen (HLA) complex. Two classes exist: MHC class I molecules are expressed on all nucleated cells of the body, acting like an identity card by defining the cells' "self" identity. MHC class II molecules are nearly exclusively expressed by APC (and also by some types of epithelial cells, e.g. in the gut) for presentation of peptides from extracellular sources.[54] The genes coding for MHC class I and II molecules consist of several subclasses (polygenic) harboring an exceptionally high polymorphism (many variants/alleles per gene), so that MHC expression is specific for each individual. MHC class I molecules consist of two chains, one highly variable α chain (consisting of 3 subunits) and the noncovalently linked invariable β2 microglobulin. On the cell surface, two subunits of the α-chains (α1 and α2) provide

formation of a cleft or groove where short peptides (8 - 10 amino acids), derived from endogenous cellular proteins that have been processed by proteasomal cleavage, are presented to the cellular environment.[48,55] MHC class II molecules consist of two highly variable chains, the α- and the β-chain, each consisting of two subunits, with the peptide-binding groove being formed by the α1 and the β1 subunit. Peptide fragments presented via MHC class II are usually larger than those presented by MHC class I, ranging from 13 amino acids upwards.[48,54] APC can take up extracellular particles (e.g. pathogens, cellular debris, dying cancer cells) by phagocytosis, and, upon protein processing in the lysosome, present these protein fragments to naïve (i.e. not yet activated) T cells. Only APC like DC can also present exogenously-derived peptides via MHC class I (instead of MHC class II) in order to activate a specific cytotoxic T cell response (see below), a process referred to as cross-presentation.[55]

Activation of the adaptive immune response starts upon engulfment of extracellular pathogens or cell fragments of dying (cancer) cells by APC (primarily DC). These cells subsequently get activated, which leads - amongst other changes concerning morphology, surface receptor expression, and cytokine production - to an increased expression of MHC class I and II molecules and migration to the lymph node, where they wait for their respective T cell counterpart.[48] T cells express an antigen-specific TCR together with either the CD4 or CD8 co-receptor, defining them as $CD4^+$ T cells (T helper or T_H cells) or $CD8^+$ T cells (cytotoxic T cells or CTL), respectively. T cell activation is MHC-restricted. This means that for successful antigen recognition and T cell activation, TCR must recognize and bind the peptide presented by the APC, together with co-receptor binding to the according MHC molecule, i.e. CD4 to MHC class II and CD8 to MHC class I. Additionally to MHC-restricted antigen binding ("first" signal), T cell activation requires also adequate signals from several further co-receptor/ligand interactions as well as cytokine patterns ("second" and "third" signals, respectively). Full activation depends on the cooperative action of these three signaling arms, while inadequate activation or competing inhibitory signals (immune checkpoints) might lead to antigen-specific immune-suppression (tolerance).[48] Once fully activated, CTL can directly kill cells expressing the respective antigen (e.g. tumor cells expressing a tumor antigen), whereas T_H cells mostly support CTL and B cells via cytokine production. B cells, upon binding of BCR-specific antigen, travel to secondary lymphoid organs (lymph nodes and spleen), where they present their specific antigen upon BCR internalization and antigen processing via MHC class II to specialized T_H cells.[48] In the rare case of a corresponding antigen encounter between B cell and T_H cells ("linked recognition"), B cells get activated, proliferate, and differentiate into plasma cells that are able to secrete their (antigen-specific) Ig (antibody secretion).[56] These antibodies can coat target cells expressing the respective antigen (antibody opsonization), resulting in elimination of antibody-coated cells by various immune cells via antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP).[57] ADCC is primarily executed by NK cells but also by eosinophilic and neutrophilic granulocytes as well as DC and macrophages via their (activating) receptors for the fragment crystallizable region (Fc) of the Ig (Fcγ receptors).[57] Additionally, B cell

activation can stimulate factors of the innate immune response like the complement system.[58] Most importantly, activation of a full-blown immune attack leading to elimination of the respective "danger", requires presence of negative/regulatory feedback loops that are launched following or simultaneously with the activating signals to prevent onset of autoimmunity and to enable tissue regeneration. In the course of this, the majority of effector lymphocytes is eliminated and only a small subset of T and B lymphocytes remains (memory T and B lymphocytes), providing long-term immunological memory and rapid re-activation in case of re-challenge.[48]

Cancer cells are masterful manipulators of the immune system, abusing multiple immune-regulatory mechanisms to escape immune-mediated destruction and to promote their own growth. In the following section, we will shortly describe how the innate and adaptive immune system can be activated to elicit a tumor-specific immune response, as well as outline several escape mechanisms employed by the tumor cells.

1.2.2. Innate factors in cancer immune recognition and evasion

Innate immune cells are usually the first to encounter (pre)malignant cells (Figure 1). Prominent among these are, besides cytotoxic NK cells, also phagocytic cells types such as macrophages, neutrophilic granulocytes (neutrophils) and DC that patrol through tissues.[29,59] In frame of the organism's comprehensive surveillance program against infection and malignant transformation, NK cells constitute a central unit in innate immunity.[60,61] Binding of NK cells to MHC class I via various inhibitory members of e.g. the killer-cell immunoglobulin (Ig)-like receptor (KIR) and the NKG2 receptor families signals NK cells to remain inactivated.[60,62] Viral infections and malignant transformation often result in downregulation of MHC class I presentation to evade recognition and destruction by an adaptive antitumor T cell response (see below).[63,64] However, in case a NK cell encounters lack of MHC class I on target cells it gets activated. On the one hand, activated NK cells proceed to kill target cells via death receptor-mediated apoptosis by FAS ligand (FASL) or tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) as well as via perforin-mediated plasma membrane perforation and injection of cytotoxic granzyme.[65] On the other hand, diminished MHC class I binding renders NK cells more sensitive towards activation by an array of stimulatory receptors including natural cytotoxicity receptors (NKp46, NKp30, and NKp44), C-type lectin-like receptors (NKG2D, NKG2C), 2B4, DNAX-accessory molecule-1 (DNAM-1), NTB-A, NKp80, CD59, and CD16 among others,[60] that recognize stress signals displayed by target cells. For instance, the activating NK cell surface receptor NKG2D recognizes membrane-bound ligands belonging to the MHC class I-related chain A/B (MICA/MICB) family on the surface of transformed cells. As NK cell activity is based on a balance between activating and inhibitory signals, massive stress-induced activating signals may even overrule the presence of MHC class I molecules on the cancer cell surface, allowing MHC class I-independent cancer cell death induction.[66] In turn, cancer cells may evade detection by innate immune cells by hiding or downregulating activating NK cell ligands, e.g. stress-induced MICA/MICB family proteins (Figure 2).[67-69]

This mechanism, together with release of diffusible forms of MICA/MICB into the extracellular matrix (ECM) to act as decoy ligands, inhibits proper NK cell activation.[70] Furthermore, intratumoral NK levels can be decreased due to downregulated levels of respective chemo-attractants (e.g. CXCL2).[65]

In addition to NK cells, phagocytic cell types of the innate immune system are involved in cancer immune-surveillance. During their patrols, macrophages, neutrophils and DC may sense altered molecular patterns in the (pre)malignant tissue, summarized as damage-associated molecular patterns (DAMP) or alarmins.[71,72] DAMP sensors on immune cells, termed surface pattern recognition receptors (PRR), include Toll-like receptors (TLR), retinoid acid-inducible gene I (RIG-I)-like receptors (RLR), C-type lectins (CLR), nucleotide-binding oligomerization domain (NOD)-like receptors (NLR), absent in melanoma 2 (AIM2)-like receptors (ALR), and oligoadenylate synthase (OAS)-like receptors (OLR).[73-75] PRR typically mediate recognition and, in turn, killing/phagocytosis of aberrant cells. In addition to PRR interaction, phagocytosis of (dying) cancer cells may be triggered via antibody and complement opsonization of the cell surface (compare below).[58,76,77] Besides engulfment of aberrant cells, predominantly macrophages and neutrophils release pro-inflammatory cytokines (TNF-α, interferon-γ (IFN-γ), interleukins (e.g. IL-1α/β, IL-6), chemokines (e.g. chemokine ligands CCL1 and CCL2),[2,78,79] reactive oxygen species (ROS) and matrix-remodeling enzymes into the microenvironment, creating a pro-inflammatory milieu and attracting further immune cells.[80] This also mediates blood vessel dilation and expression of endothelial surface adhesion proteins for leukocyte adhesion and invasion.[61,81] Additionally, IFN-γ stimulates cancer cells to display more MHC class I on their surface.[69] A further aspect of DAMP-mediated immune activation is the so-called inflammasome initiation inside the phagocytes. Upon detection of DAMP, multiprotein complexes are formed including the involved PRR like NLR or ALR.[82] These complexes serve as a scaffold for caspase 1 that subsequently activates IL-1β and IL-18. Depending on the PRR involved, various forms of inflammasomes exist, e.g. the NLRP3 inflammasome.[83] Disease, cell type, and the stimulus triggering inflammasome formation further determine the specific nature of the immune response.[84] In cancer, the role of the inflammasome is not well established and both pro- and anti-tumorigenic effects have been reported.[83,85] Application of different chemotherapeutics including metal drugs might influence and modulate inflammasome-mediated immune functions.[82,84,85] Formation of the inflammasome is also of central importance in the recently described influence of the gut microbiome on the efficacy of anticancer therapy (compare chapter 5).[86] The above described mechanisms may initially promote successful immune-mediated eradication of malignant cells. However, in case of persistent inflammation and activation of immunosuppressive checkpoints, these pro-inflammatory mechanisms can distinctly promote malignant progression.[71]

Among phagocytes, macrophages are characterized by a broad spectrum of tumor-promoting to -inhibitory activities based on phenotypic plasticity (a process called macrophage polarization).[87] They can roughly be divided into two subtypes designated as M1 and M2. The M1 ‘classical’ subtype constitutes the pro-

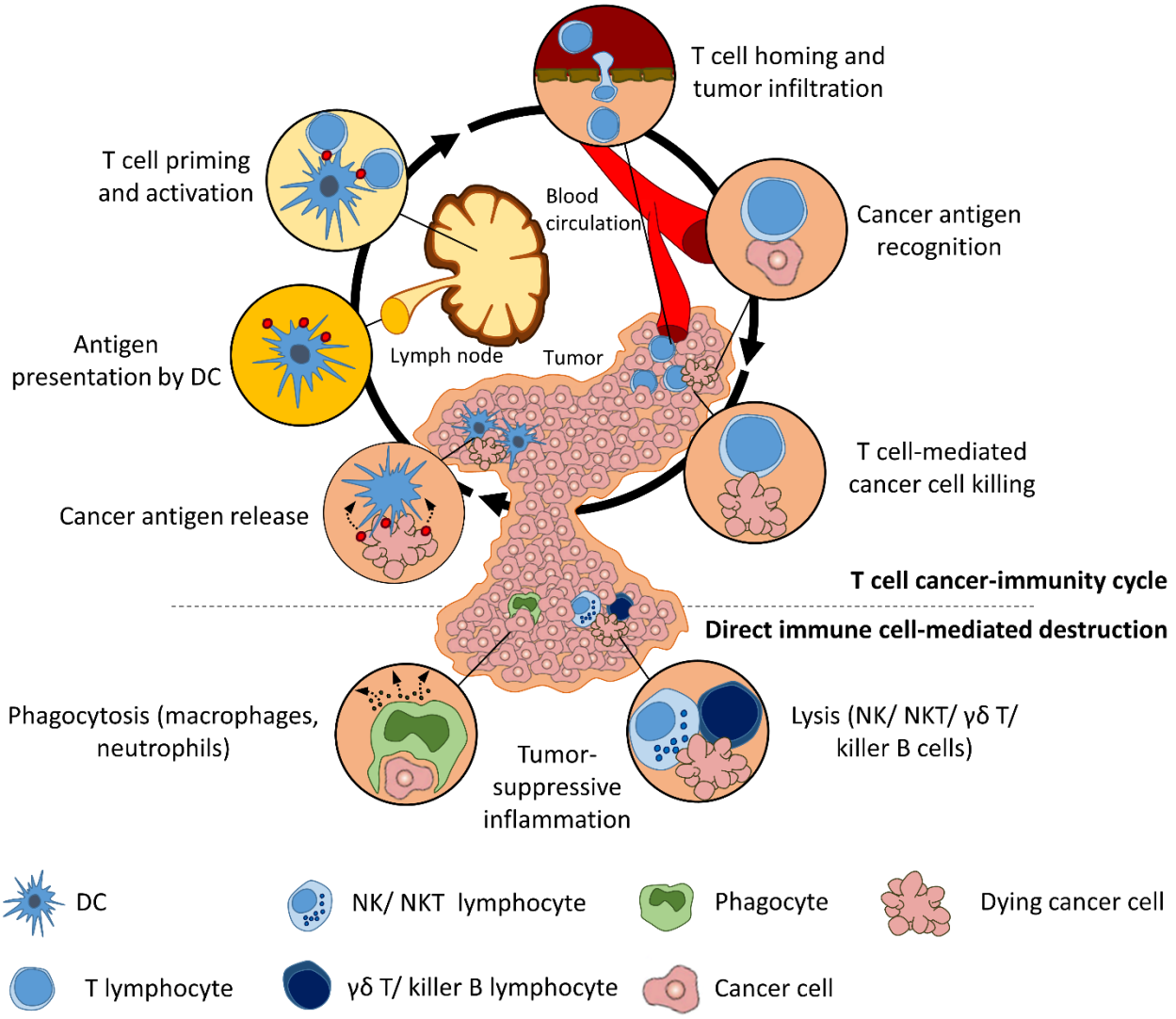

Figure 1. Most important anticancer immune surveillance mechanisms. Cancer immune surveillance is mediated by direct immune cell-mediated destruction as well as by the T cell-based anticancer immunity cycle. Direct immune cell-mediated destruction comprises phagocytosis (mainly by macrophages and neutrophils), as well as direct lysis by NK cells and several other specialized lymphocyte subpopulations (NKT, γδ T, killer B cells), both accompanied by immune-stimulatory inflammation. The T cell cancer-immunity cycle is a self-propagating and self-amplifying process, leading to antigen-specific antitumor T cell responses. This process comprises tumor (neo)antigen engulfment by APC (majorily DC) in the (pre)neoplastic tissue, traveling of APC to lymph nodes, presentation of tumor antigens to naïve T lymphocytes, traveling of activated T lymphocytes via the bloodstream to the (pre)malignant tissue, followed by specific destruction of aberrant (pre)malignant cells expressing the respective (neo)antigen. For detailed description see text. APC, antigen-presenting cell; NK cell, natural killer cell; NKT, natural killer T cell; DC, dendritic cell; (adapted from Chen and Mellman, 2013[2]).

inflammatory phenotype and is activated by PRR such as TLR together with pro-inflammatory cytokines including IFN-γ, TNF-α and IL-1β.[59,88] PRR activation of M1 macrophages typically induces MyD88 and MyD88 adapter-like (Mal/Tirap)-dependent downstream effector cascades.[89] IFN-γ is the main M1-stimulating cytokine produced by other immune cells as well as by macrophages themselves.[59] In contrast, M2 macrophages are generally associated with an immune-repressive, protumorigenic phenotype.[59] Physiologically, M2 macrophages are mainly involved in tissue homeostasis, remodeling and wound healing

by removing cellular debris created upon "tolerogenic" cell death (compare chapter 2.2.5). M2 polarization of macrophages is mediated primarily by IL-4 but also by other anti-inflammatory cytokines such as TGF-β, IL-10, IL-13, and prostaglandin (PG) E_2.[29,87] These immunosuppressive mediators are also produced by M2 macrophages themselves, together with vascular endothelial growth factor (VEGF), and matrix-degrading enzymes.[59,90] Recently, besides these main subtypes, the existence of regulatory macrophages (M_{reg}) exerting primarily anti-inflammatory and T cell-inhibitory activities by secreting IL-10 has been described.[88,91]

During tumor progression, cancer cells can deactivate phagocytic and immune-stimulatory programs and support, in cooperation with regulatory adaptive immune cells (see below), a tumor microenvironment (TME) which typically homes a heterogeneous population of mostly immunosuppressive myeloid cell types, including tumor-associated macrophages (TAM), tumor-associated neutrophils (TAN), and myeloid-derived suppressor cells (MDSC) (Figure 2).[92-96] Furthermore, cancer cells may upregulate "don't eat me" signals such as CD47 on their surface to evade phagocytosis by macrophages (compare chapter 2.2.4).[97] TAM are attracted by CCL2 and can reach high tumor abundance.[29,98,99] The chemokine/cytokine profile (e.g. IL-4, IL-10, IL-13, TGF-β) released by local regulatory immune cells favors macrophage polarization into a M2 phenotype.[98,100,101] M2 TAM, in turn, are believed to tolerize DC and to blunt adaptive (T cell-mediated) immune responses towards neoplastic cells. The unifying term MDSC describes another regulatory tumor-infiltrating innate immune cell subset, which comprises a heterogeneous collective of immature myeloid cell types (macrophages, neutrophils, and others). The most prominent suppressive functions exerted by MDSC involve release of immunosuppressive cytokines, expression of arginase-1 (compare chapter 1.2.5) and nitric oxide (NO), as well as production of ROS and PGE2.[102-105]

1.2.3. The anticancer immune cycle in cancer cell recognition and evasion

An effective, adaptive anticancer immune response has been designated as the cancer immune cycle and interconnects both innate and adaptive arms of immunity in a highly complex fashion (Figure 1).[2] According to this model, cancer cell-specific molecular patterns are recognized and elicit a potent and specific, predominantly T cell-mediated immune response.[106] With regard to activation of a specific anticancer immune response as depicted in the immune cycle, professional APC, especially DC, play a central role.[107] DC roam tissues in search for DAMP released by (dying) cancer cells. Activated DC then migrate to tumor-draining lymph nodes and present tumor antigens via MHC class I or MHC class II complexes to naïve $CD8^+$ or $CD4^+$ T lymphocytes, respectively (signal 1). MHC-restricted T cell activation leads to differentiation into effector T cells, namely CTL or T_H cells.[108,109] T_H cells can be further categorized into several subgroups like T_H1, T_H2, and T_H17 cells, as well as immunosuppressive regulatory T cells ($CD4^+/FOXP3^+$ T_{reg}, see below), characterized by different cytokine profiles and targeting distinct groups of effector cells. As already described, differentiation into effector T cells additionally depends on activation

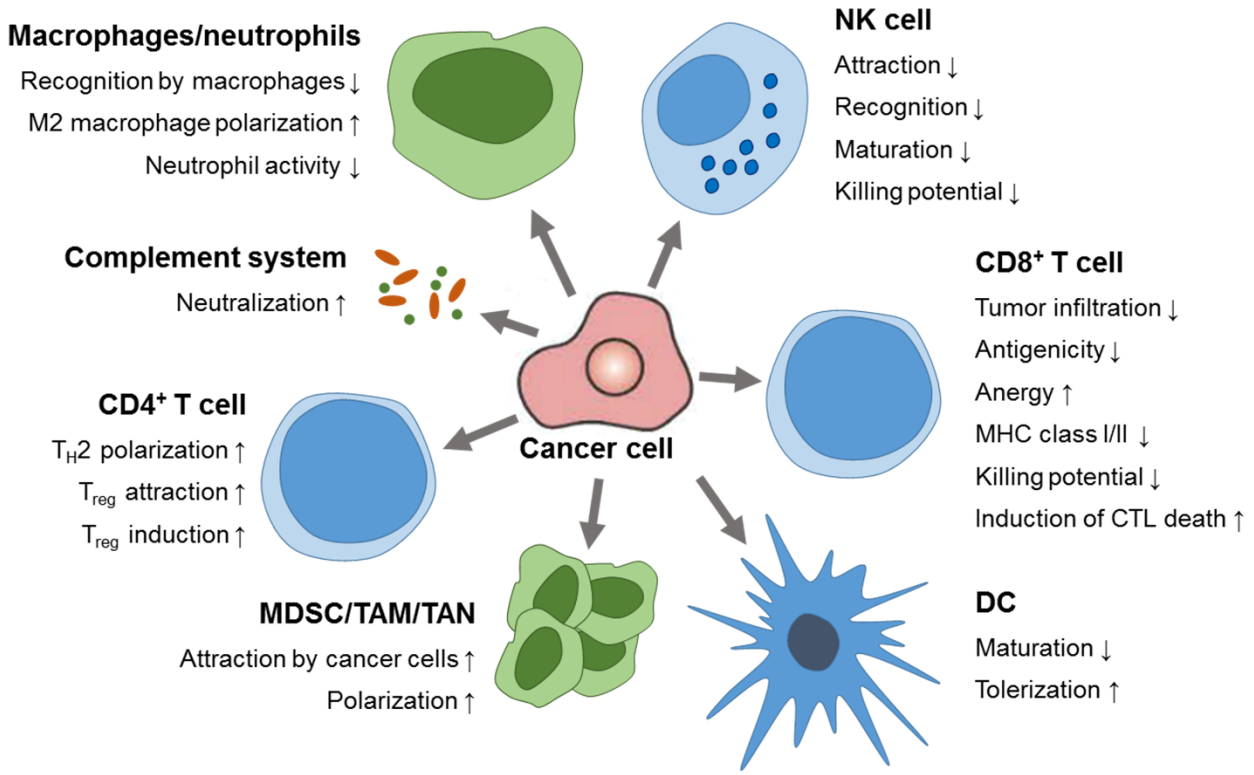

Figure 2. Major cancer immune evasion mechanisms. Cancer cells evolve multiple mechanisms to elude detection and destruction by the innate and adaptive immune system. Malignant cells evade recognition by innate immune cells (e.g. macrophages, neutrophils) via upregulation of "don't eat me" signals (e.g. CD47, CD73). Attraction of and recognition by NK cells is reduced via downregulation of chemokines (e.g. CXCL2) and stress ligands (activating NKG2 ligands such as MICA/MICB). Complement system-mediated cytotoxicity is counteracted by expression of neutralizing complement regulatory proteins (mCRP, predominantly CD46/55/59). Low cancer cell antigenicity (subclonal/non-immunogenic mutations) hampers the propensity for antitumor CTL priming. In addition, ECM- and tumor endothelium remodeling (e.g. by selectin/ICAM/VCAM downregulation) reduces immune cell infiltration into the TME. Cancer cell recognition by CTL is hampered by decreased antigen display (via e.g. MHC class I downregulation). CTL and NK cell killing potentials are diminished by cancer cell apoptosis resistance (via upregulation of IAP and/or downregulation of pro-apoptotic factors). Cancer cells attract regulatory immune cell types (T_{reg}, MDSC) to the TME by production of immunosuppressive chemokines (including CCL2, CCL22). Furthermore, cancer cells polarize immune cells into immunosuppressive phenotypes, including M2 macrophages (by CSF-1), T_H2 cells and T_{reg} cells (by TGF-β, IL-10) or impair maturation/induce anergy of DC and CTL by immunosuppressive cytokines (including TGF-β, IL-10, M-CSF) or metabolic enzymes (IDO, arginase) and, in coorperation with regulatory immune cells, via expression of immune checkpoint molecules (e.g. PD-L1/2). In addition, cancer cells induce immune cell death (e.g. CTL, NK cells) by release of cell death receptor ligands (e.g. FasL counterattack). CXCL, C-X-C motif ligand; NK cell, natural killer cell; MICA/MICB, MHC class I-related chain A/B; mCRP, membrane-bound complement regulatory protein; CTL, cytotoxic T lymphocyte; ECM, extracellular matrix; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; TME, tumor microenvironment; MHC, major histocompatibility complex; IAP, inhibitor of apoptosis; T_{reg}, regulatory T cell; MDSC, myeloid-derived suppressor cell; CCL, chemokine ligand; CSF, colony stimulating factor; TGF-β, transforming growth factor-β; IL, interleukin; M-CSF, macrophage colony stimulating factor; IDO, indoleamine 2,3-dioxygenase; PD-L1/2, programmed death-ligand 1/2; FasL; Fas ligand; For details and references see text.

and quality of co-stimulatory signals (signal 2), comprising interaction of the B7-type CD80/86 receptor on DC with CD28 on naïve T lymphocytes,[110] and the impact of cytokines (e.g. the activating cytokines IL-2 and IL-12, signal 3).[107,111] Co-stimulatory receptors like CD28 are competing with inhibitory receptors like cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on the surface of T cells,[112] providing an essential negative feedback loop to avoid immune hyperstimulation. Blocking of inhibitory receptors and ligands by so-called immune checkpoint inhibitors represents one of the most successful therapeutic strategies in

current anticancer immunotherapy so far (compare chapter 8).[113] Differentiation into effector CTL can be achieved by highly activated DC alone. However, it is in most cases supported by T_H cells, together with primarily T cell-derived IL-2 production.[28,114,115] Effector CTL then leave the lymph nodes and traffic to tumor sites,[116,117] where they infiltrate into the tumor bed and recognize their respective antigen target on cancer cells. Analogous to the above-described NK cell-mediated cell killing, CTL directly induce target cell death in two ways, death receptor- as well as membrane perforation-mediated destruction. As an overarching mechanism of immune evasion, cancer cells are able to acquire general resistance to immune cell-induced apoptosis e.g. by truncating the death receptor cascade or by upregulating inhibitors of apoptosis (IAP) (Figure 2).[118] This renders them unresponsive to the killing attempts of CTL and NK cells by death receptors as well as granzyme injection. In fact, cancer cells may turn the tables and induce e.g. FASL expression to activate apoptosis of attacking immune cells, a mechanism termed "FAS counterattack".[119]

In addition to those T cells described above (CTL, T_H, T_{reg}), which express a TCR composed of highly variable α and ß chain heterodimers, there exists another T cell subtype implicated in an antitumor immune response. These cells, named γδ T cells after their TCR consisting of invariant γ and δ chain heterodimers, play a central role in cancer immune surveillance.[120,121] Unlike conventional αβ T cells that are activated by APC in a MHC-restricted manner, γδ T cells are able to recognize tumor antigens directly and MHC-independently, and exert potent lytic activity against tumor cells.[122] A further T cell variant are the so-called natural killer T (NKT) cells. These cells share properties of both T and NK cells and increase antitumor responses by efficient production of cytokines like IFN-γ.[123] NKT cells also carry an invariant TCR, constituted of α and β chains with limited diversity, which recognizes glycolipid antigens presented by the MHC class I-like molecule CD1d on cancer cells.[124]

Regarding escape from adaptive immune responses, it needs to be considered that oncogenic driver mutations and other genomic alterations like translocations are rarely immunogenic.[125] This suggests efficient eradication of all (pre)malignant cells harboring immunogenic mutations in the process of immunosurveillance. Alternatively to non-immunogenic driver mutations, T cell detection can be avoided by downregulating antigen presentation on the cell surface.[106] Underlying mechanisms include the transcriptional silencing of MHC class I expression, loss of the ß2 microglobulin chain blocking MHC class I translocation to the plasma membrane, or impaired MHC class I-antigen loading in the endoplasmic reticulum (ER) e.g. by downregulation of the transfer proteins TAP1/2.[106,126,127] In addition, tumor cells may attract, together with the above-mentioned regulatory myeloid cell types, immunosuppressive T_{reg} cells or release immunosuppressive mediators such as TGF-ß or IL-10, inducing effector T_H cell transdifferentiation into so-called induced T_{reg} (iT_{reg}) cells and establishing tumor-promoting "safety" zones (Figure 2).[128-132] Moreover, tumor cells may secrete CCL22, which attracts circulating T_{reg} via their chemokine receptor 4 (CCR4).[133] T_{reg} cells further attenuate immune attack by deactivating CTL and shifting the T_H cell balance

from T_H1 to a more anti-inflammatory T_H2 phenotype via soluble mediators including TGF-ß and IL-10.[52,134,135] TGF-ß can additionally repress MHC class II expression on phagocytes and induce apoptosis of APC or effector T cells.[29,132] In human cancer, T_{reg} form a considerable part of tumor-infiltrating lymphocytes (TIL) and may constitute 10% to 30% of a patient's $CD4^+$ T lymphocyte population.[136] Additionally, also $CD8^+$ T_{reg} cells have been described.[137] In recent years, a subset of T_{reg} cells, that are negative for the T_{reg}-specific marker FOXP3, termed type 1 regulatory T (Tr1) cells, has received increasing attention.[138] Tr1 cells might play an important role in the chemotherapy-associated anticancer immune response.[139] As mentioned above, TAM are thought to tolerize not only DC, but also CTL towards tumor neoantigens, causing CTL anergy and T_{reg} induction.[140] In addition to the more systemic immune checkpoint function of CTLA-4, another crucial but more peripheral immune checkpoint is executed by the programmed-death/programmed-death ligand 1 (PD1/PD-L1) system, mainly acting directly within the tumor tissue.[111] Binding of PD-L1 expressed on the surface of cancer cells as well as T_{reg} and TAM to the inactivating receptor PD1 on CTL induces their deactivation and T cell anergy (Figure 2).[141-144] Besides cancer cells, T_{reg}, and TAM, also MDSC may express PD-L1 and exert a general inhibitory activity on various antitumor immune responses, including, but not restricted to, blockade of T cell functions and T_{reg} induction.[102,145]

1.2.4. Role of B cells in cancer progression

Despite the focus especially of immunotherapeutic strategies on the T cell arm of adaptive immunity, also B cells should be mentioned as they are gaining enhanced attention in the field of onco-immunology.[146] However, with regard to the diverse states of conventional but also memory and regulatory B cells (B_{reg}), a dichotomous picture emerges. Besides distinctly anti-tumorigenic activities, an important tumor-promoting role of this major lymphocytic branch of humoral adaptive immunity - partly even based on antibody secretion - becomes more and more evident.[146,147] Commonly, however, upon antigen recognition via the BCR, B cells present the processed antigen via MHC class II to T_H cells in secondary lymphoid organs.[148] Following linked recognition, B cells proliferate and, with the help of T_H cells, generate a germinal center for class switching and V-region somatic hypermutation of the BCR to optimize antigen affinity.[148] Full B cell activation for antibody production[56] requires the co-stimulatory binding of CD40L of follicular T_H (T_{FH}) cells to the CD40 receptor on the B cell surface as well as IL-4 and IL-21 secretion.[149,150] Coating of cancer cell surfaces by antibodies of the proper subclass (mainly IgG1 in humans) elicits multiple cell-killing mechanisms like ADCC and ADCP (compare chapter 1.2.1).[57,151] Antibody-binding additionally attracts components of the complement system, further stimulating engulfment by phagocytes via complement receptors such as C3b receptor.[58] Independently of a cellular response, late complement components introduce a pore-forming membrane attack complex (MAC), directly inducing (cancer) cell lysis. In frame of immune escape, complement activation on antibody-bound cancer cell surfaces may be counteracted by

overexpression of membrane-bound complement-regulatory proteins (mCRP), mainly CD46/55/59, neutralizing the classical complement pathway-mediated cell killing.[152]

Interestingly, the production of antibodies is not the only way of peripheral B cells to exert their functions also in the context of malignancy.[146,153] Several studies have dissected that B cells can be important for an optimal CTL response based on antigen presentation by B cells to T cells in the lymph node.[153,154] Accordingly, mice lacking B cells exert a defective antigen-induced T cell proliferation.[155] Additionally, so-called killer B cells (Figure 1) might be able to induce - analogous to CTL - cancer cell death via death receptor ligands like FASL or TRAIL but also directly via lytic molecules including TNF-α and granzyme.[154,156] Also in case of B cells, multiple lines of evidence exist that these important mediators of humoral immune defense might not only support anticancer immunity, but, context-dependently, also strongly support malignant progression and cancer therapy resistance.[147,157] Accordingly, several murine cancer models work less efficiently in mice lacking B cells.[154] The underlying mechanisms might be Ig-dependent or -independent.[157] Regarding the first situation, it has been demonstrated that circulating antibody immune complexes (CIC), also involving conjugated complement C1q, are deposited into the tumor bed based on the well-known enhanced permeability and retention (EPR) effect of fenestrated blood vessels.[158] Interaction of tumor-resident myeloid cells with CIC via their Fcγ or C5a receptors polarizes them towards immunosuppressive phenotypes like M2 in case of macrophages.[159,160] This induces secretion of suppressive cytokines including IL-4 and IL-10 from the myeloid compartment, leading - together with suppressive cytokines derived from B cell subpopulations - to reprogramming of $CD4^+$ T cells from T_H1 towards T_H2 differentiation.[147] As a final consequence, CTL are inactivated while tumor-promoting molecules like remodeling enzymes and pro-angiogenic factors are overexpressed.[147] Additionally, smaller B_{reg} subsets in malignant tissues are capable of exerting massive immunosuppressive effects even in the absence of an Ig response.[146] This is based on the secretion of regulatory cytokines like IL-10, IL-4 and TGF-β and induction of immune checkpoint molecules like CTLA-4 or PD-L1 on other immune cells, further promoting anergy especially of $CD4^+$ T cells.[157]

1.2.5. Additional factors in cancer immune evasion

In addition, several specific traits of the TME may promote cancer immune evasion (Figure 2). Hence, metabolic features of either cancer cells or regulatory immune cells might support an immunosuppressive cancer environment. One important example is overexpression of the enzyme indoleamine-2, 3-dioxygenase (IDO) by MDSC, TAM or cancer cells, degrading tryptophane to the immunosuppressive metabolite kynurenine, depriving T cells of an essential nutrient and in parallel inducing T_{reg} expansion.[118,161-163] Additionally, overexpression of arginase-1 in MDSC and M2 macrophages mediates T and NK cell inactivation based on L-arginine depletion.[164,165] Secretion of VEGF by cancer cells may, via the production of NO, diminish leucocyte homing into the tumor bed by downregulating adhesion molecules including p-

selectin, intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) on endothelial cells lining the tumor vasculature.[81] Furthermore, cancer cells may generate immune-privileged sites characterized by a dense surrounding ECM, creating a physical barrier impeding immune cell infiltration.[166,167]

In the following chapters, it will be outlined how anticancer metal drugs might interfere with the described mechanisms of cancer immune evasion.

2. Anticancer metal drugs and the immune system: general aspects

2.1 Metal drugs in experimental and clinical cancer therapy

Metal drugs including gold (Au) and arsenic (As) compounds belong to the oldest remedies of humans and have been used from ancient Egyptians, Greeks and Chinese to modern societies to fight a broad array of diseases including cancer.[168-170] Moreover, synthesis and evaluation of novel anticancer metal drugs is still one of the most active fields in inorganic medicinal chemistry.[23-27,171] Surprisingly, only few metal drugs have been approved for clinical use in oncology (Figure 3) which, however, still represent central components of systemic therapeutic interventions for many cancer types.[172,173] Hence, at the side of Pt, cisplatin (Figure 3A) and/or the less toxic and less active second generation Pt drug carboplatin[19,174] (Figure 3B) are approved worldwide for treatment of lung, bladder, cervical, ovarian and testicular cancer (in the latter often with curative outcome) as well as mesothelioma, but are also used in several rare diseases like osteosarcoma and childhood brain tumors, mostly within multidrug combination schemes.[19,175,176] Oxaliplatin (Figure 3C), the only third generation Pt drug approved for clinical use so far, forms a different and bulkier diaminocyclohexane adduct at the identical DNA site and is active against cisplatin-resistant models.[173] The underlying mechanisms include differences in the recognition of the respective DNA adducts by mismatch repair (MMR) mechanisms, supporting only in case of cisplatin and carboplatin, but not oxaliplatin, cell death induction.[177] Moreover, interaction with HMG proteins like HMGB1 differs between these Pt adducts, which might also underlie the clearly enhanced activity of oxaliplatin in notoriously HMGB1-overexpressing colorectal cancers.[178] Oxaliplatin is nowadays used primarily within therapeutic regimens for colorectal and pancreatic cancer patients.[179,180] Additional Pt(II) compounds with clinical approvals in selected countries are nedaplatin (Japan) (Figure 3D), lobaplatin (China) (Figure 3E) and heptaplatin (South Korea) (Figure 3F), while Pt(IV) compounds including satraplatin have not reached clinical approval so far.[181] In addition to these Pt drugs, only the As compound As trioxide (ATO, Figure 3G) is currently approved for clinical use against acute promyelocytic leukemia (APL).[182] The approval in 2000 was based on the finding that ATO allows selective APL cell differentiation based on direct targeting of the promyelocytic leukemia (PML) moiety of the respective fusion protein with the retinoic acid receptor-alpha (PML/RARα) for sumoylation and consequent proteasomal degradation (see chapter 3.2).[169] Thus, a

combination of ATO and all-trans retinoic acid (ATRA) induces cure in the majority of APL patients.[183] Furthermore, ATO has been tested against a variety of solid tumors with disparate results but has not reached approval in any other indication than APL so far.[170]

In addition to these clinically approved drugs, several Pt, ruthenium (Ru), titanium (Ti), gallium (Ga) and Au compounds have entered the stage of clinical evaluation (see www.clinicaltrials.gov for current studies).[184-187] For example the Ru complexes (compare chapter 3.3) imidazolium trans-[tetrachlorido(dimethylsulfoxide)imidazoleruthenate(III)] (NAMI-A) and indazolium trans-[tetrachloridobis(1H-indazole)ruthenate(III)] (KP1019) and its sodium analog KP1339 have been investigated in phase 1 or combined phase 1/2 clinical studies.[188-192] Especially for KP1339 (clinically termed IT-139), interesting long-lasting responses in some heavily pretreated patients with solid tumors have been reported recently, warranting further evaluation in defined tumor types.[188,193] In contrast, clinical evaluation of NAMI-A failed, based on a relatively limited activity combined with severe adverse effects at least in combination with gemcitabine.[190] This again indicates the necessity for understanding the exact basis of metal drug anticancer activity and side effects to support clinical development and avoid failure during this process.

So, what makes metal drugs especially interesting as therapeutic remedies in modern oncology? And, as the other side of the coin, why is - despite synthesis of multiple and highly diverse complexes - only a handful of them approved for clinical use? Metallodrugs are unique in that they contain both a central metal ion coordinated to frequently bioactive inorganic or organic ligands which, after release, might exhibit anticancer activities on their own. Metal ions undergo ligand exchange reactions to form covalent bonds with nucleophilic donor atoms readily available in important biomolecules as e.g. DNA or proteins. Many metallodrugs contain transition metal centers and, thus, are frequently able to undergo redox reactions.[194] This enables switches between two to up to several oxidation states characterized by differing redox potentials as well as altered reactivity. Moreover, redox reactions are prone to enhance the level of reactive oxygen species (ROS). This might specifically harm malignant tissues known to frequently harbor a disturbed redox homeostasis caused by enhanced metabolism, shortage of nutrients and oxygen (hypoxia), as well as lowered pH.[194,195] Based on these various coordination states of the metal centers, metal complexes are by far more versatile as compared to pure organic molecules for adopting diverse geometries allowing also a greater variety of stereoisomeric conformations. This versatility allows fine-tuning of the metallodrug to optimize a broad range of biological interactions but also pharmacological characteristics like organ distribution and passage through the tumor cell membranes. These features open an array of strategies to interfere with molecular targets and driver mechanisms of malignant cells but also bear the risk of toxicity and massive adverse effects (compare chapter 6).[19,196,197]

Also with regard to the cancerous tissue, the molecular targets of anticancer metal drugs might not only be present in the cancer cells themselves, but also within components of the TME, including e.g. endothelial

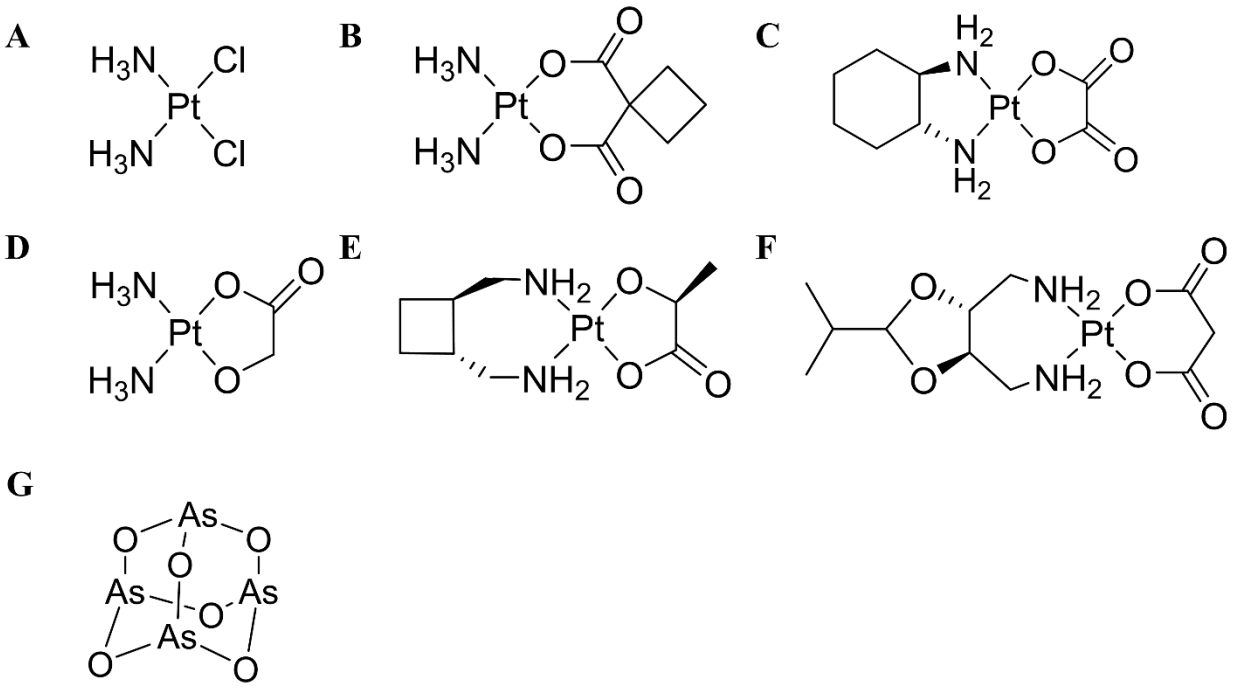

Figure 3. Chemical structures of clinically approved (A-F) Pt- and (G) arsenic-based metal anticancer complexes. (A) cisplatin, (B) carboplatin, (C) oxaliplatin, (D) nedaplatin, (E) lobaplatin, (F) heptaplatin, (G) arsenic trioxide (ATO).

cells of the tumor vasculature,[198] cancer-associated fibroblasts,[199] as well as the diverse components of the immune network allowing cancer immune escape. Accordingly, for several families of metallodrugs, anti-angiogenic[200-204] and anti-metastatic[204-207] mechanisms have been suggested as major modes of action partly by targeting primarily cells of the TME including immunocytes. However, as mentioned above, a systematic evaluation of the interaction of clinically approved and experimental anticancer metal compounds with the immune cell compartment and immunological mechanisms in the tumor tissue is widely missing. This is surprising considering the fact that already Barnett Rosenberg, shortly after reporting the anticancer activity of cisplatin as early as 1969,[208] suggested that immunological effects might underlie the cancer-selective activities of cisplatin. At that time, he considered that cisplatin might change the immunogenicity of cancer cells by removing immunosuppressive molecules from the cancer cell surface.[209,210] Even between the three clinically used Pt compounds, namely cisplatin, carboplatin and oxaliplatin, a fundamental difference regarding interactions with the immune system exists. Hence, we and others have shown that oxaliplatin is active against the identical murine colon cancer allograft model only in an immune-competent background but completely inactive in severe combined immunodeficient (SCID) or nude mice lacking T cells (Figure 4).[211,212] Methyl-substituted oxaliplatin derivatives were interestingly less affected with significant but still strongly reduced activity in the SCID background proving the delicate nature of the metal drug-immune interface in anticancer therapy response.[211] Also cisplatin is active in both backgrounds, albeit at a distinctly reduced potency in the immunocompromised situation as demonstrated by several groups using different

transplantable and chemically-induced murine tumor cell models and mouse strains.[213-215] This is surprising, considering that DNA damage is believed to represent the major mode of action for all of these drugs and direct immunological effects are still not mentioned in recent, comprehensive Pt drug reviews.[27,216] Obviously, the activity loss in mice lacking adaptive immunity suggests that an - in case of oxaliplatin even dominating - layer of immune responses is interacting with the primary cancer cell damage via DNA strand break induction. These interactions need to be dissected in detail, if successful novel generation anticancer metal drugs, especially for combination with immunotherapeutic strategies, should be developed. In that regard, it needs to be considered that preclinical evaluation of anticancer (metal) drugs is predominantly based on cytotoxicity tests *in vitro* or - if in animal models - mostly in an immunodeficient xenograft background. As an example, oxaliplatin was - in contrast to irinotecan - widely inactive against a large panel of patient-derived colorectal cancer xenografts,[217] a situation certainly not reflecting the clinical situation.[218] At that basis, oxaliplatin would have presumably missed clinical approval in this cancer entity. Conversely, one might hypothesize that at least some of the numerous experimental anticancer metal drugs not further developed based on low efficacy in initial xenograft models, could exert high therapeutic potential in an immune-proficient situation.

Additionally, it needs to be mentioned that many publications on the interaction of anticancer metal drugs (but also other therapeutic approaches) with the immune system are based on *in vitro,* i.e. cell culture data, or data of animal models. This should be considered when trying to predict the clinical situation in cancer patients. Moreover, several immune cell models, like e.g. murine RAW264.7 cells and human THP1 or U937 cells, both widely-used macrophage models, were established from a malignant background.[219] This implicates that these cell lines, although very helpful and widely resembling the monocyte/macrophage compartment, might well harbor deregulated signaling modules not entirely representative for an *in vivo* macrophage response to cancer therapy. In addition, immune cell subtype models may contain immune cell mixtures not fully reflecting the real-life situation. As one example, so-called cytokine-induced killer cells (CIK) derived from peripheral blood mononuclear cells (PBMC) by sequential incubation with CD3 monoclonal antibody and activating cytokines *in vitro* may harbor heterologous populations of $CD3^+$ T cells, $CD3^+CD56^+$ NK cell-, and $CD3^+CD56^-$ CTL-like sub-compartments, together exerting strong anticancer activity.[220] Similarly, lymphokine-activated killer (LAK) cells (lymphocytes, which were stimulated with IL-2 *in vitro*[221]) or studies using macrophage populations isolated by peritoneal lavage of untreated mice (peritoneal macrophages[222]) usually consist not only of one cell type but a rather undefined cell mixture. Consequently, whether any comparable cell type mixtures occur in the TME of cancer patients and, hence, results derived from such models can be directly conferred to the clinical situation is unclear and speculative. However, this might change in frame of adoptive therapeutic approaches, as e.g. CIK cells represent innovative tools in cancer immunotherapy in both autologous and allogeneic settings especially in hematological malignancies.[220]

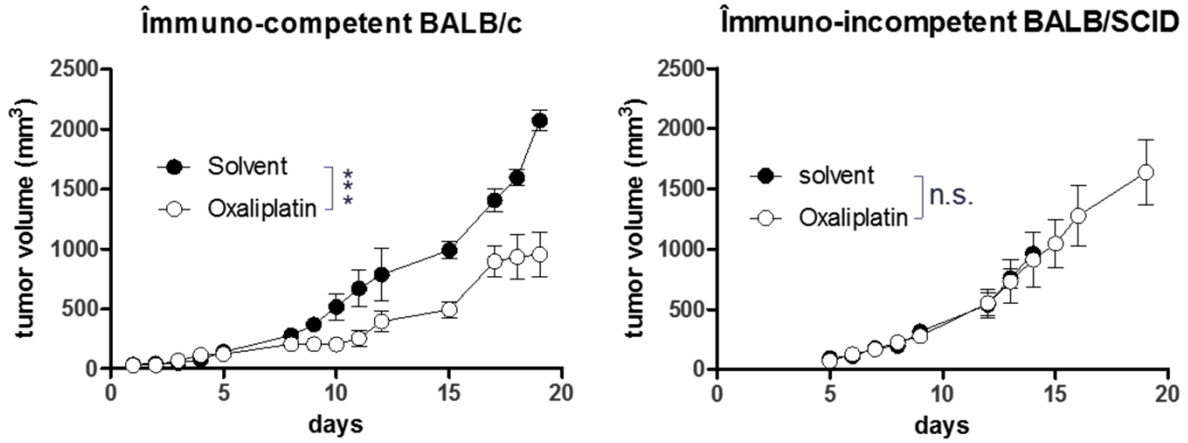

Figure 4. Immune-dependency of oxaliplatin-mediated anticancer responses. Oxaliplatin completely loses its anticancer activity against the identical CT26 murine colon cancer allograft model in an immunodeficient SCID background lacking B and T cells when compared to the immuno-competent BALB/c strain. All experimental settings and the injected cancer cell population were identical.

So, what are the main mechanistic aspects that need to be considered, as to how the immune system might contribute to the anticancer activity of metal drugs? Or, asked the other way around, how do metal drugs support immune-mediated cancer eradication and, thus, might be harnessed as ideal partners for immunotherapeutic approaches? The following chapters summarize how anticancer metal drugs might alter visibility for and sensitivity to attacks by the patient's innate and adaptive immune system (Figure 5).

2.2. Impact of anticancer metal drugs on cancer cells

2.2.1. Metal drugs alter visibility of cancer cells to innate immunity

As outlined in chapter 1.2, (pre)malignant cells are recognized by NK cells primarily by loss of MHC class I expression, leading to inactivation of inhibitory receptors of the KIR and NKG2 families and hypersensitivity towards stimulation of activating receptors like for example NKG2D and DNAM-1.[29,60,61] In order to get activated, these receptors need to detect their ligands on the cancer target cells, including stress surface markers (e.g. MICA/MICB family proteins) which are often lost on cancer cells to avoid NK cell attack despite MHC class I downregulation. So, how do metal drugs impact on tumor cell recognition by NK cells but also by circulating DC, macrophages and neutrophils? Data suggest that metal compounds can synergistically prepare tumor cells for full immune recognition not only via a DC/$CD4^+$ T_H cell/CTL immune cycle (like in frame of an immunogenic cell death (ICD) as described in chapter 2.2.6) but also via enhanced detection by NK cells and macrophages (Figure 5).[67] Hence, numerous metal compounds have been demonstrated to massively upregulate diverse stress signals on the surface of cancer cells visible to the NK cell compartment like expression of NKG2D and DNAM-1 ligands including MICA/MICB members,[223] ULBP1[224] or the NKp30 ligand B7-H6F.[225] Besides NK cells, NKG2D may be also highly expressed by several T cell subsets, including NKT and γδT cells as well as activated CTL, and exert direct cytotoxic or

co-stimulatory functions in a cell type-dependent manner.[226] NKG2D and DNAM-1 ligands on cancer cells are generally upregulated by DNA damage response (DDR) signaling via the ATM/ATR pathways.[227] These signal circuits are induced by most anticancer metal drugs either based on direct DNA damage or indirect processes like generation of redox products including ROS.[228-231] Considering the broad expression of e.g. NKG2D, DDR activation would render metal drug-treated cancer cells generally more responsive to several cell compartments of both innate and adaptive immunity in an MHC-independent manner. Additionally, not only DC but also NK cells, neutrophils and macrophages are likely to sense certain alarmins/DAMP, including HMGB1 and heat shock proteins (HSP70, HSP90) on the cancer cell surface[232] to cooperate in case of NK cells with NKG2 ligands to fully activate cytotoxicity.[223] Metal drugs can support tumor cell recognition by NK cells due to upregulation of these DAMP on the cancer cell surface[233-235] that interact with certain TLR and receptor for advanced glycation end products (RAGE) molecules on NK cells.[223,236] Such alarmin-mediated effects have been reported for multiple anticancer metal drugs[235,237] and should - at least in case of HMGB1 - not only support NK cell activation but also contribute to initiation of ICD via DC activation and T cell priming (compare chapters 2.2.5 and 2.2.6).[12,22,43] Additionally, metal drugs might downregulate immune checkpoint ligands like PD-L2 on cancer cells.[238] This might have direct consequences on the activation state not only of neoantigen-specific CTL, but also of innate immune cells like NK cells as well as monocytes/macrophages expressing the respective PD-1 receptor.[239,240]

2.2.2. Metal drugs alter susceptibility of cancer cells to immune cell-mediated cell death

Full activation of NK cells finally leads to upregulation of cell death mediators including perforin/granzyme, expression of death ligands like FASL and TRAIL on the cell surface, and production of cytotoxic cytokines like IFN-γ and TNF-α.[241] These mechanisms are shared by several cytotoxic lymphocytic cell types (CTL, NKT, γδT cells) involved in killing of target cells via perforin/granzyme-mediated cell death. Multiple data suggest that metal drug-induced alterations of cancer cells render them hypersensitive to immune-mediated killing processes. These alterations might affect several steps of the respective extrinsic cell death signaling axis from initiation to execution. One of the most important aspects is the upregulation of death receptor molecules on cancer cells, e.g. death receptor (DR)4, DR5, and FAS receptor/CD95, by metal drugs for the respective ligands (TRAIL, FASL) generated by activated cytotoxic immune cells like CTL and NK cells.[242,243] During ligand interaction, these death receptor molecules recruit at the cytosolic side of the plasma membrane a death-inducing signaling complex (DISC), activating primarily initiator caspase 8.[244] Several of these death receptors are DDR genes and activated by cellular stress factors like redox imbalance and ROS generation.[245] Moreover, it has been hypothesized that especially Pt drugs might directly stabilize death receptors like FAS, DR5, and TNFR1 based on protein adduct formation at the cell membrane (compare chapter 3.1.3).[246,247] Hence, it is not surprising that multiple anticancer metal compounds including also novel metal complexes enhance death receptor membrane expression, support downstream death

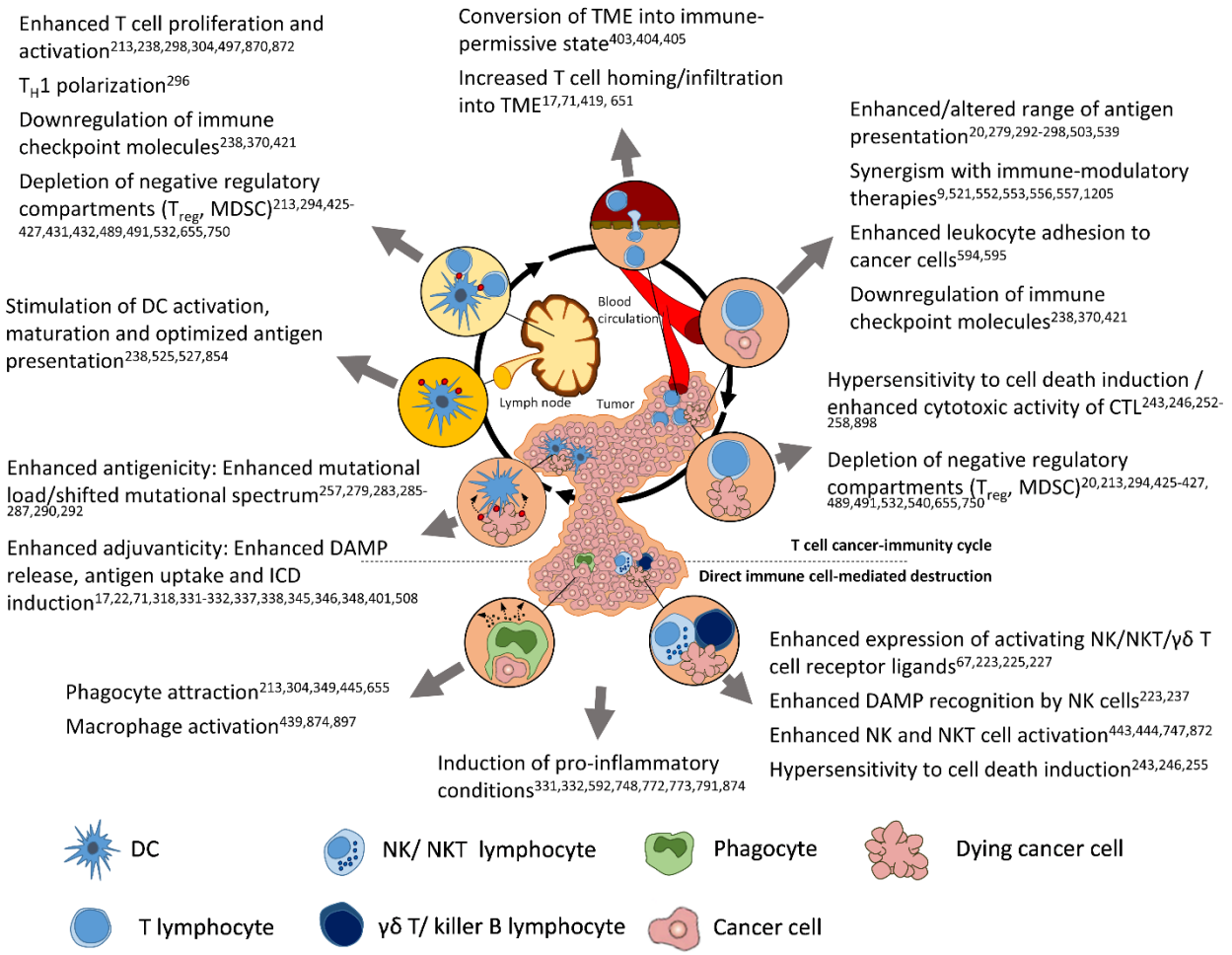

Figure 5. Key immune-stimulatory mechanisms of anticancer metal compounds implicated in the modulation of specific aspects of direct immune cell-based destruction and the T cell cancer-immunity cycle (compare Figure 1). An overview of specific mechanisms is given adjacent to each position. Selected references to research papers and review articles discussed in the text for corresponding mechanisms are given. For details see chapters 2 and 3.

signals, and synergize with death ligands like FASL and TRAIL.[225,248-252] Moreover, out of several chemotherapeutic combination approaches tested, metal-based schemes were of paramount efficacy to boost a long-peptide human papillomavirus (HPV) vaccination. Interestingly, this was less based on specific CTL-mediated target cell killing by antigen recognition than on hypersensitivity towards CTL-secreted TNF-α.[253] In addition to death receptors and their downstream signaling cascades, metal drugs might also enhance cancer cell sensitivity towards perforin/granzyme-mediated cell lysis by CTL, as for example shown for cisplatin-treated murine cervical cancer within an adoptive HPV vaccination setting (compare chapter 3.1.4).[254] Accordingly, Ramakrishnan et al. attributed a comparably enhanced sensitivity of colon cancer cells to CTL-based immunotherapeutic strategies in combination with e.g. cisplatin to enhanced granzyme uptake by a mechanism involving the cation-independent mannose-6-phosphate (M6P) receptor, also known as insulin growth factor receptor II. Interestingly, this effect was independent of FAS or perforin and also allowed killing of adjacent tumor cells lacking the CTL-specific epitopes.[255]

However, not only upregulation of death receptor expression on the cancer cell membrane in response to subtoxic metal drug doses was underlying enhanced cancer cell killing by innate and adaptive immune cell compartments. Hence, Bergmann-Leiner et al. observed that enforced CTL killing in response to e.g. cisplatin (termed here "chemomodulation") was based on both FAS-dependent and -independent mechanisms both involving caspase signaling paralleled by upregulation of the cell adhesion molecule ICAM-1.[256] Additionally, low-dose metal drug pretreatment of head and neck cancer cells in combination with irradiation enhanced the propensity to undergo CTL-mediated apoptosis.[257] This effect was based on marked reduction of the antiapoptotic proteins of the bcl-2 family, an effect also observed in NSCLC cell models treated with a mixture of cisplatin and vinorelbine (compare chapter 3.1.4).[258] The respective mechanisms do not work exclusively but in many cases cooperatively. Hence, enhanced susceptibility of lung cancer cells, pretreated with sublethal cisplatin doses, to T cell-mediated killing was depending - besides enhanced MHC class I expression (compare chapter 2.2.4) - also on hypersensitivity against perforin-mediated apoptosis and enhanced expression of pro-apoptotic genes like BBC3 (coding for the p53 downstream target PUMA), and was accompanied by reduced secretion of TGF-β and upregulation of pro-inflammatory IL-8 and the chemokine ligand CXCL5.[258] This is just one example demonstrating the layers of complexity on how metal drugs may change tumor cells for enhanced immune-mediated eradication.

2.2.3. Metal drugs alter visibility of cancer cells to adaptive immunity

Metal drugs might not only enhance the visibility of cancer cells to innate, but also to adaptive immune cells and promote successful re-establishment of a functional anticancer immune cycle at multiple stages (Figure 5). In general, the alterations can be categorized into three major functions: 1) enhanced antigenicity, meaning that cancer cells expose more neoantigens in response to metal drugs; 2) enhanced adjuvanticity, meaning that chemotherapy helps the adaptive immune system to recognize cancer cells primarily by priming recognition by DC; 3) alterations in negative feedback inhibition by immuno-suppressive mechanisms like immune checkpoint molecules often overexpressed as a consequence of cancer-promoting chronic inflammation.[3] The principles of these three major immune-regulatory functions of chemotherapy and the respective crosstalk between cancer cells and immunocytes are outlined in the next chapters.

2.2.4. Metal drugs support cancer cell antigenicity

In the classical model of immune defense (compare chapter 1.2), components of the innate and subsequently also the adaptive immunity are able to distinguish between "self" and "non-self" to allow eradication of foreign pathogens with "non-self" antigens, at the same time shielding the healthy tissues from auto-immune attacks.[259] In case of T cells, this is elaborated by a sequence of autoreactivity checkpoints in primary and secondary lymphoid organs to eliminate or deactivate autoreactive T cells, mechanisms termed "central and peripheral tolerance". Central tolerance refers to the eradication or anergy - the latter connected to $FOXP3^+$

T_{reg} induction - of inappropriately self-antigen-reactive thymic T cells by a mechanism involving medullary thymic epithelial cells (mTEC) and DC.[47,260] Self-recognition is achieved by autoimmune regulator (AIRE) protein-mediated thymic expression and extensive splicing of genes encoding tissue-specific antigens,[261] a process known as "promiscuous gene expression".[262] However, central tolerance might not deplete all auto-reactive T cell clones based on e.g. low TCR avidity or incomplete self-antigen representation in the thymus.[263] Surprisingly, a certain level of autoreactivity is even central for proper function of peripheral immunity by supporting T cell survival.[264] Consequently, several immunosuppressive mechanisms summarized as "peripheral tolerance" are essential to avoid excess auto-reactivity and induction of autoimmune diseases. This peripheral tolerance may be driven by cellular or humoral factors aiming to deactivate or kill autoreactive T cells or even convert active into regulatory immune cells like T_{reg}, in turn mediating a broader immunosuppressive environment themselves. Main players in that context are immunosuppressive cytokines and those checkpoint mechanisms serving as central hubs in successful anticancer therapy including CTLA-4, PD-1/PD-L1, lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin and mucin-3 (TIM-3), T cell immunoglobulin and ITIM domain (TIGIT), V-domain Ig-containing suppressor of T cell activation (VISTA), as well as metabolic immunosuppressive enzymes like IDO and arginase.[16,265-267] These are all mechanisms driving immune evasion during cancer development also supported by chronic inflammatory signals within the malignant tissues.[3]

Considering that cancer is derived from "perverted" cell clones of our own organism, not only cancer therapy researchers, but also the body´s own immune defense is facing an enormous problem to unequivocally distinguish cancer from healthy cells. Hence, immune recognition to initiate an effective and, in the best case, long-lasting T cell response and tumor rejection is dependent on MHC class I presentation of either viral peptides (in virus-induced cancer) or of mutation-/fusion protein-derived immunogenic neo-antigens by the malignant cells.[268] This is of strong relevance for anticancer metal drugs which are in many cases considered as mutagenic. For sure, an enhanced number of mutations and translocations would help the immune system to recognize a malignant cell clone as "non-self".[269] This fits well to the observation that cancer immunotherapy with checkpoint inhibitors is successfully used in tumors harboring a very high tumor mutation burden (TMB) including melanoma, NSCLC and urothelial carcinomas.[125,164] However, it needs to be considered that classical driver mutations of cancer with an immunogenic potential are extremely rare, as impressively summarized for highly mutated malignant melanoma by Schumacher and Schreiber.[125] Instead, successful tumor eradication due to CTL activation e.g. by checkpoint blockade is mainly based on immune recognition of very few random bystander mutations as has been recently elucidated e.g. in NSCLC.[38] In this setting, a strong mutational heterogeneity between tumor subclones (termed "subclonal" or "private" in contrast to "clonal" mutations found in the majority or all cancer cells) was associated with failure of immune therapy. With regard to oncogenic driver alterations, however, it needs to be considered that - despite their low antigenicity - the genetic make-up of tumors and especially the loss of tumor-

suppressor genes seems to be a main factor in shaping a tumor's immune landscape.[270] This is based on the intricate crosstalk of cancer cell signaling and metabolic circuits with the tumor immune-environment. Consequently, the predictive power of defined genetic signatures (despite not representing neo-antigens) for cancer immunotherapy response is intensely investigated in the field of immunogenomics.[269,270]

Unexpectedly, it has been suggested recently that - in contrast to a high TMB - presence of severe chromosomal instability (CIN) and multiple structural chromosomal aberrations is clearly associated with reduced activity of cancer immunotherapy,[271] adding an additional layer of complexity to cancer immune-recognition. Surprisingly, the group of Guido Kroemer demonstrated that tetraploidy (induced by transient nocodazole exposure) was facilitating cancer immune recognition.[272] However, despite enhanced peptide MHC class I presentation of tetraploid cells, no enhanced antigenicity could be detected, suggesting rather enhanced adjuvanticity via DAMP release as underlying mechanism[273] (compare chapter 2.2.5). As tetraploidization was accompanied by ER stress signals and calreticulin (CRT) exposure, the authors interpreted the above-mentioned immune-resistance of CIN-positive cells as selection against tetraploidization-mediated immune eradication.[273] With regard to metal drugs, it needs to be kept in mind, that chromosomal aberrations might be induced especially during resistance development[274-276] that could distinctly influence recognition by anticancer immune responses. In contrast to sensitization against immune eradication, tetraploidization was leading to distinct resistance against DNA damage-inducing therapeutics like oxaliplatin.[277] Hence, combination with immunotherapy e.g. by checkpoint inhibitors might represent a feasible strategy to overcome polyploidy-induced metal drug resistance.

In addition to hard-core "non-self" tumor neoantigens, the immune system is able to identify cancer cells based on the detection of "self" antigens with de-regulated expression including differentiation (e.g. gp100, MART-1) and germ cell (e.g. MAGE, CEA) antigens summarized under the term "tumor-associated antigens" (TAA).[278] Expression of those TAA may be stimulated by metal drugs supporting enhanced antigenicity.[252,257,279] These antigens were a long time considered as very attractive especially in vaccination and adoptive cell transfer strategies, as they are shared by larger patient subgroups as compared to the very specific mutational patterns concerning neo-antigens.[280]

Observations regarding the narrow ridge of cancer antigenicity have direct implications on the use of frequently mutagenic (metal) drugs[281] as tumor antigenicity promoters, especially as partners for immunotherapeutic settings. For alkylating agents like dacarbazine, such an effect has been worked out in detail and was summarized under the term "drug-induced xenogenization".[282] Indeed, metal drug therapy upregulated mutation frequency and changed mutational signatures (characterized by enriched C:G to A:T transversions) as well as clonal heterogeneity in some studies.[38,283,284] In a recent whole-genome sequencing analysis, cisplatin induced by far the highest mutation rates within eight frequently used chemotherapeutic agents in a chicken lymphoblast cell line model.[285] This enhanced mutation rate should - at least theoretically - strongly enhance abundance of neo-antigens and - based on deregulated gene expression control - probably

also TAA, together leading to enhanced antigenicity. Pre- or post-treatment mutational heterogeneity was often associated with Pt therapy-resistant recurrence and worse prognosis[38,284] which would again argue for combination with immunotherapy probably benefiting from mutational load. Accordingly, the group of Robert S. Kerbel reported recently that murine models selected against cisplatin or cyclophosphamide displayed for both drugs an enhanced mutational load but improved response to immune checkpoint inhibition only in case of the metal compound.[286] These observations would implicate a possible role of immunological factors in metal drug therapy response. However, the situation might also in this case be more complicated, and an enhanced induction of mutational load by metal drug therapy has not been demonstrated in all cases. Additionally, regarding mutational clonality, the results are contradictory and probably influenced by the genomic background and DNA repair capacity of the tumor type. In a melanoma cohort, chemotherapy-enhanced mutational load was accompanied by increased heterogeneity and mutational subclonality paralleled by resistance to CTLA-4 inhibitors.[38] Concerning Pt-based combination regimens, enhanced TMB with frequent inactivation of DNA repair genes was found in post-treatment as compared to pre-treatment samples of ovarian cancers using next-generation sequencing technology.[287] However, in a subsequent analysis of this dataset using algorithms to detect antigenicity, only a minor contribution of specific Pt mutation signatures but more a selection process during recurrence were predicted to enhance neoantigen expression at relapsed disease.[288] Accordingly, several recent reports did not even find reproducible altered mutation rates but more likely shifted mutational spectra following Pt-containing therapy.[289,290] Liu et al. have investigated mutational patterns of clinical urothelial cancer samples before and after Pt-containing standard therapy.[290] Pt chemotherapy did not upregulate the overall mutational load but induced a specific cisplatin mutation signature also supporting immune evasion by upregulation of PD-L1 and PD-L2 as well as of the "don't eat me" molecule CD47.[291] Interestingly, despite no clear-cut alterations in TMB, in this study "clonal" mutations were demonstrated to be enriched in urothelial carcinoma patients following Pt-based chemotherapy resistance.[292]

In addition to induction of novel mutations and, hence, neoantigens or deregulated expression of TAA, metal-based chemotherapy might enhance tumor antigenicity by supporting antigen presentation by tumor cells.[20] Accordingly, several studies have indicated that metal-based therapies might enhance MHC class I expression in cancer cells.[257,258,279,293-295] This is surprising considering the fact that cancer cells - during immune evasion - tend to shut down MHC class I presentation to avoid neo-antigen or TAA detection by tumor-specific CTL clones. The corresponding observations concern an upregulation of total MHC class I levels in diverse cancer models *in vitro* and *in vivo*, including monotherapy with low-dose Pt drugs but also combination regimens (compare chapter 3.1.4).[258,279,293-296] Metal-containing chemo-radiation therapy was associated with enhanced susceptibility to MHC-restricted, antigen-specific T cell killing.[257,294] In line with these preclinical data, enhanced MHC class I levels were recently detected in ovarian cancer progressing after Pt-containing chemotherapy.[297] In addition to enhanced robustness of antigen presentation via

increased MHC class I, metal-based chemotherapy may also broaden the range and change the hierarchy of antigens recognized by a CTL response.[298]

The question remains what underlies the massive upregulation of MHC class I presentation by anticancer metal drugs. The highly polymorphic, classical MHC class I molecules (HLA-A, -B, and -C) are primarily involved in detection of virus-infected and malignant cells, while the less polymorphic non-classical MHC class I molecules (HLA-E, -F, and -G) are mainly responsible for NK and even T cell inhibition.[299] Constitutive MHC class I gene transcription is controlled by a TATAA box, an Inr-like motif, and by the Sp1 transcription factor binding to a CA/GT-rich motif.[299] Additionally, inducible elements in the MHC class I and β-2 microglobulin promoters comprise e.g. enhancer A, IFN-stimulated regulatory element (ISRE), and SXY modules mediating responsiveness e.g. to cytokine- and stress-induced signal pathways like NF-κB and IFN-regulatory factors IRF1 and IRF3.[299,300] Accordingly, the MHC class I promoters are strongly responsive to pro-immunogenic cytokines like type I and type II IFN. Additionally, they should be activated by cell-internal stress signals as derived from viral nucleic acid species- as well as from DNA damage-mediated NF-κB pathway activation,[301] e.g. in response to anticancer metal drugs.[302,303] Interestingly, several studies demonstrated that DNA-damaging anticancer drugs like metal compounds induce cell autonomous production of pro-inflammatory cytokines in cancer cells, including type I IFN[293,304] and respective IFN-induced expression signatures.[305] The underlying mechanisms are not completely clear today but are believed to involve activation by DAMP via PRR, whose increasing importance in chemo/immunotherapy of cancer also with metal compounds is outlined in the following chapter.

2.2.5. Metal drugs enhance adjuvanticity of cancer cells to initiate an anticancer immune cycle

The classical model of adaptive immune activation solely based on detection of “non-self” patterns or antigens - already challenged by “self” TAA recognition in frame of an effective anticancer immune response - has recently been extended towards a more comprehensive model of “danger-recognition” with major implications for pharmacological cancer therapy. This model was first established by Polly Matzinger during the 1990s mainly to explain observations in transplantation and autoimmune biology.[71,306] It implicates that immunity is not only driven by “non-self” recognition but also by discrimination of “safe” and “non-safe”, the latter meaning “dangerous” and often associated with “damage”.[34] This model naturally requires a strong contribution of innate immunity. It is based on the assumption that dangerous molecules or structures, summarized as DAMP or alarmins, are - comparable to pathogen-associated molecular patters (PAMP) - recognized by PRR, leading to activation of primarily immune-stimulatory responses. Such, it is not surprising that there exists a strong overlap between danger recognition of drug-induced cancer damage via DAMP and pathogen- and virus-infected cells via PAMP.[71] PRR can reside inside the damaged cell or are activated via released DAMP on adjacent cells like DC to simulate maturation and efficacy of antigen presentation.[80] Just to mention one prominent example representatively: activation of cell-internal nucleic

acid-sensing PRR like TLR3/7/8/9 in endolysosomes or cyclic GMP-AMP synthase-stimulator of IFN genes (cGAS-STING), RIG-I, and AIM2 in the cytosol of phagocytes by single- or double-stranded DNA or RNA ligands may induce inflammasome activation and secretion of immune-stimulatory molecules like IFN and IL-1 family members.[80] While this is considered an evolutionarily conserved mechanism for sensing of foreign genetic material by cells of the innate immune system, damage induced by anticancer drugs may enforce comparable mechanisms. As one example, necroptosis (a regulated form of necrosis) induction in cancer cells is enhanced by activation of STING based on release of mitochondrial DNA into the cytosol.[307] Considering the multiple impacts of approved and experimental metal drugs on nucleic acid and mitochondrial integrity,[27,308-310] these sensor mechanisms are surprisingly unexplored. This is even more astonishing as e.g. agonists of TLR3 or TLR9 enhance chemotherapy efficacy of metal drugs when applied as single agents or within combination regimens *in vitro* and *in vivo*.[311-313]

So, what are the implications of this "danger model" for immune-oncology in general and the use of anticancer metal drugs in particular? For sure, interactions of DAMP with respective PRR molecules are already enhanced in malignant tissues before any therapeutic intervention based on increased spontaneous cell death, hypoxia and deregulated metabolic conditions as well as necrosis in the inner part of a solid tumor mass.[314] TLR might be activated in tumor cells but also cells of the microenvironment like immune cells, endothelial cells and cancer-associated fibroblasts.[314-316] DAMP/TLR interactions are even suggested to dictate the fate of the tumor by regulating tissue architecture, cell trafficking, neovascularization and immune infiltration.[316] Additionally, TLR-mediated signals are involved in metabolic reprogramming of the TME.[317] Presence and release of DAMP are strongly bolstered by cell death induction by anticancer therapy including the one with metal complexes.[22,71,318] However, also in case of cancer therapy, DAMP released from dying cancer cells may exert not only strong immune-stimulatory but also immune-inhibiting functions. The balance and composition of these factors seems to dictate whether a given drug exerts a mainly "tolerogenic", "inflammatory", or "immunogenic" cell death - the latter known as ICD.[71] Originally, cancer cell death was for quite some time differentiated solely into active apoptosis or passive necrosis.[319] Apoptosis induction was considered as the desired impact of systemic cancer therapy[320] to avoid inflammatory responses due to release of cytosolic content during necrosis ("inflammatory cell death"). Under physiological conditions, programmed removal of cells by apoptosis needs to be rather immunologically silent than inflammatory.[321] This is mediated by the exposure or release of immunosuppressive DAMP and mediators by the apoptotic cells ("tolerogenic cell death").[71] Consequently, the question arose, if drug- or radiation-induced cancer cell apoptosis also is uniformly immunosuppressive and, further asked, what the consequences regarding an anticancer immune response are.[322] During the last decades, it has been uncovered that cell death induced by anticancer drugs may result in profound and complex alterations of the immunological constitution of the TME.[12,17,318,323,324] The balance between immune-activating and -suppressing signals emitted from the dying cancer cells including DAMP and

cytokines/chemokines drives attraction or exclusion of the respective immune cell types and seems to dictate frequently the extent of therapy success.[12,71,325] In selected cases, the eradication of tumor tissue by chemotherapy or other treatment modalities is mediated by a cell death allowing activation of an entire anticancer immune cycle (compare Figure 1, Figure 5), leading even to a persistent immunological antitumor memory. This ideal form of cancer therapy-initiated cell death was termed ICD (Figure 6).[1,326] Concerning the different forms of cell death, ICD is for sure a controlled, active mechanism fulfilling morphologically the hallmarks of apoptosis as "programmed cell death".[325] In case of ICD, however, apoptosis is, based on the altered DAMP dosages and release chronology, resulting in immune-stimulatory rather than immunosuppressive effects.[71] Nevertheless, it should be mentioned, that not all forms of ICD exhibit the morphotype of apoptosis. For example, cells dying by necroptosis exhibit a strong immune-stimulating activity and allow establishment of an adaptive anticancer immune response.[327] Necroptotic cell death is taking place frequently in stress conditions with impaired apoptosis, hence being central to the elimination of virus-infected cells.[328] Necroptosis involves sequential activation of receptor-interacting serine/threonine kinase (RIPK)1 as well as RIPK3 and, in turn, mixed lineage kinase domain-like pseudokinase (MLKL).[327] This is triggered by several perturbations and stress situations via either ligand-mediated activation of death receptors like FAS and TNFR1, or DAMP-mediated activation of PRR like TLR3 (by dsRNA in endosomes) and TLR4 (by several DAMP at the cell surface).[325] Release of those DAMP being key in ICD induction (compare below) is inhibited by loss of RIPK3 or MLKL, demonstrating the central importance of this signal module in ICD induction.[329] Interestingly, necroptotic cancer cell death in response to several metals and anticancer metal drugs, including cadmium and cobalt (Co) salts[330,331] as well as Au[332,333] and Pt[334] compounds and metal-based nanoparticles (NP)[335] has been observed. Summarizing, it can be postulated that the effect of dying tumor cells as immune adjuvants is for sure supporting in many cases the success of anticancer metal drugs, as impressively demonstrated for oxaliplatin[336,337] but also others[22,338] (compare chapter 3).

2.2.6. Hallmarks of immunogenic cell death (ICD)

What is unique to drugs inducing ICD allowing them to generate, besides a rather non-inflammatory form of cell death, even a specific, long-lasting immunological memory?[1,339] Collectively, ICD enables revision of several important aspects of immune evasion (compare chapter 1.2 and Figure 2), allowing re-induction of immune recognition of cancer cells.[340] ICD is a cooperative sequence of events comprising both innate and adaptive immune functions, combining cellular and humoral factors as well as both antigenicity and adjuvanticity aspects discussed above.[324] The concept of ICD was first established in 2005 upon observation of tumor regression and even effective vaccination of mice injected with *in vitro* anthracycline-treated, dying tumor cells.[326] Subsequently, ICD has been found to be activated in response to drugs and therapeutic interventions like oncolytic viruses, cardiac glycosides, photodynamic therapy and (structurally unrelated)

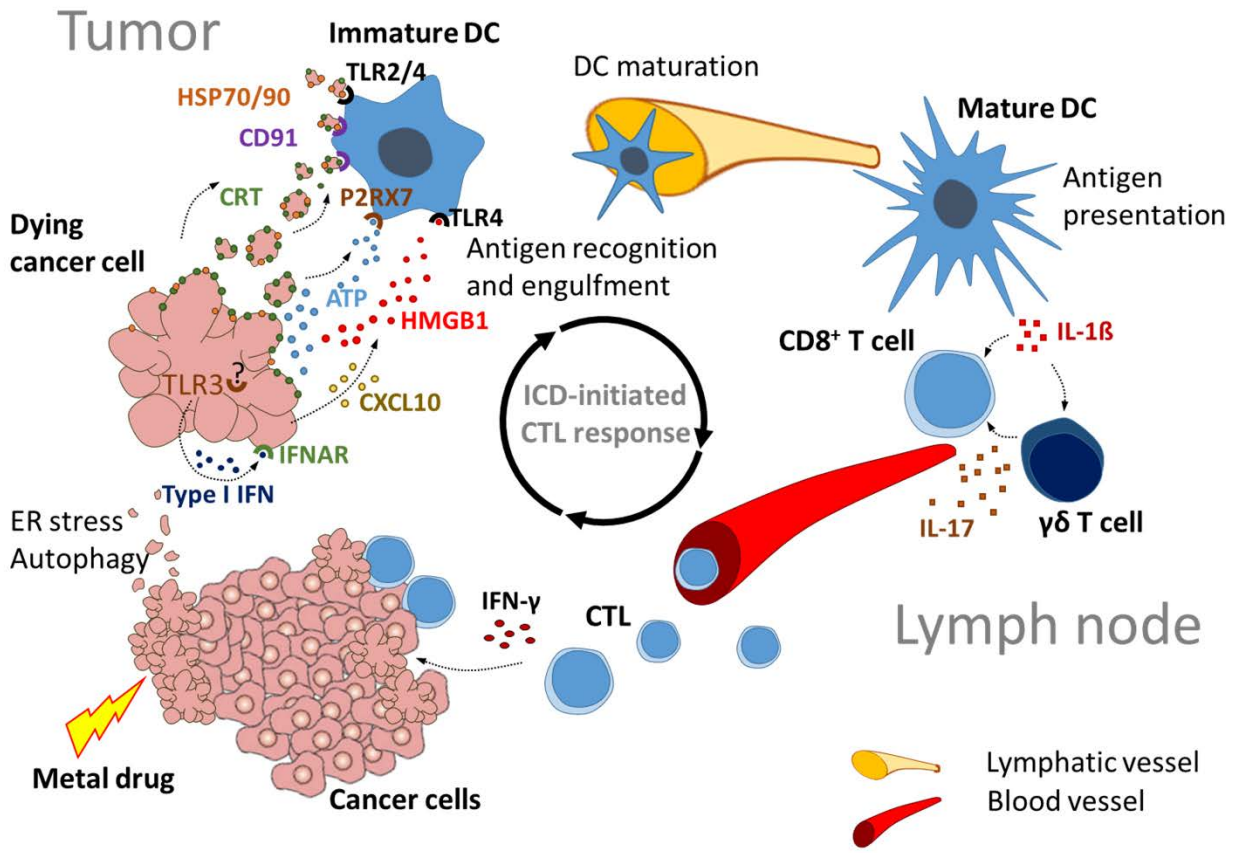

Figure 6. Molecular hallmarks of ICD. Certain drugs, including selected metal compounds, induce a form of cell death which, based on ER-stress- and autophagy-mediated exposure or release of DAMP molecules like CRT, HSP70/90, ATP, and HMGB1, induce a tumor antigen-specific CTL response. DAMP activate respective receptors on the DC plasma membrane like TLR, CD91 (LRP1), and the purinergic receptor P2X7. The process is additionally driven at multiple points by specific cytokines, e.g. IFN, IL, and chemokines (e.g. CXCL10) via interaction with their specific receptors as indicated. ICD, immunogenic cell death; DAMP, damage-associated molecular pattern; CRT, calreticulin; HSP70/90, heat-shock protein 70/90; ATP, adenosine triphosphate; HMGB1, high-mobility group box 1; CTL, cytotoxic T-lymphocyte; DC, dendritic cell; TLR, toll-like receptor; IFN, interferon; IL, interleukin; CXCL, C-X-C motif ligand. For detailed description see text. (Adapted from Kroemer et al., 2013[1])

chemotherapeutic agents.[341] ICD can also be induced by irradiation, where it might explain at least in part the rarely observed abscopal effect, i.e. a systemic anticancer effect following local tumor irradiation.[342] Consequently, the hallmarks of ICD and the repository of ICD-inducing drugs were progressively established during the following years and are still constantly increasing (Figure 6).[343] The knowledge to which extent metal drugs induce either complete or partial ICD is - though surprisingly limited - currently starting to manifest, as reviewed by us recently.[22]

In order to facilitate understanding of the complex sequence of events in ICD, the main molecular players will be described here before discussing the initiating events in the therapy-damaged cancer cells. Cells undergoing ICD expose or release specific DAMP for interaction with their particular PRR molecules primarily on immune cells of the microenvironment.[344] The key DAMP for full ICD induction are the protein chaperones CRT[345,346] and HSP70/90,[347] the DNA damage sensitizer HMGB1,[215,348] and the nucleotide ATP.[349] However, multiple other danger signals might support the process of ICD at different stages

including the release of dsDNA or RNA,[17] as well as annexin A1 (ANXA1).[344] Additionally, the process is driven by the induction of immune-stimulatory cytokines (e.g. IFN type I[350,351] and type II,[352] IL-1β,[352] and IL-17[353]) and chemokines (e.g. CXCL8[354] and CXCL10[351]) derived from several cell types including - besides dying cancer cells - also DC, CTL and γδ T cells (Figure 6).[17,323,353] Functionally, ICD includes the exposure of "find-me" signals by the apoptotic cells for attracting cells of the innate immunity combined with "eat me" signals allowing efficient engulfment of the apoptotic bodies and antigen presentation. Massive inflammation or distinct immune-suppression would be both detrimental at that stage. Consequently, clearly both "find me" and "eat me" signals are optimized to allow strong attraction of DC but also - at least to some extent - rejection of e.g. proinflammatory neutrophils and monocytes/macrophages (the latter preferentially attracted by "tolerogenic" cell death).[71]

As a first step in ICD induction, CRT is actively translocated from the ER to the surface of early apoptotic cells (termed ecto-CRT)[355] via an exocytotic process (compare below) even before exposure of the immunosuppressive phosphatidylserine.[345] Ecto-CRT functions as an "eat-me" signal primarily to DC via binding to its receptor CD91 (low-density lipoprotein receptor 1, LRP1) on APC.[356] This induces - besides proper engulfment of dying cells to allow efficient neoantigen presentation on DC - also release of pro-inflammatory cytokines like IL-6 and TNF-α, supporting a T_H1 and T_H17 cell polarization.[71] Consequently, ecto-CRT counteracts the ubiquitous "don't eat me" function of CD47 (integrin-associated protein) and binding to its receptor signal-regulatory protein α (SIRPα) on the APC surface.[356] Hence, ecto-CRT serves as a central switch towards antigenicity of programmed cell death.[97]

Ecto-CRT, however, is not sufficient to induce ICD but needs cooperation with at least extracellular ATP and HMGB1. Besides functioning as central cellular energy source, ATP and other nucleotides are known to exert multiple extracellular messenger functions (termed purinergic signaling system) also connected to mechanical or chemical stress and tissue damage.[357] This highly sensitive and versatile signaling module is enabled by the steep decline of intracellular to extracellular concentrations of ATP (approximately 10 mM to 10 nM, respectively) in unstressed tissue. Under stress, like tissue damage and inflammation, extracellular ATP levels can increase rapidly and massively. There, it mediates concentration- and receptor-dependent messenger functions by interaction with a high number of nucleotide receptors on target cells including primarily immune cells.[3,17,349] Several release mechanisms for nucleotides have been suggested including exocytotic, vesicle-driven processes as well as efflux by anion channels (ATP is negatively charged) and ABC transporters.[358] During ICD induction, ATP is actively released from pre-apoptotic cells by an autophagic process[349,359] (compare below) and functions as an efficient "find me" signal for several myeloid cell types including primarily DC but also macrophages and, in the nervous system, microglia.[253] Attraction and activation of immune cells is elaborated by binding to a multitude of purinergic receptors,[17,357] e.g. at the DC cell membrane.[360] Persistent low ATP levels stimulate solely the high affinity metabotropic P_2Y receptor family, leading to DC maturation favoring a T_H2 immune response. At higher ATP concentrations

- like the ones functional in ICD induction - low-affinity P2X receptors including P2X7 are activated especially close to the dying cancer cells. This leads to a tumor-resident DC-mediated T_H1 response.[361] Functionally, ATP binding to P2X7 activates the NLRP3 inflammasome (compare chapter 1.2.2) with caspase 1-mediated secretion of IL-1β, essential for priming an efficient CTL response during ICD.[352] The antigen-specific CTL activity against cancer cells is based on massive IFN-γ production,[352] thus closing the ICD-initiated anticancer immune cycle. This step is promoted by specific γδ T cells (termed γδ T17 cells) arriving after ICD induction in the tumor bed before the respective CTL and essentially supporting the chemotherapy-mediated CTL antitumor response by secreting primarily IL-17A[353] (Figure 6). Interestingly, IL-17A is also contributing to cisplatin nephrotoxicity. However, in that case this cytokine is not produced by γδ T cells, but rather by innate effector cells based on TLR2 ligand production and activation of the apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC)-dependent inflammasome complex[362] (compare chapter 6.2). Concerning anticancer therapy, diverse chemotherapeutics including metal compounds[363,364] but also heavy metals ions themselves[365] and metal-containing NP[9] have been shown to mediate ATP release and purinergic signaling. However, this issue has not been investigated systematically so far.

The third key DAMP molecule essential for promotion of ICD induction is HMGB1, a widely expressed and incredibly multifunctional non-chromatin DNA chaperon stabilizing the nucleosome and supporting diverse DNA-involving mechanisms like replication, transcription, and repair.[366] Surprisingly, HMGB1 in cancer cell nuclei was suggested to enhance Pt drug-induced DNA damage by shielding the DNA lesion from efficient nucleotide excision repair (compare chapter 3.1.6).[367] Passive release of HMGB1 from necrotic cells is a main mediator of damage-induced inflammation but - e.g. in case of ICD - also active HMGB1 export mechanisms have been suggested, involving autophagy (see below) and probably also the NLRP3 inflammasome.[368] Both release and extracellular functionality of HMGB1 seem to be tightly regulated by acetylation as well as - especially with regard to immune functions - oxidation state at three cysteine residues.[318] While inactive in its fully oxidized form, HMGB1 mainly acts as immune cell attractor in its reduced form and induces pro-inflammatory cytokine release after forming disulfide bridges.[369] This is of central interest with respect to anticancer metal drugs considering their massive impact on the intracellular and extracellular redox status of cancer cells and tissues.[194] Extracellular HMGB1 acts as DAMP by binding to several receptors on innate immune cells. One of them is RAGE, a multifunctional immunoglobulin superfamily receptor, central in the pathophysiology of inflammatory and degenerative diseases, but also cancer.[370] HMGB1 binding to RAGE induces cell migration and invasiveness based on NF-κB and MAPK signaling[371] and cooperates with the CXCL12/CXCR4 chemokine axis in inflammatory cell recruitment.[372] Additionally, and even more important for ICD, HMGB1 functions as ligand primarily for TLR4 (the classical TLR for lipopolysaccharide, LPS) on APC, mediating activation of the MyD88 signaling pathway essential for proper tumor antigen presentation by DC.[215] Accordingly, the vaccination

effect of cancer cells dying via ICD was lost in TLR4-knock-out mice and following downregulation of extracellular HMGB1 levels.[215] Likewise, blockade of ICD-mediated vaccination due to loss of HMGB1 release was recovered by application of TLR4 agonists.[348] Together, this suggests that HMGB1 might be important for the anticancer activity of metal drugs by at least two independent mechanisms, i.e. intracellularly by blocking drug-mediated DNA damage repair[367] and extracellularly by supporting the anticancer immune response by boosting neoantigen presentation by DC. However, the picture seems to be more complex. Generally, also tumor-promoting effects of HGMB1 have been described, especially in the context of persistent inflammation.[373] Accordingly, RAGE-mediated inflammation was identified as a central player in tumor growth and metastasis[374] and HMGB1 release from necrotic cells promoted aggressive recurrence after ICD-inducing chemotherapy.[375] Additionally, in several studies enhanced HMGB1 levels in cancer cells were connected rather to resistance than sensitivity of cancer cells also against anticancer metal drugs *in vitro* and *in vivo*. This unwanted effect was based on the anti-apoptotic impacts of MyD88 activation in cooperation with Ras/ERK signaling.[376] Hence, inhibition of HMGB1 and MyD88 was suggested as strategy for anticancer therapies especially of Ras-driven tumors.[376,377] However, considering the central role in ICD induction such interventions and the combination with ICD-inducing chemotherapy need to be elaborated carefully.

So how is the above-mentioned sequence of ICD induced and how are the critical DAMP released? The initiation of ICD - as a cooperative process between cancer cells and the microenvironment - is depending on several distinct molecular mechanisms in the dying cancer cells. The most important process, also involved in the release of the above-mentioned “eat-me” DAMP - like ecto-CRT and ecto-HSP70/90 - is ER stress, based on an unfolded protein response (UPR).[378] Activation of protein kinase R-like ER kinase (PERK)-mediated axis of ER stress drives phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α).[379,380] This initiates, depending on the ICD inducer, a signal cascade involving activation of the apoptosis-promoting bcl-2 family members BAX/BAK, calcium signaling, and caspase 8-mediated cleavage of the ER protein BAP31.[355] ICD involves mainly caspase 8 - and not caspase 3 - as initiator caspase, however, in case of e.g. hydrostatic pressure-induced ICD, also caspase 2.[381] The final step is activation of the secretory pathway and translocation of CRT in association with the disulfide ER-resident isomerase ERp57 onto the surface of the dying cells, even before appearance of phosphatidylinositol as key immunosuppressive DAMP.[355] In many if not all cases, ICD-related ER stress induction is linked to oxidative burst and the overproduction of ROS.[324,382] ER calcium release might further promote ROS production and seems to be a prerequisite for ROS-connected ICD.[323] Several of the DAMP driving ICD, like CRT and HSP70/90, function physiologically as chaperons for injured or misfolded proteins in the ER. The central role of ER stress in ICD induction has also massive implications for the activity of anticancer metal compounds. Although full ICD induction based on UPR has been reported mainly for oxaliplatin so far[337], multiple other metal complexes are known to induce massive ER stress also via the PERK axis. In

case of the promising Ru compound KP1339 such ER stress contributed together with caspase 8 to cell death induction in several cancer cell lines.[383,384] This fits well with the chaperon and UPR regulator GRP78, residing upstream of PERK, in ER stress protection[385] as one major target of this Ru(III) compound.[188,386]

In addition to or in cooperation with ER stress, autophagy - a process capturing intracellular components in autophagosomes and delivering them to lysosomes[387] - seems to be essential for ICD induction in cancer cells.[349,388] Autophagy is a catabolic "self-eating" process of cell constituents and even whole organelles (in the latter case termed macro-autophagy) either as starvation rescue to generate metabolic precursors or to remove damaged components like misfolded proteins e.g. in response to chemotherapy.[389] Accordingly, autophagy often functions context-dependently either as resistance mechanism against therapy-induced cell death or, in case of excessive activation, as inducer of so-called autophagic cell death. Consequently, autophagy induction and inhibition have both been suggested as therapeutic strategies against cancer.[390] Besides this cancer cell-autonomous effects, autophagy also regulates the TME by activating the immune system in response to therapeutic interventions like irradiation and drug treatment.[391] Autophagic processes have been demonstrated to drive metal- and metal complex-induced cancer cell death but also to mediate adverse and carcinogenic effects.[392,393] For multiple anticancer metal drugs, either protection from or induction of cell death by autophagy has been reported, primarily based on data derived from *in vitro* experiments.[394] However, the contribution of metal drug-induced autophagy to immunogenic anticancer events *in vivo* is widely unexplored.

In fact, several factors of anticancer immunity are supported by autophagic processes in cancer but also immune cells[395] including - via lysosomal hydrolysis - efficient loading and presentation of intracellular tumor antigens by DC to T_H cells via MHC class II.[396] Additionally, autophagy regulates the repertoire of MHC class I epitopes also of cancer cells, probably based on the interaction with protein translation initiation, and - in addition - supports CTL survival.[388] With regard to ICD induction, the main contribution of cancer cell autophagy seems to be the release of ATP to the extracellular space.[349] This process involves, also in response to metal drugs, caspase-dependent plasma membrane translocation of lysosomal-associated protein 1 (LAMP1)[349,359] and pannexin-1 as a non-selective, large-pore ATP release channel.[397] However, not only ATP, but also HMGB1 release into the extracellular space is supported by autophagy, the latter - in a feedback fashion - further accelerating cellular autophagy.[398] Interestingly, also ICD-inhibitory roles of autophagy have been suggested based on reduced CRT exposure following ATG5 knockdown in frame of ICD-inducing photodynamic therapy.[382] In case of metal drug-induced cell death, however, autophagy was promoting CRT exposure during early but inhibiting during late stages, and only combination of ER stress with autophagy was leading to ecto-CRT exposure.[399] Together, these data suggest essential and cooperative roles of ER stress/UPR and autophagy in ICD induction based on a delicate balance between different ICD-promoting and -inhibiting activities. These factors also seem to be of central importance in anticancer immune responses initiated by metal drugs.[399]

The deeper knowledge on the central role of ROS-mediated ER stress and UPR in ICD induction led to the classification of *bona fide* ICD inducers into two categories.[324,343,400] Type I inducers exert diverse cytotoxic mechanisms and trigger ER stress indirectly as a secondary mode of action. This category includes, besides irradiation, with respect to clinically approved anticancer agents e.g. the anthracyclines doxorubicin, idarubicin and epirubicin (antibiotics), the taxanes paclitaxel and docetraxel (microtubule poisons), the anthracendione mitroxantrone, the proteasome inhibitor bortezomib, the glycopeptide bleomycin and the alkylating agent cyclophosphamide (a nitrogen mustard).[22,336] Type II ICD inducers mediate ROS-related ER-stress with CRT exposure directly as main mode of action and include some forms of photodynamic therapy and oncolytic viruses.[400] With regard to metal drugs, oxaliplatin represents a classical type I ICD inducer[337] but also the very rare category of type II ICD inducers have been identified or in other cases assumed in the world of preclinical metal compounds (compare chapter 3.1.5).[338,401] Considering the multiple roles of ROS and protein damage to induce UPR and ER stress as mode of action for several metal drug families, the existence of by far more metal-containing type I and type II ICD inducers can be considered highly probable. However, also a critical view on this issue is necessary. For sure, induction of ICD by anticancer drugs is desirable, leading to a supposedly synergistic activity between the induced cancer cell damage (as DNA damage in case of many metal drugs) and profound activation of an anticancer immune response. Nevertheless, it needs to be mentioned that *bona fide* ICD inducers are rare. Based on library screening approaches, below 10% of clinically used and experimental anticancer compounds within FDA and NCI panels were found to elicit ICD-inducing activities.[343] This implicates that in the clinical situation by far not all successfully used anticancer drugs are *bona fide* ICD inducers. Hence, also cisplatin, per definition postulated as "tolerogenic" cell death inducer[71], is, as mentioned above, distinctly more active in an immuno-proficient background and was not replaced by the classical ICD inducer oxaliplatin in many cancer indications.[173] Alternatively, the ICD inducer oxaliplatin triggers apoptosis characterized by massive exposure of phosphatidylserine at the cell surface, a well-known immunosuppressive DAMP.[402] This indicates that the exact contribution of the immune system and the balance between immune-stimulatory and immunosuppressive mechanisms during systemic cancer therapy with metal drugs needs to be dissected in detail to allow optimization of the therapeutic outcome.

2.3. Impact of anticancer metal drugs on immune cells

2.3.1. Metal drugs may support proliferation and homing of innate and adaptive effector compartments

The immune-potentiating effects of metal-containing cancer therapeutics described so far are predominantly initiated by the damage inflicted on cancer cells. Accordingly, primarily the release of the above described "find-me" signals by the drug-exposed tumor tissue leads to an enhanced recruitment of effector immune

cells into the cancer lesions, thus counteracting the immune-resistant milieu of the TME.[17,71] This might even transfer immunologically "cold" tumors, keeping immunocytes, and especially CTL, out of the malignant tissue, into "hot" tumors, a concept currently heavily discussed to overcome intrinsic immunotherapy resistance.[403] This adjuvant effect might also be strongly involved in metal drug response and prime the tumor for diverse immunotherapeutic interventions.[404] Hence, it was for example shown that metal-based chemotherapy fully supplanted other adjuvants to convert the TME of HPV oncogene-driven T71 lung cancer and CT26 colon cancer models to a highly immune-permissive state for successful peptide-based vaccination approaches.[405] The observed immune stimulation was dependent on TLR4 and IFN type I signaling and based on enhanced DC and CTL infiltration into the tumor lesions. Interestingly, the applied peptide for vaccination needed to be injected intratumorally (and not subcutaneously) proving the central role of the TME in CTL stimulation. In frame of such observations, one might hypothesize that not only metal drug-mediated alterations in the cancer cells but also directly in immune cells could have an impact on the anticancer immune response. This seems likely, as several transition metals utilized in anticancer metal drugs are able (at least in their ionic form) to exert an innate immune response, including nickel (Ni) and Co via TLR4[406-408] and Au via TLR3 activation on the DC surface[409]. Indeed, several *in vitro* studies have analyzed the effect of metal salts and metal complexes (primarily Pt drugs) on immune cells, like macrophages, DC or T cell subtypes, and found a broad and sometimes contradictory panel of effects including activating, de-activating and even cytotoxic activities (compare chapter 3). The latter observation led to the suggestion of metal drugs as anti-inflammatory remedies e.g. in rheumatoid and autoimmune diseases.[410] The variable effects on immune cells *in vitro* might be a consequence of the metal drug type, dose, regimen and combination differences and again underlines the necessity for a precise elucidation of the diverse interactions of metal drug chemotherapy with immune functions to develop optimized cancer treatment regimens. Nevertheless, it has to be mentioned that the *in vivo* relevance of these *in vitro* findings is so far widely unclear. A surplus of immune-stimulatory processes of metal drugs and enhanced recruitment of effector cells into the malignant tissues have been reported both in mouse models and patient tissues. In that case, however, it is widely impossible to dissect the immune-stimulatory effects of damaged (cancer) cells from direct impacts on the immune cell compartments. The strong immune component of metal drug-induced adverse effects (compare chapter 6)[197,411,412] argues against a dominant immune cell cytotoxicity, but rather supports direct or indirect immune-stimulation by diverse metal drugs both in healthy and malignant tissues. The contribution of immune reactions to the adverse effects especially of Pt drugs is currently reinvigorating efforts to develop prodrugs and tumor delivery strategies with selective and tumor-specific release of the active metal compound[19,27,413,414] to avoid systemic (immune) adverse effects. This is especially important considering combining metal-containing chemotherapy with immunotherapeutic approaches, a strategy even enforcing the risk for systemic immune hyper-activation.

With regard to the combination with immune checkpoint inhibitors another activity at least of Pt drugs needs to be considered. By combining *in vitro* and clinical observations, the study of Lesterhuis et al.[238] delivered strong indication for a direct immune cell-activating function of metal-based chemotherapy. The authors suggested that pretreatment of DC with metal drugs enhanced their potential to stimulate T cell proliferation and activation to produce IFN-γ and IL-2. This was mainly based on downregulation of the immune checkpoint receptor ligand PD-L2 as a consequence of STAT6 blockade by the metal compounds. In a more recent work, the authors demonstrated - in agreement with other groups[415] - that e.g. Pt drugs generally represent STAT inhibitors including supposedly pro-tumorigenic (STAT3, STAT5, STAT6) as well as anti-tumorigenic (STAT1) members by directly binding to the Src homology 2 (SH2) domains of these proteins.[416] This interaction is not restricted to cancer cells but for sure also happens in immune cells. Considering the central role of STAT signaling in immune cell biology, IFN responses, and immune checkpoint regulation,[417] a massive - though yet not dissected - impact of metal drugs on this immune-regulatory axis might be anticipated. Obviously, IFN-γ signaling via JAK/STAT is a major regulator of e.g. PD-L1 expression in both cancer and regulatory immune cells.[418] The general STAT-inhibitory function would consequently ascribe an immune checkpoint-inhibitory activity to the metal drugs. Accordingly, combinations with several other drugs but not metal-containing regimens enhanced PD-L1 expression within a preclinical chemo-radiation setting.[365] Additionally, PD-L1 expression was reduced in lung cancer patients following metal-based chemotherapy.[370] In contrast, a cisplatin-containing induction chemotherapy in advanced head and neck squamous cell carcinoma (SCCHN) enhanced CTL infiltration was paralleled by increased PD-L1 expression on TME immune cells,[419] probably reflecting a later stage following successful immune response activation. Additionally, DNA-damaging agents like metal drugs might enhance PD-L1 expression on cancer cells based on stress-mediated MAPK signaling.[420] Hence , a competition of PD-L1 expression-activating (MAPK) and -inactivating (STAT3/5/6) signals can be assumed for metal drugs. This hypothesis in corroborated by observations in gastric cancer where both induction and loss of PD-L1 expression during Pt-based therapy was observed in patient subsets. Interstingly, down-modulation was more frequent and correlated better responses and progression-free survival (PFS).[421] In addition, STAT activation by cisplatin seems to play a central role in adverse effects like ototoxicity (compare chapter 6.4). Together these data might shed light on the profound activity of checkpoint inhibitor antibodies in combination with Pt drugs either preclinically[422] or in clinical studies especially of NSCLC, an observation currently revolutionizing treatment of this highly aggressive disease (compare chapter 8.3.1).[9]

2.3.2. Anticancer metal drugs target regulatory immune cell populations

However, though sounding paradoxical at first, not only activating but also direct cytotoxic effects of anticancer metal drugs on immunocytes might support immune-reactivation of the TME. So how can that

work? To recapitulate from the chapters above, cancer cell clones have - during immune escape - undergone several rounds of selection against immune-recognition (outlined in chapter 1.2) based on diverse "abused" functions of the immune system. These may include - in addition to the already discussed alterations of antigenicity and adjuvanticity - adaptation of an immune-privileged environment by a so called "homeostatic feedback inhibition". In simple words, this inhibitory safeguard function is physiologically important to avoid immune-mediated tissue damage and allow resolution of inflammation during e.g. wound healing.[3] This beneficiary and multifaceted feedback inhibition, mainly targeting T cell inactivation (causing T cell exhaustion, generating T_{ex} cells)[423] may be deregulated in pathological situations like persistent antigen exposure or chronic inflammation as e.g. in frame of cancer. Thereby, several mechanisms are evoked to block or reverse immune cell activity (with a focus on CTL, but also concerning NK cells and others), widely summarized as immune checkpoints. These immunosuppressive activities are - in the malignant situation - frequently accomplished by cancer cells but also by regulatory immune cells including T_{reg}, MDSC, M2-type TAM and B_{reg} (compare chapter 1.2.4).[424] These cell types cooperate with the cancer cells to establish the immunosuppressive microenvironment by blocking a CTL-mediated anticancer immune response involving secretion of immunosuppressive cytokines, overexpression of immune checkpoint receptor ligands (e.g. PD-L1, PD-L2) either on the cell surface or via microvesicle shedding, and production of immunosuppressive metabolites.[16]

So the question arises, how these regulatory immune cell compartments might be specifically targeted to reverse the immune-restrictive cancer microenvironment and re-establish immune surveillance. Surprisingly, solid evidence exists that regulatory immune cell compartments might be highly sensitive against cytotoxic chemotherapy especially with metal drugs including Pt but also As compounds[425,426] (compare chapter 3.1 and 3.2), while unfortunately for many promising metal drug families no data about this important aspect have been established so far. The mechanisms underlying this hypersensitivity against metal drugs are not completely understood and might be multifactorial. Observations published so far include a high vulnerability of T_{reg} and MDSC to metal drug-induced proliferation arrest or apoptosis induction by deregulation of pro/antiapoptotic bcl-2 family members.[425] Furthermore, enhanced vulnerability of regulatory immune cell compartments by ROS stress might be a key factor leading to hypersensitivity against the frequently redox-active metal drugs.[194] Hence, a higher sensitivity of T_{reg} against an ATO-induced oxidative and nitrosative burst was reported with superoxide anion and peroxynitrite as key radicals.[427] Even central factors in the differentiation program towards regulatory immune cell subsets, like the FOXP3 transcription factor guiding T_{reg} cell development,[428] might be shut down by metal drugs leading to loss of their potential to release immunosuppressive cytokines and inhibit CTL functionality.[429] Nevertheless, the nature of the metal drugs seems to be crucial, as e.g. a binuclear (η^6-p-cymene)Ru(II) complex containing a bridging bis(nicotinate)-polyethylene glycol ester ligand was enhancing differentiation of T cells towards a T_{reg} phenotype (compare chapter 3.3).[430] With regard to clinical data,

multiple studies have indicated that, although in some cases the absolute number of T cells was reduced, an enhanced ratio CTL/T_{reg}[431] and/or a reduced number of tumor-resident MDSC[432] was observed (compare chapter 8.2). This strongly supports reduced CTL deactivation and induction of a permissive TME based on the preferential killing of regulatory immune cells by anticancer metal therapy also in the clinical situation. Summing up, metal drugs might influence the immune TME in multiple and also contradictory ways depending on the drugs used, their modes of action, the doses applied and the combination regimens. However, the picture is more and more emerging, that at lease Pt-based chemotherapy can be considered as being mainly permissive for re-establishment of cancer immune surveillance. This strongly implicates that, for sure, metal drugs should be big players in the search for the ideal combination partner for immunotherapy in cancer. This entails dawn of novel challenges for chemistry to synthesize smart and innovative metal complexes allowing selective release into the cancer tissues to optimize the therapeutic window and minimize adverse effects within such immune-stimulatory combination settings. The data available are elaborated in detail in the next chapters with regard to specific metals in anticancer drug development.

3. Immunological effects of specific metal drugs

3.1. Platinum (Pt)

3.1.1. The immune perspective of Pt drugs

Pt drugs are by far the most successful anticancer metal compounds with three representatives approved for clinical use worldwide and three further in selective countries (compare Figure 3). Hence, the by far largest body of literature concerning an interaction with an anticancer immune response exists for the clinically approved Pt drugs cisplatin and oxaliplatin, whereas surprisingly little information is available for carboplatin. From a clinical perspective, systemic immune supression in patients treated with chemotherapeutics such as Pt drugs is of major concern. For instance, a prominent dose-limiting side effect of carboplatin is myelotoxicity (compare chapter 6.1).[433,434] This issue has even been exploited as cytoreductive therapy, an approach to mobilize peripheral-blood progenitor cells in hematological malignancies such as non-Hodgkin's lymphoma.[435] However, as outlined in chapter 2.1, as early as the discovery of cisplatin as anticancer agent by Rosenberg in the late 1960s,[208] a discussion concerning the contribution of immunological effects was initiated and basically persists until today.[20,21,210] Interestingly, stimulated by the remarkable success of Pt-containing chemotherapy with immune checkpoint inhibitors in highly aggressive solid tumors,[9,18] the field is currently experiencing an unexpected and urging interest also from the side of pharmaceutical companies. The discussion on immune effects of Pt drugs evolved in parallel with major discoveries in the field of immunology like identification of various immune cell subtypes (e.g. T cell subsets during the 1960s), the MHC restriction (1974), or genes encoding BCR and TCR (1985 and 1987, respectively). For summarizing data on Pt drug-immune interactions published in early studies, this

needs to be kept in mind, meaning that certain terminologies, functional descriptions and cell subtypes reflect the knowledge of that time rather than the current consensus on immunological functions and models. For instance, early work on e.g. cisplatin postulates an interaction with natural cytotoxicity (NC) cells as compared to NK cells, a concept not pursued further since the 2000s, where natural cytotoxicity was attributed to TNF-α produced by NK and NKT cell subsets.[436] Consequently, we aim in the following section to give an overview on the data available for effects of Pt compounds on several aspects of innate and adaptive anticancer immunity and, if necessary, mention the background of publication date.

As already outlined above, Pt drugs (like many other conventional chemotherapeutics) have traditionally been assumed to result in a generalized supression of immune responses.[437,438] However, there is (and always was) strong evidence that, indeed, even the opposite might hold true. With regard to innate immune cells, cisplatin has been demonstrated as potent activator of isolated murine peritoneal macrophages,[439,440] human monocytes,[441,442] as well as NK cells.[443,444] These observations will be outlined in the following sections.

3.1.2. Impact of clinically approved Pt drugs on macrophages

Several studies demonstrated increased amounts of intratumoral macrophages after cisplatin treatment *in vivo* (e.g. in mice bearing TC1 lung cancer[445] or ID8 and RHM-1 ovarian carcinoma allografts[213]). With regard to the effects of Pt drugs on macrophages, a lot of early cell culture research has been performed using lavage-isolated peritoneal macrophages from untreated Balb/c mice.[222] Such, already in the 1990s, two successive publications demonstrated increased release of cytolytic factors (e.g. IL-1α, TNF-α, ROS, lysozyme) from macrophages incubated with cisplatin or carboplatin *in vitro* to mediate cancer cell killing.[446,447] Corroboratively, in 1998, Shishodia et al. found that murine cisplatin-treated macrophages acquire an enhanced capacity to lyse tumor cells as well as produce increased amounts of IL-1, TNF, ROS, reactive nitrogen intermediates, lysozyme and arginase.[440] Macrophage activation by cisplatin was dependent on protein kinase C (PKC)- and tyrosine kinase-induced MAPK signaling as well as Ca^{2+}/calmodulin signaling.[439] Moreover, peritoneal macrophages rapidly activated lyn kinase (a Src kinase family member) upon cisplatin treatment.[448] Accordingly, another report showed that murine peritoneal macrophages overexpress various TLR (TLR2-TLR9) upon contact with cisplatin in cell culture, leading to induction of downstream effectors (MyD88, IRAK1, TRAF6, MAPK, NF-κB), and - upon ligand binding (e.g. polyI:C, CpG DNA, LPS) - enhanced production of pro-inflammatory cytokines as well as inducible nitric oxide synthase (iNOS).[449] Cisplatin induced rapid, Ca^{2+}-dependent translocation of NF-κB into the nucleus.[450] Furthermore, production of oncostatin M (a cytostatic gp130 cytokine[451]) by the treated macrophages was already detected 15 minutes after incubation with cisplatin.[452] Cisplatin stimulation of macrophages resulted in contact-dependent tumoricidal activity mediated by leucocyte function-associated antigen 1 (LFA-1, i.e. an integrin heterodimer constituted of the α-chain CD11a and the β-chain CD18) binding to the well-known cell adhesion molecules ICAM1-3.[453] Upregulation of LFA-1 by cisplatin was

Ca^{2+}/calmodulin/calmodulin-dependent kinase (CamK)- and PKC-dependent and mediated binding of the macrophages to target cells.[454,455] However, antibody-based blockade of LFA-1 did not reduce cisplatin-induced overproduction of antitumor cytotoxic mediators like IL-1, TNF and NO. Additionally, treatment of peritoneal macrophages with cisplatin resulted in biphasic antigen presentation to keyhole limpet haemocyanin (KLH)-primed T cells, where the second peak was based on an autocrine feedback loop due to release of IL-1, TNF-α and NO, again in a PKC- and Ca^{2+}/calmodulin-dependent manner.[456]

However, as mentioned in chapter 2.1, it has to be considered that peritoneal macrophages are constituted by a heterogeneous mixture of immunocytes with different biological functions.[222] This makes exact assessments regarding the roles of defined cell types with respect to immune-modulatory functions of Pt drugs difficult. Comparably to peritoneal macrophages, cisplatin treatment of M-CSF-stimulated bone marrow-derived macrophages (BMDM) also led to rapid excretion of oncostatin M.[457] Moreover, cisplatin treatment resulted in release of reactive nitrogen intermediates (probably NO) in GM-CSF- and M-CSF-stimulated macrophages (later used for *in vitro* polarization of macrophages into M1 and M2 phenotypes, respectively[458]) and enhanced their anti-P815 mastocytoma activity.[440,459]

As already described in chapter 1.2.2, M1-type macrophages are considered as favorable subtype exerting antitumor activity, while M2 macrophages have been frequently associated with pro-tumorigenic functions based on their anti-inflammatory cytokine profile, which also leads to T_{reg} proliferation.[460] Consequently, it is of interest that M-CSF-induced M2 and M2-like macrophages were found to be more sensitive to cisplatin and carboplatin treatment than M1 macrophages and DC.[461] However, in this study, treatment with these Pt drugs also resulted in the NF-κB-mediated release of PGE2 and IL-6 from some cancer cell types.[461] This, in turn, could induce differentiation of tumor-promoting M2-like macrophages from monocytes[461] and subsequently result in enhanced tumor growth. Also with regard to oxaliplatin, the role of macrophages seems to be complex. On the one hand, oxaliplatin treatment stimulated release of TNF-α, IL-10 and IL-8 (but not IL-1β) in serum-differentiated human monocyte-derived macrophages and significantly enhanced their cytotoxic activity against CaCo-2 colon cancer cells.[462] On the other hand, it was shown that TAM of the M2 type protect hepatoma cells from oxaliplatin-induced autophagy (and apoptosis) via IL-17 secretion by the tumor cells.[463,464] With regard to carboplatin, however, addition of BMDM was not able to protect cancer cells from this drug, while being very efficient in case of taxol.[465]

In conclusion, although there is distinct evidence that clinically approved Pt drugs can impact on the interplay between macrophages and tumor cells, the sensitive balance between different macrophage subtypes, which profoundly differ in their impact on tumor growth, is difficult to assess and urgently demands more in-depth investigation. Here, it is worth noting that also macrophages can express TLR (and this expression can be stimulated by cisplatin treatment).[449,461] Consequently, it is likely that chemotherapy-mediated release of e.g. HMGB1 or dsDNA may not only activate DC but also local tumor-promoting macrophages.[461]

3.1.3. Pt drugs and NK cell activity

As described above, NK cells are an important part of the innate immune system's attack against cancer. Meanwhile, besides several cell culture and *in vivo* animal studies,[225,443,466,467] there is even clinical evidence that Pt drugs impact on cancer cell recognition by NK cells. For example, NK cells isolated from patients after treatment with a cisplatin-containing regimen showed enhanced activity against K562 cells *in vitro.*[468] Moreover, combination of cisplatin with gemcitabine and 5-FU in patients with advanced pancreatic carcinoma resulted, despite reduction of absolute lymphocyte counts in most patients, in increased levels of IL-12p70- (the active dimer of IL-12) and IFN-γ-producing NK cells.[221] The cytolytic activity as well as number of NK cells (derived from PBMC) from patients with advanced cervix carcinoma significantly correlated with their response to a cisplatin-containing therapy regimen.[469] Interestingly, these effects seem to be based more on enhanced recognition of Pt drug-exposed cancer cells than on a direct activation of NK cells, as *in vitro* treatment of NK cells with oxaliplatin or carboplatin did not enhance their lytic activity against K562 cells.[470]

As mentioned above, NK cell-mediated cell lysis and apoptosis induction is a cell contact-dependent process based on interaction between activating NK cell receptors (such as NKG2D, DNAM-1 and NKp30) and target cell ligands, including for example MICA/MICB, ULBP 1-3, CD112, CD155 and B7-H6.[471] Noteworthy, among the strongest stimuli for the expression of NKG2D- and DNAM-1-activating ligands is activation of the DDR via ATM/ATR and CHK2.[227,472,473] Consequently, it is not surprising that cisplatin-induced DNA damage leads to upregulation of diverse NKG2D receptor-activating ligands (including MICB, Rae1 and MULT1) in an ATR-dependent manner.[473-475] In addition, also B7-H6, the membrane-bound NKp30 ligand, was upregulated by cisplatin[476] which in turn sensitized cancer cells to lysis by NK cells.[225] Also, oxaliplatin treatment resulted in higher expression of the NKG2D- and DNAM-1-activating ligands MICA/MICB, ULBP-3, and CD155, but not of B7-H6.[471] Activation of NK cells by ligand binding subsequently results in upregulation of cell death mediators including perforin/granzyme, FASL and TRAIL.[241] In addition to stimulation of NK cell-activating signaling, treatment with all clinically approved Pt drugs was shown to render cancer cells also hypersensitive to NK cell cytotoxicity based on upregulation of diverse death receptors like FAS[477-481] and DR4/5.[467,471,482] Interestingly - besides the probable involvement of drug-induced death receptor gene expression stimulation - also a direct stabilization of the proteins by Pt adducts probably to FAS and its cofactor ezrin at the plasma membrane was postulated.[246] This assumption was based on the fact that phosphaplatins, which are suggested not to form DNA adducts, readily upregulated these death receptors on cancer cell membranes.[247] An overview of the impact of cisplatin on the recognition of cancer cells by NK cells is given in Figure 7.

Besides MHC class I expression, also other "self"-signaling, inhibitory ligands for NK cell receptors exist including the Clr-b ligand for the NKR-P1B receptor. Fine et al. convincingly demonstrated that cisplatin

treatment resulted in down-regulation of Clr-b in several cancer cell lines.[475] This again enables the recognition of "missing-self" signals by NK cells. Interestingly, in contrast to the NKG2D axis, Clr-b downregulation was independent from ATM/ATR signaling and seems to be regulated by changes in the proteosomal degradation of this molecule.[475] Noteworthy, the importance of NK cells in the anticancer activity of cisplatin was also supported by the *in vitro* observation that cisplatin-resistant lung cancer cell lines were unresponsive to NK cell-mediated cytotoxicity based on upregulation of PD-L1 and down-regulation of several NKG2D ligands (mainly via MEK-ERK signaling).[483] This, in turn, resulted in enhanced expression of PD-1 together with reduced levels of NKG2D on primary NK cells. Accordingly, co-treatment with a PD-L1-blocking antibody was able to re-sensitize cells with acquired cisplatin resistance to NK cell lysis.[483]

Given the potent cytotoxic activity of NK cells against cancer cells, there have been attempts to exploit their potential also in immunotherapy by using LAK cells. Investigation of LAK cells dates back until 1982 and is based on the finding that *in vitro*, lymphocytes can be stimulated by IL-2 to kill tumor cells otherwise insensitive to CTL or NK cell-mediated destruction. Meanwhile, LAK cells are considered mainly as mixture of NK cells as well as CTL expanded upon IL-2-stimulation.[221] In the 1990s -2000, there has been intense research on the impact of cisplatin on the effectivity of LAK cell-based immunotherapy in cell culture[484] but also in clinical trials.[221,467,485,486] In line with the studies on NK cells, cisplatin also rendered cancer cells from different tissues more susceptible to LAK cells.[477-480,484] Noteworthy, in most cases, this was primarily based on the induction of FAS expression by cisplatin, while the Ca^{2+}-dependent, granzyme-mediated pathway of cell lysis was not affected. In addition, cisplatin had also direct effects on LAK cells, as treatment increased LAK cell-mediated secretion of TNF and IL-1, resulting in TNF-mediated lysis of L929 lymphoma cells.[487] Moreover, combination of cisplatin with gemcitabine and 5-FU in patients with advanced pancreas carcinoma resulted in increased LAK cell activity.[221]

With regard to CIK cells, cisplatin treatment enhanced the sensitivity of murine mastocytoma cells to lysis by this mixture of $CD3^+$ NK, CTL-like killer cells, and anti-CD3-activated killer-T cells in cell culture via the FASL but not the granzyme pathway.[488] In line with this, synergistic activity of this combination was also seen against B16 melanoma *in vitro*, where treatment resulted in enhanced intratumoral T cell levels.[489] Similar results were observed in LL3-[490] and CT26[491]-bearing mice. Within a clinical study on late stage gastric cancer, combination of CIK cells with the FOLFOX (5-FU, folic acid, oxaliplatin) scheme improved the short-term curative effect (16% increase in total remission rate).[492] However, the benefit was only transient, as no difference in the 2-year overall survival was observed.

3.1.4. Impact of Pt drugs on cancer cell recognition by CTL

As already described regarding interactions with NK cells, treatment with cisplatin can result in the activation of several damage signals, which in turn attract immune cells including, besides NK cells, also

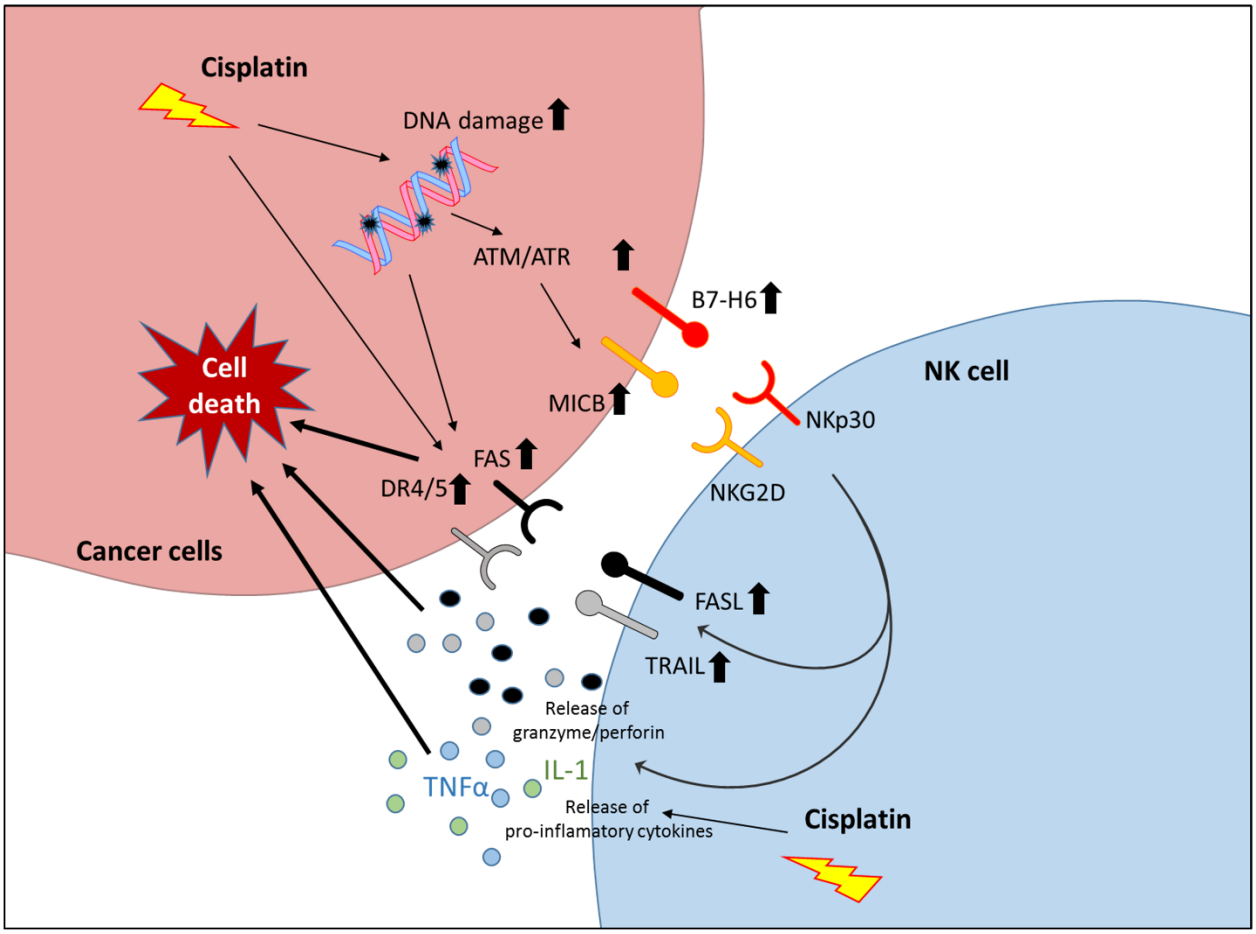

Figure 7. Effects of cisplatin on cancer cell visibility and killing propensity by NK cells. Cisplatin-induced DNA damage response (ATM/ATR) leads to the expression of NK cell-stimulating ligands such as MICB or B7-H6. Upon binding to the respective receptors NKG2D and NKp30, NK cells are triggered, on the one hand, to release cytotoxic proteins such as granzymes and perforins and, on the other hand, to express apoptosis-inducing signals such as TRAIL and FASL. These ligands, in turn, bind to DR4/5 and FAS on the cancer cell surface, which are upregulated upon cisplatin treatment. Together with the cisplatin-stimulated release of TNF-α and IL-1 by the NK cell, this subsequently leads to apoptosis of the cancer cell. NK, Natural killer cell; MICB, MHC class I polypeptide-related sequence B; NKG2D, natural killer group 2D; TRAIL, TNF-related apoptosis-inducing ligand; FASL, FAS ligand; DR4/5, death receptor 4/5; TNF-α, tumor necrosis factor α; IL-1, interleukin 1. For details and references see text.

CTL.[480] The importance of CTL in the anticancer activity of Pt drugs was nicely shown in two animal models (RHM-1[213] and AE-17[298] allograft-bearing mice), where co-treatment with a CD8-depleting antibody strongly diminished the anticancer activity of cisplatin against tumors *in vivo*, while antibodies against CD4 and NK cells had no significant impact. Moreover, cisplatin treatment amplified CTL response to subdominant tumor antigens of murine mesothelioma cells *in vivo* and CTL isolated from these animals were characterized by proliferation to specific antigen stimulation and by IFN-γ secretion.[298] Accordingly, higher numbers of CTL, secreting enhanced levels of IFN-γ and IL-2, were detected in the peritoneal cavity of cisplatin-treated ID8-bearing mice.[213]

In addition to changes in cancer antigenicity by Pt treatment, also “stress signals” have been shown to contribute to cancer cell recognition and sensitivity towards killing by the immune system. Such, increased

FAS expression on prostate carcinoma cells after cisplatin treatment sensitized cancer cells to CTL- and IL-mediated apoptosis.[478] Similar results were observed in experiments with LLC lung[493] as well as SCCHN cells[494] *in vitro* and *in vivo*. However, no FAS upregulation by cisplatin was observed in murine thymoma and colon carcinoma cell lines EL4 and MC38, respectively.[255] In contrast, drug treatment resulted here in substantial increase of intracellular granzyme levels.[255]

As outlined in chapter 2.2.2, in addition to death receptor signaling, metal drugs might also enhance cancer cell sensitivity to granzyme/perforin-mediated cell lysis. Hence, synergism between an adoptive papillomavirus E7 antigen-specific CTL therapy and cisplatin was not dependent on FAS expression (or on the upregulation of MHC class I), but was reversed by blocking the perforin/granzyme pathway via ethylene glycol tetraacetic acid (EGTA).[254] Interestingly, the Ca^{2+}-dependent granzyme pathway of CTL-mediated activity was not affected in the above-mentioned study on prostate cancer cells,[478] indicating that there might exist tissue-dependent differences in the stress response of cancer cells to cisplatin treatment. With regard to the underlying mechanisms, the authors suggested that this increase was probably mediated by perforin and the cation-independent M6P receptor (compare chapter 2.2.2). In line with this hypothesis, M6P receptor expression was stimulated by cisplatin treatment on the tumor cells tested in this study.[255] Moreover, Gameiro et al.[258] showed that treatment with cisplatin/vinorelbine (in addition to increased levels of FAS, MHC class I and ICAM-1) resulted in increased levels of carcinoembryonic antigen (CEA) expression in diverse human lung cancer cell lines (including A549). This, in turn, led to increased tumor cell lysis by HLA-A2-restricted CEA-specific $CD8^+$ T cells. Besides cell death initiation, cisplatin seems to contribute to cancer cell sensitivity to immune cell killing by promoting partly FAS-independent activity of effector caspases[256] and altering the balance of pro- and anti-apoptotic signaling molecules including bcl-2 family members.[257,258]

As outlined in chapter 1.2.3, downregulation of MHC class I molecules is a frequently observed mechanism of cancer cells to avoid CTL recognition.[21,495] Hence, the restoration of MHC class I molecule expression by anticancer therapy is important to redirect CTL against cancer cells. There are multiple reports that treatment with clinically approved Pt drugs effectively stimulated MHC class I expression *in vitro* (e.g. in human COLO201 and murine CT26 colon carcinoma,[279] ID8 ovarian carcinoma,[481] or EL-4 thymoma cells,[496]) as well as *in vivo* (e.g. murine colon CT26,[279] lymphoblastic TC1,[296] or myeloma MOPC-104E grafts[295]). Upregulation of MHC class I in turn led to induction of tumor-specific immunity after chemotherapy with cisplatin, e.g. in case of the MOPC-104E model, based on enhanced expression of the MHC class I antigens H-2Dd and H-2Kd (but not class II antigens I-Ad and I-Ed).[295] Also in case of CTL isolated from mice after intratumoral treatment with a combination of cisplatin, 5-FU, and bone marrow-derived (GM-CSF- and IL-4-stimulated) DC (BMDC), an anti-MHC class I antibody was similarly effective in inhibiting cytolytic activity as was T cell depletion by anti-CD8 antibodies.[497] However, comparably to FAS expression, also responsiveness of MHC class I expression levels to Pt treatment seems to be cell type-

dependent, as cisplatin had no impact on K562 lymphoma[466] or DLD-1 colon cancer cells.[279] Also in case of oxaliplatin, no impact on HLA-class I expression of diverse human cancer cells (HCT116, Caki2, PC3) *in vitro* and HCT116 cells *in vivo* was found.[498]

Reflecting these controversial experimental data, also the clinical picture remains inconsistent. On the one hand, high expression of MHC class I was associated with prolonged overall survival in cisplatin-treated ovarian carcinoma patients.[499,500] Especially upregulation of the non-classical HLA-G was described as independent predictor for improved survival upon Pt-based therapy, while loss of classical HLA-A was associated with shorter progression-free survival.[501] On the other hand, in the study by Mariya et al. HLA class I expression did not correlate with resistance to Pt drugs in the same tumor type[502] and even enhanced MHC class I levels were detected in tumors progressing after Pt-containing chemotherapy.[297] This is in accordance with an immunoproteomics report on a cisplatin-resistant ovarian carcinoma cell line (SKOV3-A2), which displayed higher levels of several MHC class I presented peptides and an altered resistance-specific peptide repertoire. Selected resistance-associated epitopes were significantly better recognized by CTL in the cisplatin-resistant subline as compared to the sensitive parental ovarian carcinoma cell line (OVCAR3).[503] Together, this might indicate that Pt treatment often results in higher MHC class I expression and even better CTL recognition,[213,504] but eventually cancer cells develop resistance also against the attracted CTL and, consequently, disease progression occurs.[297,502]

The ability of the adaptive immune system to fight cancer is also dependent on the capacity of various immune cell types (e.g. APC and CTL) to home to and infiltrate into the malignant tissue.[21] For instance, Beyranvand Nejad and colleagues showed that cisplatin enhanced APC infiltration into HPV-associated tumor grafts (TC1 and C3).[304] These APC, including DC, were characterized by high expression levels of T cell co-stimulatory ligands such as CD80 and CD86. Interestingly, the authors suggested a functional role of these ligands in drug-induced anticancer immune response, as mice deficient in CD80/86 on APC exhibited reduced cisplatin antitumor efficacy.

Independently of MHC recognition by immune cells, clinical data have suggested that the expression levels of immune-modulatory factors (e.g. CCL5 and IDO) in cancer tissue are able to predict response to carboplatin treatment.[504] Another study found an association of IL-2, IFN-γ and CTL levels with the response rate to carboplatin-containing chemotherapy of ovarian cancer.[213] Furthermore, one clinical study revealed decreased levels of IL-10, VEGF, TGF-β and arginase-1 after treatment with carboplatin.[505]

3.1.5. Pt drugs and the anticancer immune cycle: ICD and beyond

As outlined in chapter 2.2.6, ICD induction reflects an optimal synergism between chemotherapy and the anticancer immune response. However, ICD inducers are rare and concern only a small subgroup of clinically approved anticancer agents.[343] With regard to metal drugs, oxaliplatin is one of the best-investigated model compounds.[337,506] Oxaliplatin is considered to represent a typical ICD inducer type I

based on the major mode of action as DNA damaging agent.[309,350] Consequently, various studies have described induction of virtually all ICD hallmarks following oxaliplatin treatment including vaccination of immunocompetent mice by dying cancer cells treated with lethal oxaliplatin concentrations.[337,506] This vaccination potential, considered as gold standard to define ICD-inducing agents, is surprisingly weak to non-existent in case of cisplatin and carboplatin.[337,507] However, this does not mean that these drugs lack any impact on the anticancer immune cycle and cannot support CTL activation. This is also shown for example in the study of Kim et al.,[507] where cisplatin-treated murine ovarian surface epithelial cells (MOSEC) stimulated IL-6 and IL-10 expression in BMDC and enhanced the amount of $IFN^+/CD4^+$ splenocytes resulting in significant prolongation of overall survival of MOSEC-bearing mice. However, also in this study no memory effect was observed after injection of cisplatin-treated MOSEC cells.[507] The differences observed seem to rely on the potency of the Pt drugs to induce an appropriate and complete DAMP panel essential for ICD induction (compare chapter 2.2.6). The following chapters 3.1.6 - 3.1.8 aim to summarize the available data regarding differences in the induction of ICD hallmarks by Pt drug treatment and its effects on tumor cell recognition by DC.

3.1.6. Pt drug-mediated DAMP exposure and release

With regard to DAMP, oxaliplatin induces the entire panel of essential ICD drivers resulting in DC differentiation and maturation via signaling to diverse receptors including TLR.[21,355,508] Such, it was shown that supernatants from oxaliplatin-treated cancer cells induced DC differentiation and subsequently T cell proliferation and enhanced CTL cytotoxicity against cancer cells *in vitro*.[498] Oxaliplatin treatment results in surface localization of ER proteins including most prominently CRT at the cancer cell membrane via the PERK/eIF2α axis (compare chapter 2.2.6).[337,355,506,509] However, the situation is not so clear with cisplatin. On the one hand, there are several reports that no CRT exposure of (mainly murine) cancer cells exposed to cisplatin is found,[337,355,509] which was subsequently attributed to insufficient ER stress induction.[509] Consequently, it was shown that combinations of cisplatin with recombinant CRT[337] or ER stress inducers like thapsigargin and tunicamycin,[509] as well as with genetic CRT release by reticulon-1c overexpression[510] resulted in more pronounced induction of CRT exposure *in vitro* and vaccination of mice against cancer cells *in vivo*. However, on the other hand, a recent study by Di Blasio et al. reported that in several human cancer cell models, CRT exposure after cisplatin treatment was similar to (or even stronger than) the one by oxaliplatin.[363] In addition, both drugs induced similar levels of ecto-Hsp70, another important DAMP, in several cell lines of murine and human origin.[363] Subsequently, this resulted in enhanced uptake of tumor-derived particles, especially in $CD1^+$ and $CD16^+$ DC of myeloid origin via CD91 (while plasmacytoid DC - a type I INF-secreting, specialized DC subset predominantly functional in antiviral responses[511] - were distinctly less efficient).[363] In addition, upregulation of several co-stimulatory molecules (e.g. CD80 and

CD86) and a changed cytokine profile were found, which subsequently resulted in enhanced T cell proliferation.[363] Also Wong et al. reported CRT exposure of CT26 cells after cisplatin treatment.[338]

In contrast to CRT, there are consistent reports that both cis- and oxaliplatin induced efficiently and at comparable levels release of ATP and HMGB1.[337,349,364] However, with regard to HMGB1, it is worth mentioning that the role of this protein in the activity of Pt drugs has been already intensely investigated in a completely different context. Indeed, HMGB1 is a multi-faceted protein with diverse biological functions, which also include, besides acting as an architectural protein in chromosomes, distinct DNA chaperone activities.[512] Noteworthy, HMGB1 binds to DNA with structure-specificity (not sequence-specificity) and, consequently, plays an important role in regulating DNA-related processes based on recognition of altered DNA structure. HMGB family proteins (including HMGB1 and 2) are characterized by a DNA-binding domain which has an especially high affinity for a structural motif on DNA platinated by cisplatin but not by polynuclear Pt compounds.[513-515] Especially 1,2-intrastrand cross-linking adducts are recognized, which account for ~80% of total Pt-induced DNA adducts. Initially, it was assumed that this binding of HMGB proteins results in increased recognition of DNA damage repair mechanisms. However, more recent work revealed even the opposite, as binding of HMGB proteins, on the one hand, increased the distortion of platinated DNA and, on the other hand, shielded these lesions from the repair machinery.[516,517] Interestingly, HMG-domain family proteins have been also shown to bind more tightly to cisplatin than to oxaliplatin adducts.[178] Pt drugs inducing DNA lesions, which were not shielded by HMGB proteins, were distinctly less effective against cancer cells.[513,517] This points towards an important role of HMGB proteins in the mode of action at least of some anticancer Pt drugs. Accordingly, cisplatin adducts are especially well shielded by HMGB4, a recently discovered new member of the HMGB protein family.[516,517] Interestingly, this protein is preferentially expressed in testis, and malignant outgrowth from this tissue is known to bear exceptional sensitivity to cisplatin treatment leading to cure in the majority of patients.[518] The high efficacy of HMGB4 to sensitize cells against cisplatin seems to be based on its lack of the long acidic C-terminal tail, which is found in all other HMGB family proteins.[516,517] Consequently, while HMGB1 reduced nucleotide excision repair effectivity against 1,2-intrastrand cross-links by 45% compared to control, HMGB4 resulted in 90% inhibition at the same concentrations.[516] However, overall, it is unknown whether the DNA damage shielding by HMGB family proteins has a physiological function. Also, it is completely unknown, whether HMGB1 released from Pt-killed cancer cells is still associated to (platinated) DNA fragments, as multiple ways of (active and passive) HMGB1 release have been described,[512] and whether the differences in binding efficiency of HMGB1 to the Pt-DNA adducts of individual Pt drugs reflect differences in their respective ICD-inducing potentials.

Considering the amount of data on oxaliplatin, cisplatin and carboplatin regarding ICD induction, it is rather surprising that the scientific literature on this topic in case of other Pt compounds is very sparse. Neither for the only regionally approved Pt(II) compounds nedaplatin, lobaplatin and heptaplatin, nor for the lead Pt(IV)

compounds like satraplatin (Figure 8A) any data regarding activation of ICD are available. Nevertheless, Wong et al. have compared in their landmark publication on ICD induction several Pt(II) compounds and Pt(IV) prodrugs (Figure 8A-J) together with cisplatin, oxaliplatin and carboplatin (compare Figure 3). Their analysis started with a screening approach employing cancer cell phagocytosis by the J774 murine macrophage cell line as model for APC activation during ICD.[338] In their hands, Pt-NHC (Figure 8B), a cyclometalated complex supposed to target the ER, was most potent in inducing CT26 cancer cell phagocytosis by J774 cells. Interestingly, cisplatin, satraplatin, picoplatin (Figure 8C) and phenanthriplatin (Figure 8D) were scored positive concerning this parameter while oxaliplatin and carboplatin failed. The authors further showed that Pt-NHC-promoted phagocytosis was mediated by CRT exposure together with occurrence of several other ICD hallmarks such as ATP and HMGB1 release. As Pt-NHC represents a unique cyclometalated complex that selectively targets the ER and induces massive ER stress involving ROS production,[519] this substance would fulfill all criteria of a type II ICD inducer.[350] The contrasting results by Wong et al. with regard to oxaliplatin as compared to earlier reports[337] are enigmatic and again emphasize the complexity of these immune-stimulating processes.

Regarding Pt(IV), Arsenijevic et al. evaluated the cytotoxic activity of a series of $[PtCl_4(en)]$ (Figure 8K) and $[Pt(1R,2R\text{-DACH})Cl_4]$ (Figure 8L) complexes in comparison to several Pt(II) complexes.[520] The most active drug $[PtCl_4(en)]$ was further evaluated concerning the impact on the anticancer immune response against the orthotopically growing murine Lewis lung cancer LLC1 model. Treatment with $[PtCl_4(en)]$ increased the total number of $F4/80^+$ macrophages, DC, NKT, as well as $CD4^+$ T cells in the lungs of tumor-bearing mice. Moreover, $[PtCl_4(en)]$ significantly attracted perforin-positive CTL into the tumors. Accordingly, splenocytes isolated from tumor-bearing, $[PtCl_4(en)]$-treated animals showed enhanced cytotoxic activity against LLC1 cells *in vitro*, indicating that the strong anti-metastatic efficacy of $[PtCl_4(en)]$ could at least in part be based on an ICD-mediated, CTL response.

3.1.7. Pt drugs and TLR

DAMP release from dying cancer cells in response to Pt drugs activates PPR on the immune cell surface including several TLR. By far most data are available for activation of TLR4 by HMGB1 (compare chapter 2.2.6). Interestingly, one of the most convincing proofs that ICD indeed contributes also to the clinical efficacy of oxaliplatin comes from the side of TLR4 and concerns a study analyzing 338 patients with colorectal cancer of a randomized phase 3 trial.[337] Comparison of upfront oxaliplatin-based combination therapy vs. sequential chemotherapy revealed that patients with a loss-of-function mutation of TLR4 displayed a distinctly shorter progression-free and overall survival under oxaliplatin-containing therapy.[337] Based on the finding that exogenous HMGB1 is recognized by TLR4 on DC, activating a MyD88-dependent signaling pathway, also the combination of oxaliplatin with a TLR4 agonist (Dendrophilin, a standardized, highly purified formulations of S- and R-LPS) was investigated. This combination was characterized by a

highly synergistic activity via MyD88 against murine EL4 thymoma cells.[348] This is in good agreement with our own study using bacterial ghosts as potent adjuvant for oxaliplatin. Here, we could show that these immunogenic empty bacteria envelopes, produced by expression of the lysis gene E, were able to potently enhance ICD by oxaliplatin and subsequently increase the anticancer activity via stimulation of $CD8^+$ T cells.[521] The enhanced anticancer activity observed for oxaliplatin by stimulation of TLR4 signaling seems to be in contrast to a recent study with cisplatin by Kim and colleagues.[139] Here, the authors uncovered that cisplatin treatment of LPS-stimulated $CD11c^+$ BMDC significantly decreased the LPS-induced expression of CD80, CD86, and MHC class I and II in a dose-dependent manner.[139] Stimulation of cisplatin-treated DC with agonists for TLR2, TLR3, TLR4, TLR7, or TLR9 led in all cases to high production of anti-inflammatory IL-10, an effect also seen upon treatment with LPS. This IL-10 secretion was mediated via p38 MAPK and NF-κB signaling and induced development of a tolerogenic DC phenotype that was able to reduce proliferation of both $CD4^+$ and $CD8^+$ T cells. Furthermore, cisplatin/LPS co-treated DC altered differentiation and polarization of T cells, promoting T_H2 and FOXP3-negative Tr1 cells while reducing T_H1, T_H17 but also T_{reg} cells as compared to LPS-only treated controls.[139] In contrast, cisplatin treatment of murine peritoneal macrophages co-incubated with murine fibroblastic L929 cells induced a proinflammatory response, characterized by high expression of TLR2 and TLR4 and production of NO, TNF-α, IL-1β, IL-12, and IFN-γ.[522]

Considering the DNA-targeting activity of many Pt compounds (compare chapter 2.2.5), it is surprising that activation of various other TLR by nucleic acids, released form dying cancer cells, is less well explored. Nevertheless, several studies investigate combination of clinically approved Pt drugs with nucleic acid-responsive DNA/RNA-activated TLR agonists (e.g. for TLR3 and TLR9) and report strong synergistic activity *in vitro* and *in vivo*.[311-313,523] Ding et al. showed that pre-treatment of oral squamous cell carcinoma cells with the TLR3 agonist polyinosine-polycytidylic acid distinctly enhanced low-dose cisplatin-induced cancer cell death and activated spleen immunocytes while at the same time dampening several immunosuppressive cell subsets (MDSC, TAM, and tumor-associated fibroblasts) as well as adverse drug effects.[311] TLR9, a PRR that recognizes DNA with unmethylated CpG motifs, can be stimulated by synthetic oligodeoxynucleotides (ODN) expressing CpG motifs (CpG-ODN). Sommariva et al. showed that CpG-ODN downregulated DNA repair genes in murine colon carcinoma, but conversely, upon combination with cisplatin, upregulated these genes in immune cells and prolonged survival of animals as compared to experimental groups receiving single treatment only. Accordingly, ovarian cancer patients with a higher expression of CpG-ODN-modulated DNA repair genes (assessed by comparison with gene expression data derived from murine CpG-ODN-treated MC38 colon carcinoma and human IGROV-1 ovarian carcinoma xenografts) showed enhanced overall survival.[312] However, for another TLR9 agonist, the oligodeoxynucleotide PF-3512676, data are less convincing. Combination of PF-3512676 with cisplatin in a randomized clinical phase 2 trial reported promising results but also a higher frequency of hematologic

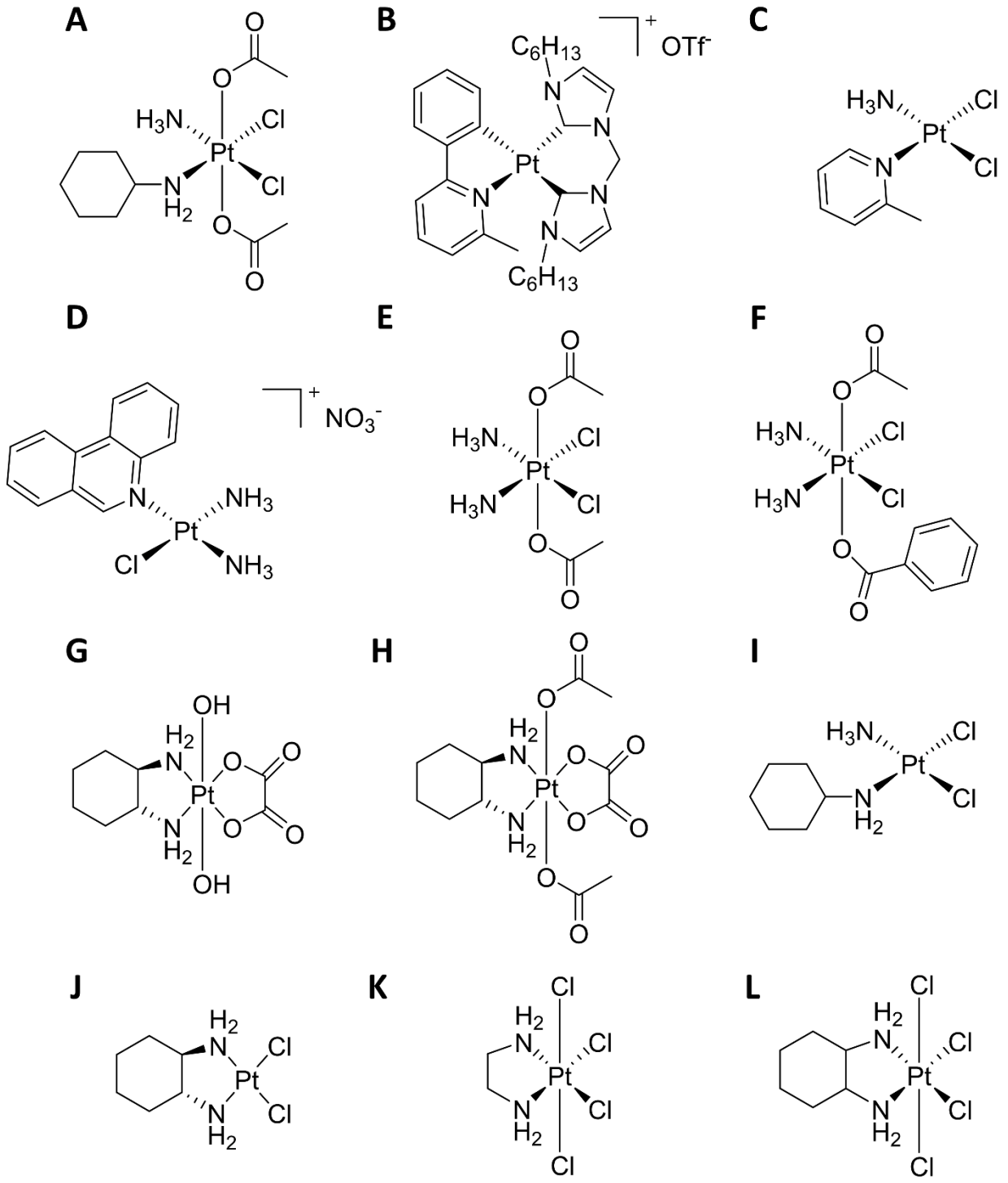

Figure 8. Chemical structures of lead preclinical anticancer Pt complexes and those with published immune-regulatory activities. (A) satraplatin, (B) Pt-NHC, (C) picoplatin, (D) phenanthriplatin, (E) cisplatin(IV)-$(OAc)_2$, (F) cisplatin(IV)-(OAc)(Obz), (G) oxaliplatin(IV)-(OH_2), (H) oxaliplatin(IV)-$(OAc)_2$, (I) JM-118, (J) [Pt(1*R*,2*R*-DACH)Cl_2], (K) [$PtCl_4$(en)] (en = ethylenediamine), (L) [Pt(1*R*,2*R*-DACH)Cl_4] (dach = (±)-trans-1,2-diaminocyclohexane). For details see text.

toxicity as compared to cisplatin alone,[313] while a following phase 3 trial showed no improvement in progression-free or overall survival together with increased toxicity in the combination setting.[524] With

regard to TLR3 activation, Van et al. demonstrated direct induction of ovarian cancer cell death by dsRNA, an effect that was synergistically enhanced by carboplatin and mediated via TLR3, RIG-I, and melanoma differentiation-associated protein 5 (a RIG-I-like receptor family member).[523] Moreover, it has to be considered that interaction with TLR- (especially TLR4-)mediated signaling also crucially contributes to adverse effects of Pt drug therapy including e.g. nephrotoxicity, peripheral neuropathy, and ototoxicity (compare chapter 6).

3.1.8. Impact of Pt drugs on DC maturation and T cell stimulation

Besides changes on the cancer cell level, Pt drugs may directly affect DC maturation and T cell interaction and these effects might be strongly dependent on the particular DC subpopulations investigated. For example, Hu et al. have described that treatment with cisplatin exerts only a minor inhibitory impact on the differentiation of human monocytes into $CD14^+$ monocycte-derived DC (moDC) as compared to other chemotherapeutics.[525] Such, cisplatin moderately delayed the downregulation of the human monocyte marker CD14, and slightly decreased the number of differentiated $CD1a^+$ cells *in vitro*.[525] Also, MHC class II expression was reduced, while no effects on CD86 and CD80 activation markers were observed. However, in subsequent assays with isolated $CD14^+$ moDC as APC and $CD4^+$ lymphocyte as responder cells unexpectedly cisplatin but not the other chemotherapeutics resulted in significantly enhanced staphylococcal enterotoxin B-induced T cell proliferation via the IFN-β pathway (while levels of IL-1β, TNF-α, IL-6 and IFN-α were unchanged).[525] In accordance, Tel et al. reported that treatment with oxaliplatin resulted in enhanced maturation of moDC and subsequently higher stimulation of T cell proliferation.[526] However, when analyzing blood-derived DC subsets in more detail, the opposite effects were observed with both TLR3- and TLR7/8-activated $CD1c^+$ myeloid DC[526] or TLR9-activated plasmacytoid DC, while no effects on TLR7/8-activated plasmacytoid DC were observed.[526] This was in all cases associated with downregulation of secretion of IL-6, TNF-α, CCL5, and CXCL10, with the exception of TLR3-activated myeloid DC, where only reduction of CXCL10 was detected and the other markers remained unchanged.[526] Furthermore, treatment of TLR9-activated plasmacytoid DC with oxaliplatin resulted in increased exposure of PD-L1 (but not PD-L2), which could explain the observed reduction in T cell stimulation by this DC subset.[526] This modulation led to increased IFN-α production, decreased inflammatory cytokine and chemokine secretion and a decrease of total STAT1 and STAT3 expression levels while total STAT2 and STAT6 as well as all phosphorylated STATs were unaffected.[526] No impact of oxaliplatin on expression of MHC class I and II of these TLR-stimulated myeloid and plasmacytoid DC was seen in this study.[526]

As mentioned in chapter 2.3.1, Pt-based therapy (cisplatin, carboplatin, oxaliplatin) downregulated PD-L2 expression in DC in a STAT6-dependent manner, which in turn resulted in enhanced T cell activation and increased tumor cell recognition comparable to PD-L2-blocking antibodies.[238] With regard to the underlying mechanism, DC exposed to the Pt drugs produced significantly higher levels of IFN-γ and IL-2. Moreover,

the T cell stimulatory capacity of matured DC via TLRs was enhanced. Interestingly, in contrast to the study by Hu et al., no significant difference in expression of MHC class I or II were observed, while in both studies expression levels of co-stimulatory molecules (CD80 and CD86) on DC remained unaffected upon Pt treatment.[238,525] Surprisingly, none of the tested Pt drugs (cisplatin, carboplatin, oxaliplatin, and nedraplatin) showed any effect on DC maturation in a extended screening study using a murine DC line stable transfected with yellow fluorescence protein under the control of the IL-1β promoter as readout.[527] These contradictory observations stress the importance of standardized cell models and drug screening conditions especially when testing interactions between cancer and immune cells.

With regard to the *in vivo* situation, a combination of ICD-inducing oxaliplatin with IL-12, a DC differentiation marker, enhanced the anticancer activity of oxaliplatin against preformed liver metastases of MC38 colon cancer cells in mice.[528] This treatment was associated with increased IFN-γ levels in the blood and an elevated ratio of cytotoxic $CD8^+$ T versus $CD25^+/FOXP3^+$ T_{reg} cells in the tumor tissue of the treated animals. In addition, monocytic MDSC ($CD11b^+/Ly6C^+/Ly6G^-$) were diminished. This indicates that the combination of oxaliplatin with IL-12 might be able to revert the immunosuppressive environment of the tumor.[528] Moreover, already first clinical trials on the combination of oxaliplatin/capecitabine with carcinoembryonic antigen (CEA)-peptide-loaded DC in colon cancer patients were performed. Indeed, good tolerability and a functional CEA-specific T cell response was observed in four out of seven patients.[529] However, also with regard to cisplatin, combination strategies with isolated, stimulated DC showed very promising effects. Thus, combination of systemically administered cisplatin with 5-FU and intratumorally applied BMDC that had been stimulated with GM-CSF- and IL-4 *in vitro* had strong synergistic activity against murine MC38 colorectal carcinoma tumors *in vivo*,[497] and combination of a Pt-containing therapy with a DC/CIK cell mixture (1:5) resulted in significantly better clinical outcome in advanced NSCLC patients.[530]

Taken together, these data provide evidence that combination of clinically approved Pt drugs with *in vitro* stimulated immune cells can have beneficial effects. However, the exact schemes and combination settings still warrant further evaluation.

3.1.9. Impact of Pt drugs on T cell and immune-regulatory subsets

Comparable to the DC compartment, several reports have addressed direct impacts of Pt drugs on isolated CTL in cell culture. Thus, in a study using both autologous EBV-specific and allogeneic CTL with lymphoblastoid cells as targets, distinct differences in the impact of the three clinically approved Pt drugs were found.[531] Surprisingly, pretreatment with oxaliplatin inhibited CTL cytotoxic potency, while this parameter was strongly enhanced upon contact with cisplatin and carboplatin, suggesting drug selectivity of this effect.[531] Additionally, Correale et al. showed that oxaliplatin treatment enhanced cytolytic activity of CEA peptide-specific CTL only when administered late (day 13) but not early (day 2) after starting *in vitro*

CTL stimulation with the respective antigen peptides. This suggests also a strong time-dependency of such immune-stimulatory effects.[532] Noteworthy, the stimulatory effect was probably based on a significant decrease of a T_{reg} cell subset, which continuously proliferates in lymphocyte cultures as a feedback response to the cyclic *in vitro* antigen stimulation.[532] These data were complemented by an *in vivo* approach using humanized HLA-A(*)02.01$^+$ transgenic (HHD) mice with thymidylate synthase-expressing autologous EL-4/HHD lymphoma cells. Interestingly, combination of an oxaliplatin-containing poly-chemotherapy regimen and a thymidylate-specific peptide vaccination exerted the strongest anticancer effects when chemotherapy was started after but not during the vaccination procedure. This was again paralleled by depletion of the T_{reg} cell subset in the tumor tissue, indicating that these cells are especially proliferative at that late timepoint of peptide-based vaccination.[532]

Several reports found that treatment with clinically approved Pt drugs results in altered ratios of diverse T lymphocyte subsets in animal models as well as in humans (for the latter also compare chapter 8.2). Accordingly, in the study of Tsai et al.[296] stimulation of primary murine lymph node cells with metronomic cisplatin resulted in IFN-γ production by CTL, which subsequently promoted differentiation of a T_H1 phenotype in CD4$^+$ T cells. The distinct activity of metronomic cisplatin against HPV-transformed murine TC1 tumors was accompanied by enhanced percentages of T_H cells (CD3$^+$/CD4$^+$) and T_{reg} (CD25$^+$/FOXP3$^+$) cells in the splenocyte cell population of the treated animals.[296] With regard to humans, administration of neoadjuvant 5-FU with cisplatin increased the number of CD4$^+$ and CD8$^+$ T cells as well as expression of HLA class I in patients with esophageal cancer, while the number of T_{reg} cells was unchanged.[533] Combination of cisplatin with gemcitabine and 5-FU in patients with advanced pancreas carcinoma resulted in a decline of absolute lymphocyte counts in most patients. Interestingly, the mean percentage of CD4$^+$ lymphoctyes and IL-12p70 and IFN-γ-producing NK cells increased after therapy (compare chapter 3.1.3).[221] Here it is worth mentioning that the increased proportion of CD4$^+$ T cells was not based on enhanced presence of the CD4$^+$/CD25$^+$/FOXP3$^+$ T_{reg} subset (which was actually decreased).[221] Accordingly, in early studies with melanoma patients the occurrence of CD4$^+$ TIL correlated with tumor regression and response to cisplatin (in combination with dacarbazine and IFN-α2b).[534] Moreover, in regressing tumor areas, CD4$^+$ cells were mainly negative for the activating co-stimulatory molecule CD28, while CD4$^+$ lymphocytes in the vicinity of living tumors cells were mainly positive for this marker. A similar picture was seen with CD8$^+$ cells.[535] On the one hand, interaction of B7 and CD28 co-stimulatory molecules has been shown to be essential for the activation of effector function mediating spontaneous tumor regression. Thus, it could be speculated that low expression of CD28 on T cells indicates down-regulation of the immune response to the tumor. On the other hand, CD28-negative T cells may reflect currently activated lymphocytes within an ongoing immune response.[536] In addition, resistance to CTL-mediated killing might be associated with expression of tumor-associated calcium signal transducer (TROP2/TACSTD2), a surface glycoprotein, which is highly expressed on various cancer cells.[537] Recently, it was shown that cisplatin

treatment resulted in further stimulation of TROP2 (as pro-survival signal) in human lung cancer cells *in vitro* and that co-culture of such cells with $CD8^+$ T cells induced apoptosis of these lymphocytes in a TROP2-dependent manner.[537]

During the last years, there is increasing evidence that chemotherapy may selectively down-modulate immunosuppressive components of the TME resulting in compromized cancer immune evasion (compare chapters 2.3.2 and 8.2).[213,538-541] One explanation for these effects might be the differential dynamics of homeostatic proliferation of T_{reg} compared to other (effector) T cell subsets in response to systemic chemotherapy, making this compartment more susceptible to cytotoxic drugs affecting DNA synthesis.[538,539] Such, it was shown that cisplatin treatment resulted in significant reduction of intratumoral and splenic T_{reg} cells in CT26[491] and RHM-1 cancer-bearing[213] mice, respectively. Moreover, while treatment with the FOLFOX regimen did neither alter total lymphocyte counts, nor percentages of $CD4^+$, $CD8^+$ or $CD3^-/CD56^+$ (NK) cells in PBMC of colon carcinoma patients, the percentage of T_{reg} ($CD4^+/FOXP3^+$) cells was reduced especially in patients with high T_{reg} numbers before treatment.[542] Using murine CT26 cells as lung and abdominal metastasis model, Gou et al. found that IL-7 (an IL-2-related immune-stimulatory cytokine) exerted anticancer activity only when combined with oxaliplatin based on enhanced CTL tumor recruitment and markedly reduced splenic T_{reg} cell levels.[543] Accordingly, T_{reg} ($CD4^+/CD25^+/FOXP3^+$) cell depletion by using a CD25 antibody inhibited regrowth of murine mesothelioma models between cyles of cisplatin-containing chemotherapy.[544] Likewise, eradication of a regulatory B cell subset, i.e. plasmocytes expressing IgA, IL-10, and PD-L1 in a TGF-β-dependent fashion, was essential for successful therapy of several murine prostate cancer models by oxaliplatin-induced ICD.[545]

There are also several indications that Pt drugs affect not only T_{reg} cells but also other immunosuppressive leukocyte compartments including - besides the already discussed M2 macrophages - also MDSC.[294,489] Chang and colleagues showed that especially dose-dense cisplatin-containing therapy massively reduced MDSC numbers in mice bearing ovarian tumors.[213] In the MBT-2 murine allograft mouse model for bladder cancer, cisplatin treatment inhibited tumor progression by effectively decreasing the proportion of granulocytic MDSC. Comparable observations were also obtained with regard to PBMC from patients where the levels of granulocytic MDSC inversely correlated with CTL amounts.[546] Cisplatin treatment selectively decreased the amounts of MDSC ($Gr\text{-}1^+/CD11b^+$) cells in the murine B16 melanoma model paralleled by loss of the MDSC marker Gr-1 and gain of the DC marker CD40 at the cell suface and loss of activity against CTL and CIK effector cells.[540] Accordingly, the immune-inhibitory functions of MDSC were distinctly reduced by cisplatin in the A375 human melanoma xenograft nude mouse model, and proliferation of CTL, together with IFN-γ production were massively enhanced when co-cultured with cisplatin-pretreated MDSC.[547] Accordingly, a combination of cisplatin with a thrombin inhibitor was highly active in reducing both MDSC and the levels of pro-tumorigenic cytokines while increasing CTL-derived IFN-γ in the ascites of ID8 ovarian cancer-bearing mice.[548] With regard to colon cancer therapeutic regimens

used in the clinics, Kanterman et al. showed impressively that while FOLFIRI (5-FU, folic acid, irinotecan) induced severe immunosuppression, FOLFOX did the opposite. This difference was based on the promotion of MDSC survival by irinotecan but suppression by the 5-FU/oxaliplatin combination in a chemically-induced colon cancer model.[549] These effects were also confirmed in peripheral blood of treated stage IV colorectal cancer patients, proving the clinical relevance of these preclinical observations.

3.1.10. Combination of Pt drugs with immune modulators

As already described above, cancer cells are known to actively inhibit immune recognition through diverse mechanisms, including loss of the antigen-presenting machinery or expression of inhibitory molecules and enzymes that induce T cell anergy or apoptosis. One such enzyme is IDO, metabolically inducing T cell suppression (compare chapter 1.2.5).[118,161,162] IDO expression has been described for several tumor types, and the interest in IDO inhibition is also reflected by the clinical development of diverse IDO inhibitors.[163] With regard to Pt drugs, there is strong evidence that the combination of cisplatin with IDO inhibition has highly synergistic activity, as inhibition of IDO by shRNA sensitized A549 and HeLa cells to cisplatin treatment.[550] Furthermore, the clinically developed IDO inhibitor indoximod had synergistic activity with cisplatin against LN2299 glioma cells *in vitro*[551] and against an autochthonous mammary gland tumor model *in vivo*.[552] Also combination of oxaliplatin with an indoximod-containing nanovesicles exerted synergistic activity against murine pancreas carcinoma. This was associated with an increased number of DC ($CD91^+/CD11b^+/CD11c^+$) as well as enhanced IFN-γ and decreased IL-10 levels in the tumors of treated animals.[553] The promising activity of this approach is also reflected by the development of several new multi-targeted therapeutics combining Pt drugs with IDO inhibitors. Of special interest are here, besides several nanoformulations (e.g. a mesoporous silica NP containing both indoximod and oxaliplatin[553] or a new nanoformulation loaded with cisplatin and new IDO inhibitor (compare chapter 4)[554]), novel Pt(IV) drugs which contain indoximod derivatives in the axial positions.[555]

Other inhibitory cell-surface receptors, which are expressed on T cells and represent promising targets for checkpoint inhibitor-based immunotherapy are the above-mentioned CTLA-4 and PD-1 together with its ligand PD-L1. Several studies indicate a strongly synergistic activity of checkpoint inhibitors with Pt drugs both in mouse models but also in the clinical situation (for the latter compare chapter 8). For example, combination of oxaliplatin with MEDI4736, an antibody with high specificity against human PD-L1, was highly synergistic against murine CT26 tumors.[556] Also, the combination of cisplatin with a CTLA-4-blocking antibody resulted in efficient antitumor effects in the murine AB-12 mesothelioma model based on enhanced infiltration of perforin- and granzyme B-positive CTL.[557] In contrast, no synergism of a comparable drug combination was found in mice bearing mesothelioma AB1-HA tumors.[558] Such discrepancies might be explained at least in part by data considering that the optimal timing of the

combination of checkpoint inhibitors is challenging. Hence, the Pt drugs might activate or downmodulate PD-L1 and PD-L2 expression in conjuction with impacts on MAPK and STAT signaling pathways as outlined in chapter 2.3.1. Considering that both PD-1/PD-L1 blockade and the immunogenic metal drug chemotherapy activate the immune system, especially immune-related adverse effects need to be followed tightly, although side effects were so far widely controllable in clinical studies (compare chapter 8.3). Nevertheless, strategies are currently developed to deliver either the metal drug or the checkpoint inhibitor topically to the malignant tissue. At the side of the metal drugs, this concerns prodrug and tumor delivery strategies with tumor-specific activation or release of the active metal compound.[19,27,413] In a very recent report, Song et al. suggested to apply oxaliplatin systemically as usual but to deliver PD-L1 inhibitors locally using a PD-L1 trap in form of the respective plasmid DNA via lipid-protamine-DNA NP. In orthotopic murine colon and breast cancer as well as melanoma models, this strategy resulted in massive synergism concerning the anticancer activity, however at the same time avoided splenic T_H17 cell accumulation as a marker of adverse systemic inflammation.[559]

Another strategy to enhance recognition of cancer cells by the immune system is the use of a CD40 agonist antibody (CP-870,893). CD40 is a cell surface member of the TNF superfamily, which is expressed in all APC subpopulations.[560] In general, CD40 signaling activates APC and triggers tumor-specific T cell responses. Based on the strong immunogenic effects of Pt drugs, it was hypothesized that CP-870,893 should exert potent synergisms in a combination setting. Consequently, already two clinical phase 1 trials of this CD40 agonist with Pt drugs have been reported: combination of CP-870,893 with carboplatin (and paclitaxel) in patients with advanced solid tumors,[560] and with cisplatin (and pemetrexed) against malignant pleural mesothelioma.[561] In both studies, the proportion of memory B cells (together with the activation marker CD86) increased. Moreover, especially, in the mesothelioma study, although the objective response rates to the combination were similar to chemotherapy alone, three patients achieved long-term survival.[561]

Another co-stimulatory approach is the combination of Pt drugs with an agonistic 4-1BB (CD137) antibody, a surface glycoprotein and member of the TNFR superfamily 9, which is expressed on activated T and NK cells.[562] Indeed, cisplatin was found to be highly synergistic with an agonistic (crosslinking) 4-1BB antibody in B16- and CT26-bearing mice.[541] In more detail, cisplatin increased the levels of 4-1BB on T cells and together with 4-1BB antibody enhanced the antitumor immune response, resulting in complete remission and $CD8^+$ T cell-mediated vaccination in several of the treated mice.[541] Interestingly, these effects were observed together with distinctly reduced cisplatin-mediated nephrotoxicity[541] (compare chapter 6.2). In murine ID8 ovarian and TC1 lung carcinoma allografts, a combination of cisplatin with 4-1BB-activating and PD-1-blocking antibodies significantly prolonged animal survial and was suggested to be even curative in some cases.[563]

Finally, in line with the above-described synergistic effects of Pt drugs with DAMP such as LPS[348] or bacterial ghosts[521] (compare chapters 3.1.6 and 3.1.7), Wong et al. recently designed the first asymmetric

Pt(IV) complexes containing formyl peptide receptor (FPR)1/2-targeting peptide ligands (such as annexin 1).[564] Interestingly, FPR1/2 are PRR not only expressed in abundance on myeloid cells, but also on various cancer cell types.[565] Accordingly, these new compounds not only enhanced the cytotoxic activity of PBMC (together with secretion of TNF-α and IFN-γ), but also exerted significant anti-proliferative activity against FPR1/2-expressing cancer cell lines.[564]

Together, these multifaceted and diverse examples of Pt drugs as promoters of an anticancer immune response indicate a yet underestimated mode of action of these metal-based compounds and support their use in conjuction with immunotherapeutic approaches.

3.2. Arsenic (As)

In contrast to most other metals discussed in this review, As is an element of the main groups directly below phosphorus. Consequently, compounds of As resemble in several respects those of phosphorus e.g. that it readily forms covalent bonds with most non-metals including carbon. The biologically common oxidation states are As(III) and As(V). The latter is significantly less toxic and its biological activity is mainly based on substitution for phosphate e.g. in ATP. Furthermore, As(V) is readily metabolized to As(III) species due to a sophisticated cascade of reductive and oxidative events reflecting the need for protection of eukaryotic cells against environmental arsenicals.[194,566] The environmental form of As(III) is As trioxide (ATO; As_2O_3; compare Figure 3) which in water forms arsenous acid ($As(OH)_3$) or the corresponding arsenite salts e.g. AsO_2^-.

Several comprehensive reviews published in recent years have dealt with the molecular mechanisms underlying the profound impact of As on the integrity of various organ systems of the body, including the integumentary, nervous, respiratory, cardiovascular, endocrine, hepatic, renal, and reproductive systems.[567] Importantly, the hematopoietic system is another compartment strongly affected by (environmental) arsenicals. Illustratively, this metal has been documented to affect manifold immunological processes, implicated in inflammation, ROS production, hypersensitivity reactions, lymphocyte activation/function as well as humoral immunity. Intriguingly, these effects are subject to marked dichotomy, being either stimulatory or inhibitory, depending on the experimental settings, cell types, or species studied (summarized comprehensively by Dangleben et al.[568] and by Zhang et al.[569]).

On the one hand, environmental exposure to As is a global public health concern, because it is widely distributed in drinking-water and dust from industrial processes and associated with increased risk for numerous diseases, including skin, lung and bladder cancer.[570,571] On the other hand, ATO (the identical compound encountered as environmental toxin) is approved for the treatment of APL, targeting the PML/RARα fusion protein as well as the chimeric transcription factor AML1/MDS1/EVI1 with high rates of complete remission (compare chapter 2.1).[572,573] In addition, the cytotoxic activity of ATO is strongly

associated with enhanced production of ROS, modulation of pro- and antiapoptotic factors, and cytoskeletal derangements.[574-577] Such, also regarding clinical application of ATO, increased NADPH oxidase (NOX) activity and elevated basal oxidative stress have been suggested to contribute to the high ATO sensitivity of APL cells.[578]

Besides ATO, other organic and inorganic arsenicals are currently being investigated in clinical trials for hematological malignancies. These include As sulfide and S-dimethylarsino-glutathione[579] (www.clinicaltrials.gov) (Figure 9A). Furthermore, several As-based compounds are in preclinical phase, including e.g. melarsoprol (originally applied for the 'sleeping disease' trypanosomiasis) (Figure 9B), S-dimethylarsino-thiosuccinic acid (Figure 9C), dipropil-S-glycerol arsenic (Figure 9D), arsenicin A (Figure 9E), dimethylarsinic acid (Figure 9F), 4-(N-(S-glutathionylacetyl)-amino)phenylarsonous acid (GSAO) (Figure 9G), and P-(N-(S-penicillaminylacetyl)amino)phenylarsonous acid (PEANO) (Figure 9H).[579]

A considerable body of literature on As compound-mediated effects on the immune compartment is derived from environmental arsenite.[568,580,581] Despite obvious differences between environmental arsenite and pharmaceutically administered ATO including dose, uptake route (ingestion, inhalation, transepithelial vs. intravenous injection, respectively), and exposure duration, the fact that the active metabolite is chemically identical in both cases (in water, ATO is converted into arsenite) makes data on immune interactions obtained from the environmentally-derived substance highly relevant for cancer therapy. For instance, Haque et al. recently reviewed the immune-modulatory role of environmental As exposure focusing on T cell function.[582] The authors conclude that As effects on the immune system tend to disrupt immunological control and increase propensity for autoimmune diseases, infection, and cancer, at least partially due to oxidative stress, inflammation and imbalanced lymphocyte activation. For instance, in 2012, Morzadec et al. have shown inhibitory activity of As(III) on IL-17A expression of T_H17 cells.[583,584] In addition to the effect on T cells, environmental ATO was demonstrated to affect macrophage maturation by disruption of cytoskeletal and phagocytic processes as well as deranged surface marker and cytokine expression (downregulation of CD11b, upregulation of the TLR4 co-receptor CD14, as well as of TNF-α and IL-8).[585,586] Contradicting these data, a study on inhaled ATO performed in mice demonstrated no impact on innate immune cell status, however, impaired humoral responses.[587] Furthermore, a DC-depleting effect of As has been described in a porcine *in vitro* model.[588]

As stated above, data on the immune impact of arsenicals are controversial, depending - amongst others - on the scientific focus of the studies presented or on applied doses. With regard to the therapeutic development of As compounds, a number of studies aimed to develop As compounds as immunosuppressive agents for diseases that require a reduction of immune effects such as autoimmune reactions, transplant rejection and chronic inflammatory conditions (standing in contrast to the desired immune stimulation in anticancer therapy). For instance, immune-compromising effects of ATO are derived from transplantology research.[589,590] Several years ago, Li and colleagues demonstrated in an elegant animal model that ATO

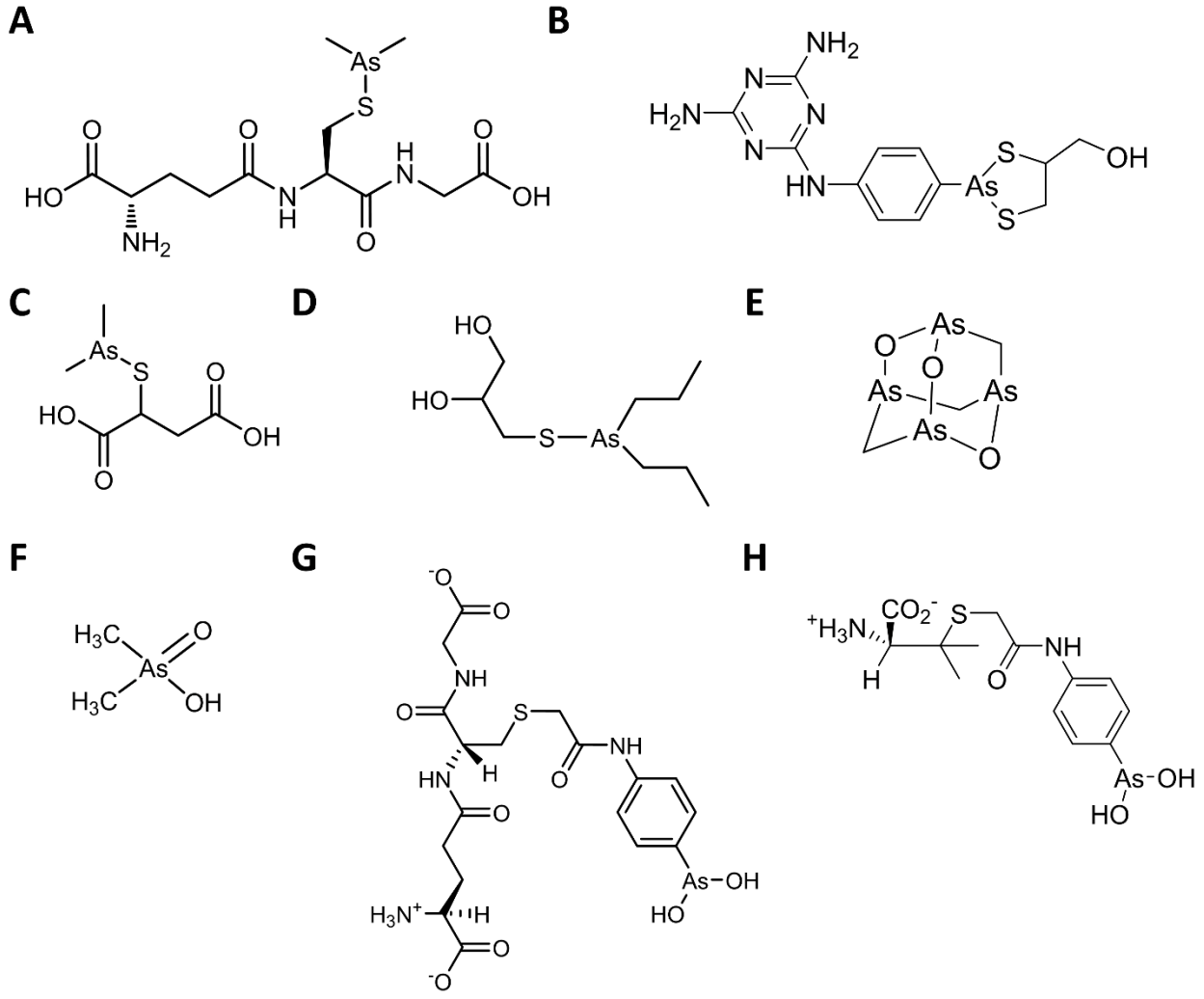

Figure 9. Chemical structures of lead anticancer arsenic-based compounds and those with published immune-regulatory activities. (A) S-dimethylarsino-glutathione (Darinaparsin, ZIO-101), (B) melarsoprol, (C) S-dimethylarsino-thiosuccinic acid (MER1), (D) dipropil-S-glycerol arsenic, (E) arsenicin A, (F) dimethylarsinic acid, (G) 4-(N-(S-glutathionylacetyl)amino) phenylarsonous acid (GSAO), (H) P-(N-(S-penicilllaminylacetyl)amino)phenyl-arsonous acid (PEANO). For details see text.

induced depletion of alloantigen-primed $CD8^+$ memory T cells, leading to reduced heart allograft rejection, accompanied by reduced levels of IL-2 and IFN-γ, as well as increased levels of IL-10 and TGF-ß within the transplants.[590] Furthermore, immune- suppressive effects of ATO have been emphasized in chronic inflammatory disorders such as asthma or psoriasis.[591] Furthermore, Li and colleagues showed asthmatic airway hyper-responsiveness to be alleviated by ATO-mediated $CD4^+$ T cell death.[591] Together, these data suggest detrimental effects of ATO on immune compartments. This would at first sight argue against immune-mediated mechanisms contributing to the anticancer efficacy of this compound and question the feasibility of combining this compound with immunotherapeutic strategies.

However, as outlined above, the deregulated immune and metabolic situation in the malignant tissue might not allow such straight-forward conclusions. Accordingly, data derived from As compounds tested as

anticancer agents suggest otherwise. Several papers show the involvement of deranged cytokine (e.g. IL-1ß, TNF-α) expression profiles and NF-κB signaling in cancer cell death induction by As compounds including ATO and phenylarsine oxide.[592,593] Already in 2001, an immunologic mechanism behind the therapeutic effect of ATO was suggested by Deaglio and colleagues.[594] The authors observed that exposure of human myeloma-like cell lines to low doses (0.5-1.0 µM) of ATO strongly increased the killing potential of LAK cells *in vitro*. This effect was accompanied by LAK cell-upregulation of CD31 and CD11a, the ligands for the myeloma cell surface markers CD38 and ICAM-1, respectively, suggesting increased cell-cell adhesion to promote tumor cell killing. A follow-up study reported that already 0.5 µM ATO increased breast cancer cell lysis by LAK cells. Here, again increased cell-cell interactions via ICAM-1 and the lymphocyte-associated integrin family member LFA-1 appeared to play a key role in tumor cell lysis.[595] In 2012, it was reported that the anticancer efficacy of ATO - besides dependency on an immune-competent background - was increased based on selective T_{reg} depletion, furthermore enhancing anticancer efficacy of adoptive splenocyte transfer in a colon cancer model in mice.[427,596] The authors attributed T_{reg} depletion to oxidative and nitrosative bursts specifically in this cell compartment. This specific effect may be attributed to an altered redox status, mediated by upregulation of pro-oxidative genes encoding iNOS, superoxide dismutase 1 (SOD1), copper chaperone for superoxide dismutase and NADPH oxidase organizer 1 (NOXO1) exclusively in T_{reg}, but not in other $CD4^+$ cells, resulting in T_{reg}-specific induction of ROS and reactive nitrogen species (RNS). Interestingly, in this study, immune-stimulatory effects were achieved at doses (0.5-1 µM) lower than would be required for cancer cell death induction *in vitro* (2 µM). Similar effects have also been demonstrated in independent reports.[594,595] Corroboratively, Xu et al. showed impaired T_{reg} function in peripheral blood of ATO-treated APL patients, although in this case, a potential involvement of altered redox stress was not investigated.[426] Furthermore, reduced pulmonary metastasis of colon carcinoma cells by ATO was accompanied by reduced T_{reg} infiltration in metastatic nodules.[597] In a follow up study by the same group, this finding was harnessed for a combination approach of ATO with adoptive T cell transfer, yielding synergistic anti-metastatic effects in the same model of colon carcinoma colonization to the lungs.[598] In addition, yet another publication by Wang et al. demonstrated that ATO altered the immune microenvironment in hepatocellular carcinoma allografts, also in this case illustrated by reduced T_{reg} infiltration, but, in parallel, also by an overall increase in intratumoral (not further specified) T cells.[599] This went hand in hand with decreased IL-10 and TGF-ß, as well as increased IFN-γ serum levels. The mechanism underlying selective T_{reg} depletion by ATO might not only be based on hypersensitivity, but even on a direct inhibition of FOXP3 transcription factor expression in naïve T cells, blocking differentiation towards a T_{reg} phenotype.[429] A comprehensive review on the immune-modulatory role of As on T_{reg} cells has been published recently.[582] In addition to T_{reg} depletion, As drugs may also exert their antitumor efficacy via inhibition of other immunosuppressive cell types. For instance, Gao and colleagues have recently shown that ATO induces MDSC differentiation, inhibits their proliferation and triggers

apoptosis induction.[425] The *in vivo* antitumor efficacy was accompanied by reduction of splenic MDSC and attenuation of their CTL-inhibitory potential. The authors identified blockade of the JAK/STAT, PI3K/AKT and MAPK signaling pathways to play key roles in the downregulation of MDSC activity, which was further associated with decreased levels of TNF-α and IL-10 in the blood as well as of VEGF, iNOS, TGF-ß, ROS and arginase-1 expression in MDSC of treated animals.

With regard to the influence of As drugs on the visibility of cancer cells for the innate immune compartment, ATO has been shown to increase transcription and surface display of NKG2D ligands (predominantly ULBP1) in chronic myeloid leukemia (CML), APL and breast cancer cell lines, leading to enhanced NK cell-mediated killing.[600]

In a combination approach with the rationale to enhance antitumor CTL responses, ATO was co-administered with an expression vector encoding B7-H3.[601] B7-H3 - a B7 family member surface marker - is expressed in non-lymphoid tissues and implicated in effector lymphocyte regulation in peripheral tissues.[602] This co-receptor exhibits a functional dualism, acting on the one hand as inhibitor of T cell activation, but has on the other hand also been shown to induce antitumor immunity by stimulating T_H1 and CTL responses.[602,603] In a study by Luo et al., *in situ* administration of a B7-H3 expression plasmid followed by (intratumoral) ATO injection led to strongly synergistic effects against a subcutaneous murine hepatocellular carcinoma model.[601] This was associated with increased IFN-γ levels in the blood and with CTL- and NK cell-mediated antitumor immunity, leading to immunologic memory and rejection of cancer cells upon systemic re-challenge.

Taken together, there is an ambiguous picture emerging concerning the impact of As compounds on the immune system. Importantly, for ATO, evidence for both immunosuppressive and -stimulatory activities on different immune compartments exists, especially when comparing environmental versus pharmacological exposures and, in the latter case, also under non-malignant versus neoplastic conditions. Nevertheless, enhanced visibility of cancer cells to immune cells by upregulation of surface stress markers and selective depletion of tumor-associated regulatory immune cell compartments in response to As remedies are encouraging and warrant clinical validation.

3.3. Ruthenium (Ru)

When Barnet Rosenberg first published the anticancer activity of some Pt compounds with cisplatin as most prominent member in 1969,[208] he cited already two reports on the anticancer activities of other metal drugs, namely Ru and rhodium (Rh) compounds. The respective Ru observation dated back to a paper published by Collier and Kraus in 1931.[604] This means that the idea of Ru as metal center for anticancer complexes is even distinctly older than the highly successful Pt. Several classes of Ru-based compounds with unique modes-of action have been studied since then in detail, ranging from purely inorganic to organometallic

compounds.[605-611] Besides systemic cancer therapy, Ru complexes have also been developed for the use in photodynamic therapy.[612] Ru compounds are believed to represent promising alternatives to anticancer Pt drugs based on several proposed advantages,[606,610] including 1) lower toxicity and selective tumor uptake by protein binding, probably involving albumin and transferrin;[613-616] 2) different resistance mechanisms than Pt compounds;[617,618] 3) easily accessible oxidation states Ru(II) and Ru(III) in biological systems, allowing Ru(III) prodrug strategies based on activation by reduction in the cancer tissue;[194] 4) interaction with protein targets as predominant mode of action.[609,619,620] The current developments in the field of anticancer Ru drugs have been reviewed extensively recently.[184,610,616,621] So the question remains why to date only three Ru complexes, namely KP1019 (Figure 10A) and it sodium analog KP1339 (Figure 10B), as well as NAMI-A (Figure 10C) have entered clinical evaluation as systemic anticancer therapeutics, while a fourth one, TLD-1433 (Figure 10D), is studied in frame of photodynamic therapy.[188-191,622] And further asked: why has no Ru compound made it to clinical approval so far? One explanation might be that several of the key advantages of Ru drugs mentioned above might have been overestimated,[192,614] leading to creation of "undemonstrated misconceptions or myths" as recently drastically stated by Enzo Alessio in a personal perspective on the field of Ru anticancer drugs.[623] Another missing piece in the puzzle might be that - in contrast to the already extensively investigated role of the immune system in the anticancer activity of Pt compounds (compare chapter 3.1) - comparable investigations on immune components of Ru complexes are still relatively sparse. Nevertheless, this picture has recently started to change also due to the observation that DNA is, in contrast to the widely used Pt agents, not always the central target of anticancer Ru compounds.[609,619,620,624,625] Additionally, the durability of the responses observed in the recent phase 1 clinical study (NCT01415297) for KP1339 (IT-139) suggests contributions of long-lasting stromal effects like activation of immune memory functions.[188]

As in case of other anticancer metal drugs, Ru compounds were a long time considered to be predominantly immunosuppressive. This assumption was mainly based on findings especially for ruthenium red (an inorganic dye; ammoniated Ru oxychloride, Figure 10E) inhibiting a multitude of ion channels including L-type calcium current.[626] Ruthenium red was demonstrated to inhibit T cell proliferation in response to several stimuli including TCR activation by viral antigens, alloantigens and also cytokines like IL-2 *in vitro*.[627] Moreover, ruthenium red blocked expansion of lymphocytes in draining lymph nodes and specific antibody production in mice immunized with cytochrome c combined with complete Freud's adjuvant.[627] Several complexes of a series of pyridine- and imidazole-substituted Ru complexes were even more active as compared to rapamycin in blocking TCR-mediated lymphocyte stimulation in the very low nanomolar range.[628] A binuclear (η (6)-p-cymene)Ru(II) complex containing a bridging bis(nicotinate)-polyethylene glycol ester ligand inhibited proinflammatory T_H1/T_H17 cell differentiation and induced a T_{reg} phenotype characterized by IL-4 and IL-10 production.[430] Furthermore, the Ru(II) complex cis-[Ru(II)(η^2-O_2CR)$(dppm)_2$]PF_6 (R = H or $(CH_2)_4COOEt$) (Figure 10F) was developed as anti-*Leishmania* agent based

on the iron (Fe)-mimetic properties of the Ru core and showed high anti-parasitic activity. This, however, went hand in hand with *in vitro* cytotoxic activity against human macrophages in the low- to submicromolar range.[629] Furthermore, Ru is also used as central metal for the synthesis of carbon monoxide (CO)-releasing molecules (CORM). CO, released in small amounts, is implicated in various biological processes, for instance protecting against inflammation or acting as vasoactive molecule for blood pressure regulation.[630,631] Endogenously, CO is generated by heme oxygenase (HO)-1 and HO-2 in frame of haem catabolism.[632] The anti-inflammatory effects of CO include, for instance, decreased production of TNF-α and NO in macrophages.[633,634] Hence, CORM, predominantly synthesized as carbonyl complexes using e.g. Ru as central transition metal, have been developed for treatment of inflammatory diseases[635] such as arthritis, colitis or sepsis, as well as for ischemic conditions including myocardial infarction,[636] peripheral vascular dysfunction,[637] and acute renal failure.[632,638] Recently, however, CORM complexes have also been analysed for their anticancer potential. These include, amongst others, tricarbonylchlorido(glycinato)Ru(II) (CORM-3) (Figure 10G) and complexes of fac-[Ru(II)$(CO)_3Cl_2L$] with L = N^3-methylbenzimidazole (MBI) or 5,6-dimethylbenzimidazole (DMBI), the latter reducing *in vivo* tumor growth in an immunocompetent allograft model.[639] For these complexes, however, it will be necessary to investigate, whether CO release may hamper anticancer activity by anti-inflammatory/immunosuppressive effects exerted directly on immune cells.[634] Correspondingly, a role of CORM-3-released CO has been described in NLRP3 inflammasome inactivation and decreased IL-1ß and IL-18 secretion in LPS- and ATP-stimulated macrophages.[640] Similarly, suppressive effects of CORM-3 on NO and IL-1ß production have been documented in LPS-stimulated macrophages.[641] In this case, inhibition of nuclear NF-κB translocation and STAT1 phosphorylation have been suggested to play a role in the anti-inflammatory effects of CORM-3. Recently, this compound was also found to induce M2 polarization of naïve rat alveolar macrophages as well as to suppress iNOS expression in M1 macrophages.[642] However, immune-cell suppressive effects of CO-releasing molecules might not be the only obstacle to successfully introduce these compounds as anticancer agents. Importantly, in an *in vitro* model, CORM-3 even protected renal cancer cells (as well as normal kidney cells) from cisplatin cytotoxicity by reducing levels of TNF-α and cleaved-caspase 3.[643] This, on the one hand, suggests CORM-3 as reno-protective agent in cisplatin anticancer therapy, but, on the other hand, undoubtedly questions a successful clinical application as single anticancer agent or within immunooncological combination schemes.

However, several observations suggested that the interaction of Ru compounds with the immune system especially in frame of cancer might be complex. Hence, one study presented an ambiguous picture of immune-regulatory effects of Ru compounds. The compound cis-(dichloro) tetraammineruthenium(III) chloride exerted cytotoxic activity towards PBMC at high concentration, while at low doses inducing their proliferation and IL-2 production.[644] Interestingly, already earlier research on the anticancer activities of NAMI-A and KP1019/KP1339 or their predecessors suggested substantial impacts of host factors including

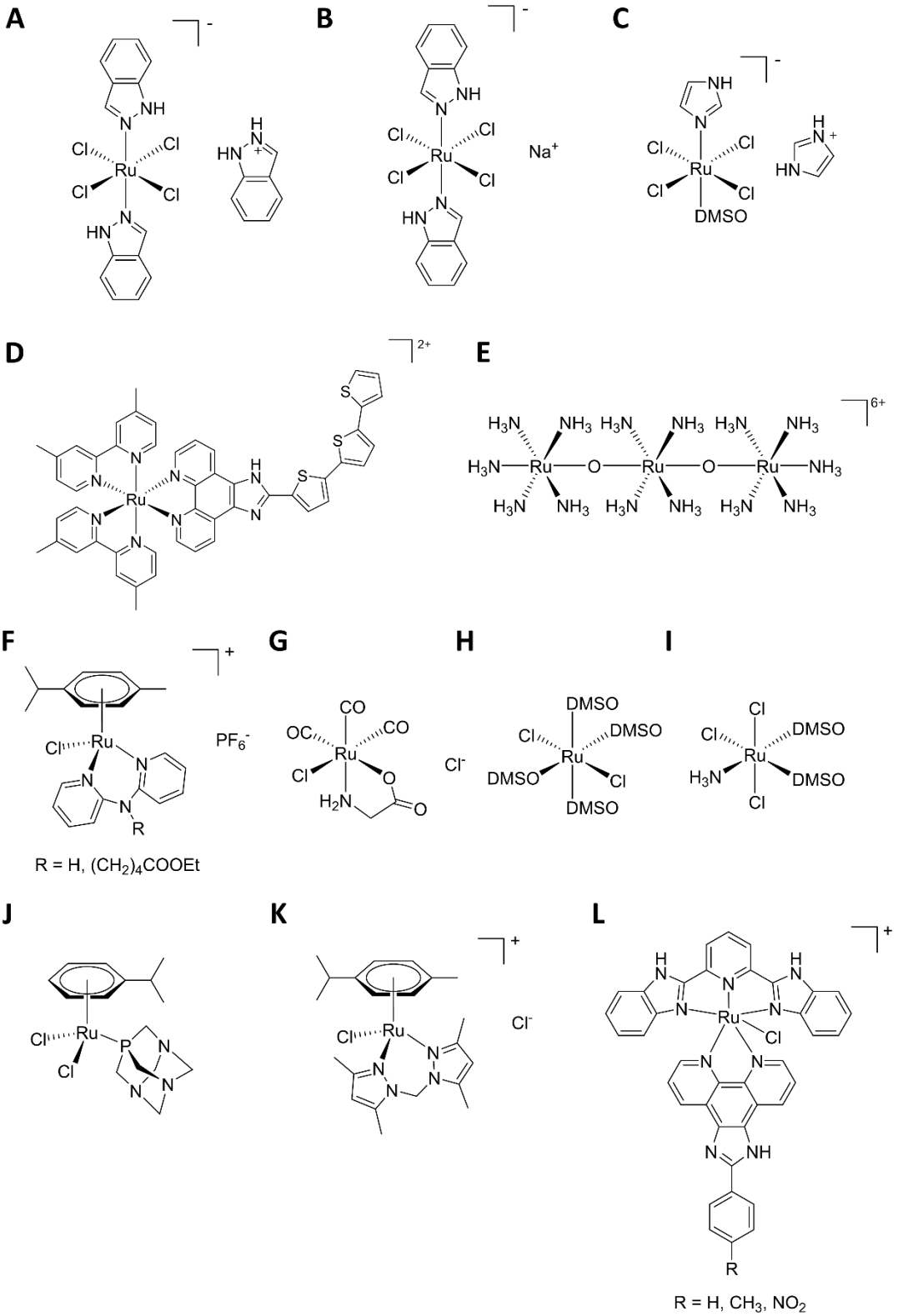

Figure 10. Chemical structures of lead anticancer ruthenium complexes and those with published immune-regulatory activities. (A) KP1019, (B) KP1339, (C) NAMI-A, (D) TLD-1433, (E) Ruthenium red, (F) cis-[Ru(II)(η^2-O_2CR)(dppm)$_2$]PF_6 (R = H or $(CH_2)_4$COOEt), (G) CORM-3, (H) trans-[$RuCl_2(DMSO)_4$], (I) mer,cis-[$RuCl_3(DMSO)_2NH_3$], (J) RAPTA-C, (K) UNICAM-1, (L) [Ru(bbp)(p-mpip)Cl]ClO_4 (complex , bbp = 2,6-bis(benzimidazolyl)pyridine; p-mpip = 2-(4-methylphenyl)imidazo [4,5-f]-1,10-phenanthroline). For details see text.

immune mechanisms.[645] Kreuser et al. have reported enhanced activities of heterocyclic Ru complexes including KP1019 against colon cancer cells after pretreatment with immune-stimulatory cytokines like type I and II IFN.[646] Agents such as trans-[$RuCl_2(DMSO)_4$] (Figure 10H) and mer,cis-[$RuCl_3(DMSO)_2NH_3$) (Figure 10I) widely lost their anticancer activity when mice were pretreated with immunosuppressive agents.[645] However, xenogenization experiments with transplantation of NAMI-A-treated MCa mammary carcinoma cells for up to 13 transplant generations did not show any signs of tumor rejection as an indicator for enhanced antigenicity, arguing against strong mutagenicity of these Ru(III) complexes.[647] Together, these data suggest that Ru compounds might have an impact on the cancer-immune crosstalk without directly altering cancer cell antigenicity.

Surprisingly, despite these early indications of an immunological contribution to Ru complex anticancer activities, this track was not systematically taken up and followed. For many newer and promising drug classes, like e.g. organometallic piano-stool 1,3,5-triaza-7-phosphaadamantane (RAPTA) complexes (Figure 10J), corresponding information is widely missing. The highest density of data has accumulated so far for the anti-metastatic Ru drug NAMI-A. For this compound, an inhibitory effect on TGF-β-induced Rho A activity has been described, leading to reduced tumor cell migration.[648] Similarly, co-culture experiments of HCT116 colorectal cancer cells with untransformed hepatocytes, mimicking a liver metastatic microenvironment, have shown the potential of NAMI-A to reduce the migratory capacity of cancer cells induced by the hepatocyte-derived chemokines and growth factors monocyte chemotactic protein-1 (MCP-1) and VEGF, respectively.[649] Besides these, also hepatocyte-mediated shedding of the pro-inflammatory markers and growth factors (IL-8, IL-12 p70, IL-6, and GM-CSF) has been identified in co-culture conditions, though direct effects on cancer cell migration were not apparent for these mediators. Nevertheless, the findings of NAMI-A to interfere with these (also strongly immune response-associated) soluble factors warrant further investigation on the effects of this compound also on the cancer - immune cell interplay. This might shed light on whether the anti-metastatic properties of NAMI-A rely on its ability to interfere with immunocyte-derived pro-metastatic signals. In parallel, the apparent disruption of extracellular factors by this Ru compound might also modulate immune cell homeostasis in the TME. Direct support for an impact of NAMI-A on cancer-immune cell interactions comes from a publication by Bacac and colleagues.[650] This work stated that NAMI-A in co-culture experiments induced splenocyte NO production and adhesion onto metastatic cancer cells, probably by upregulation of ICAM-1. This calls for careful evaluation of the balance between antitumorigenic/anti-metastatic and immune-regulatory effects of NAMI-A. In addition, there is evidence for an impact of NAMI-A on lymphocyte recruitment to the TME. Such, treatment of subcutaneous MCa mammary carcinoma grafts with this Ru compound elevated intratumoral numbers of $CD3^+$ cells, especially CTL.[651] In another study, NAMI-A treatment of mice resulted in an increase of the $CD8^+$ subset of splenic lymphocytes.[652] Furthermore, increased numbers of circulating and intratumoral (again in MCa mammary carcinoma grafts) $CD8^+$ cells were observed upon

treatment with sodium trans-[$RuCl_4$(DMSO)imidazole] (NAMI), the sodium analogue of NAMI-A.[653] This implies that NAMI-A as well as related Ru compounds do not exert toxicity on TIL and might even synergize with immunotherapeutic interventions. Interestingly, a very recent report has shown the ability of KP1339 to induce several hallmarks of ICD in a 3-dimensional *in vitro* model of colorectal carcinoma.[401] In this setting, the authors demonstrated drug-induced ER stress PERK/peIF-2α-signaling in spheroid cultures, accompanied by ATP release, CRT translocation to the membrane as well as HMGB1 depletion. This fits well with the fact that the master chaperone GRP78 is considered as one target of KP1339,[188,386] thus activating UPR by several downstream mechanisms (compare chapter 2.2.6).[385] Accordingly, KP1339-induced cell death was at least in part mediated by ROS-related ER stress induction, death receptor upregulation, and caspase 8 activation.[383,384] The role of such immune-related functions of KP1339 in the long-term disease-stabilizing effects of this Ru compound in the clinical situation[188,193] urgently needs to be dissected. ER-stress induction has also been demonstrated to underlie the cytotoxic effects of other Ru compounds in preclinical settings, like in case of the anticancer redox organoruthenium compound (RDC11).[654]

In addition, besides these potentially immunogenic effects directly on cancer cells, there exists evidence for Ru compounds to modulate also immune cell infiltration in favor of more immune-stimulatory TME conditions. Illustratively, the preclinical organometallic Ru(II) compound [Ru(p-cymene)(bis(3,5 dimethylpyrazol-1-yl)methane)Cl]Cl (UNICAM-1) (Figure 10K), tested for its potential against triple-negative breast cancer, reduced T_{reg} infiltration and increased DC and macrophage recruitment into the TME.[655] *In vitro* testing of two other Ru(II) p-cymene complexes with a 2,2'-dipyridylamine moiety showed only moderate cytotoxicity towards isolated mouse splenocytes and did not affect cytokine production (IFN-γ, IL-17, IL-10).[656] In the light of the apparent inertness with respect to immune cell activation (at least in case of splenocytes), effects of this compound on anticancer immunity are of interest but remain to be determined.

Contrary to the potential immune-stimulatory activity of Ru-based compounds such as NAMI-A or KP1339, also a negative impact of an unrelated Ru-based complex, Ru(III) polyaminocarboxylate (AMD6221), on tumor immune rejection has been documented.[657] In this report, the authors suggested NO-mediated immune activation and cancer cell death based on cancer cell NOS expression induced by immune cell-secreted cytokines. Importantly, AMD6221 blunted this cancer immune rejection via its NO-scavenging capacity. It needs to be mentioned that in this particular study, AMD6221 was not investigated for its anticancer potential, but utilized for its NO-scavenging properties to study the role of NO in anticancer immune rejection *per se*. Nevertheless, concerning the development of novel, but also classical Ru compounds, comparable properties might unwantedly exert similar immunosuppressive effects, potentially counteracting anti-neoplastic activity via down-modulation of antitumor immune activation. Opposing these arguments, however, a recent study has demonstrated anti-angiogenic and anti-metastatic activities of

potassiumchlorido (ethylendiamminotetraacetate)ruthenate(III) (RuEDTA), KP1339, and NAMI-A to be, at least partially, based on scavenging of NO released by endothelial cells.[658]

In addition, several other key observations with regard to defined Ru complexes are implicated in distinct interactions with important mechanisms of the anticancer immune cycle without explicitly stating so. Hence, several classical and novel Ru complexes were associated with antiangiogenic properties due to interaction with VEGF downstream signals. For example Ru(II) complexes containing a 2,6-bis(benzimidazolyl)pyridine moiety and especially $[Ru(bbp)(p\text{-}mpip)Cl]ClO_4$ (bbp = 2,6-bis(benzimidazolyl)pyridine; p-mpip = 2-(4-methylphenyl)imidazo[4,5-f]-1,10-phenanthroline) (Figure 10L) have been identified as highly potent VEGF/VEGF receptor (VEGFR) 2 inhibitors with distinctly stronger antiangiogenic potency as compared to NAMI-A both *in vitro* and *in vivo*.[230] This should, besides blockade of cancer blood support, also impact on immune cell and especially CTL recruitment into the cancer tissues. Moreover, recent evidence suggests that VEGF is strongly supporting an immunosuppressive milieu and that VEGF inhibition is reprogramming the TME to an immune permissive state.[659]

3.4. Gold (Au)

The use of Au in medicine reaches back to ancient times and earliest documentations by the Egyptians and Chinese are dated ~2500 BC.[660-662] Throughout history, widespread use of Au in medicine has been reported for treatment of multiple conditions like wounds and fistulas, warts, (rheumatic) fever, syphilis, tuberculosis, as well as several neurologic and psychiatric conditions like migraine, depression and epilepsy. Additionally, Au was believed to preserve youthfulness and prolong life. In 1890, Robert Koch discovered that Au cyanide was bacteriostatic against *Mycobacterium tuberculosis* cultures. This led to a short period of application of Au salts for treatment of tuberculosis around 1920 (and was then dismissed as it proved clinically ineffective) and in the 1930s stimulated introduction and development of new Au compounds for treatment of rheumatoid arthritis (RA), a chronic inflammatory autoimmune disease, as RA was thought to be caused by the tubercle bacillus. In the following time, several Au(I) compounds with reduced toxicity were developed, and the orally applicable Au(I) thiolate-triethylphosphine complex auranofin (Ridaura) was approved by the FDA for treatment of RA in 1985 (Figure 11A).[660,661] Two other Au(I) compounds clinically used for the treatment of RA, namely aurothiomalate (Aurolate) (Figure 11B) and aurothioglucose (Solganal) (Figure 11C), were meanwhile withdrawn from the U.S. market.[663] In RA patients, efficacy of auranofin is comparable to methotrexate but toxicity and adverse effects occur more frequently. However, this compound can provide an alternative for RA patients where methotrexate is contraindicated.[664]

Au compounds can be regarded as prodrugs and require activation (achieved by ligand exchange reactions) before they can develop their full pharmacological potential. The main targets seem to be proteins rather than DNA, via interaction with specific thiol- and seleno-containing peptide moieties.[169] Au(I) complexes

are thermodynamically more stable than Au(III), however an Au(I)/Au(III) redox model has been suggested, that is created under strongly oxidizing conditions like in the lysosomes of activated macrophages and granulocytes.[665] There, enzymes like myeloperoxidase (a lysosomal enzyme of primarily neutrophils, that is located in the granules, produces hypohalous acids which is released into the extracellular space during degranulation), mediate oxidation of Au(I) to Au(III). Proteins and molecules containing thiol groups and thiolethers reduce Au(III) again to Au(I), creating an Au(I)/Au(III) redox system able to scavenge ROS and denature and inactivate lysosomal enzymes of activated phagocytes. During this process, also metabolites like e.g. $Au(CN)_2^-$ are generated that can inhibit the respiratory burst of neutrophils and monocytes as well as lymphocyte proliferation.[660,666,667] Generally, Au(III) is more reactive than Au(I), and has been suggested to be responsible for high toxicity and induction of adverse effects like dermatitis.[169,660,661] Additionally, reduction to Au(0) occurs, which might be more critical for the anti-inflammatory effects of Au(I) drugs.[660] At present, besides still being investigated as anti-inflammatory drugs, Au(I), but especially Au(III) complexes have become exciting candidates for anticancer therapy.[185,194,663,665,668-671] Some of these showed promising antitumor activity combined with low toxicity, ready to enter clinical phase 1 trials,[671] however, respective outcomes have not been reported. Interesting groups among Au(III) compounds comprise mononuclear Au(III) complexes (including Au(III) dithiocarbamate complexes), Au(III) porphyrins, organogold(III) compounds, and dinuclear Au(III) complexes.[169] Additionally, development of new N-heterocyclic carbene ligand complexes of Au(I) and Au(III) for anticancer treatment are in the focus of interest.[670] However, although in many cases promising results and even higher cytotoxicity against cancer cells as compared to cisplatin are reported *in vitro*, convincing *in vivo* studies are still missing.[672] Even more surprising, the connection between the anti-inflammatory actions of primarily Au(I) derivatives and the antineoplastic activity of Au(I) and Au(III) compounds has not been established experimentally so far. Especially immune-modulatory effects of Au(III) complexes are completely unexplored, however, this information is nowadays urgently requested for preclinical development of anticancer therapeutics.

Hence, a role of immune effects in the anticancer activity of Au compounds can only be hypothesized.[673] The immune-modulating and anti-inflammatory effects of Au(I) complexes are extensively reviewed in the literature in the context of RA and also other immune-related diseases like HIV and malaria.[660,661,673] Au drugs target proliferation and differentiation of both innate (monocytes/macrophages, granulocytes, DC) as well as adaptive immune cells. However, the exact modes of action are complex and in many cases still not completely clear. Effects observed involve modulation of inflammatory cytokine responses (TNF-α, IFN-γ),[660,673,674] induction of cytoprotective proteins like HO-1,[675,676] and interference with multiple immune receptors (TLR, CD45),[409,677] antigen processing,[678] MHC molecules,[679,680] and signaling pathways (NF-κB, PKC).[681-685] Hence, aurothiomalate inhibited IFN-γ-mediated complement activation and, at high doses, MHC class II expression on monocytes.[680] Moreover, this anti-inflammatory Au(I) compound reduced production of IFN-β and NO, thus inhibiting the release of HMGB1 into the extracellular space, in murine

and human macrophages (RAW 264.7 and THP-1, respectively) *in vitro*.[686] Additionally, Au(I) compounds were shown to suppress TNF-α production by several immune cell types including macrophages[674] and neutrophils.[660,673] For example, both aurothiomalate and auranofin were able to impede TNF-α-mediated cytotoxic neutrophil effects against epithelial cells and enhanced leucocyte adhesion to endothelial cells, both important players in RA.[660,673] Also in neuroinflammation, a process central to neurodegenerative diseases like Alzheimer and Parkinson, auranofin was shown to attenuate cytotoxic cytokine secretion by microglia and microglia-like THP-1 promonocytic cells and inhibit the respiratory burst and release of TNF-α and NO by monocytic cells.[687] Auranofin can induce cytoprotective transcription factor programs in monocytic cells based on NF-E2-related factor 2 (NRF2) protein stabilization and, in turn, activation of HO-1.[676] In peritoneal macrophages, auranofin and Au thiomalate induced HO-1 and peroxiredoxin 1, two antioxidants inhibiting macrophage maturation.[675] With regard to TLR signaling, auranofin was shown to suppress LPS-induced homodimerization and downstream signaling of TLR4 in murine pre-B and monocytic cell lines.[677] Rachmawati et al., analyzing the influence of various high molecular weight complexes on innate immune cells, demonstrated that the Au compound $Na_3Au(S_2O_3)_2.2H_2O$ was able to induce moderate DC maturation via TLR3 signaling.[409] Also interference with several key transcription factors for immune cell signaling like activator protein-1 (AP-1) (by interaction with cysteines of fos and jun) and STAT3 has been reported.[688,689] NF-κB and PKC signaling pathways are central mediators for activation, differentiation and maturation of both myeloid as well as lymphatic cells.[683-685] Aurothioglucose and auranofin have both been described to inhibit NF-κB signaling by impeding binding of NF-κB to DNA and, in case of auranofin, blocking IKKβ subunit in COS-7 cells and LPS-stimulated macrophages.[681,682,690] In some cases, reduction of IL-1β and IL-6 release was underlying NF-κB inhibition by auranofin.[689,691,692] PKC activity has been modulated by auranofin and aurothiomalate in neutrophils and T cells.[693,694] In B and T cells, auranofin, aurothioglucose, and aurothiomalate also inhibited adenylyl cyclase/cyclic adenosine monophosphate (cAMP) signaling.[695] Aurothiomalate can hamper proper antigen presentation and $CD4^+$ T cell activation by binding cysteine-containing peptides and inhibit various transcription factors.[678,696] Wang et al. showed inhibition of CD45, a tyrosine phosphatase expressed on all hematopoietic cells that augments signaling via B and T cell antigen receptors, by aurothiomalate.[697] With regard to B cells, Au(I) drugs effectively inhibited activation, proliferation and antibody production, already at much lower doses than required for T cell interference.[698,699]

As mentioned above, the role of immunological and anti-inflammatory effects in the anticancer activity of Au compounds remains largely unexplored at the experimental level. Only one study by the group of Travnicek[700] investigated in parallel both anticancer and anti-inflammatory effects of Au(I) complexes of 9-deazahypoxanthine *in vitro* and *in vivo*, but also in that case not in cancer-bearing animals.[700] This lack of data is especially surprising, as even in review articles inhibition of cancer-promoting inflammation has been postulated as important player in the anti-neoplastic activity of Au compounds like auranofin.[673]

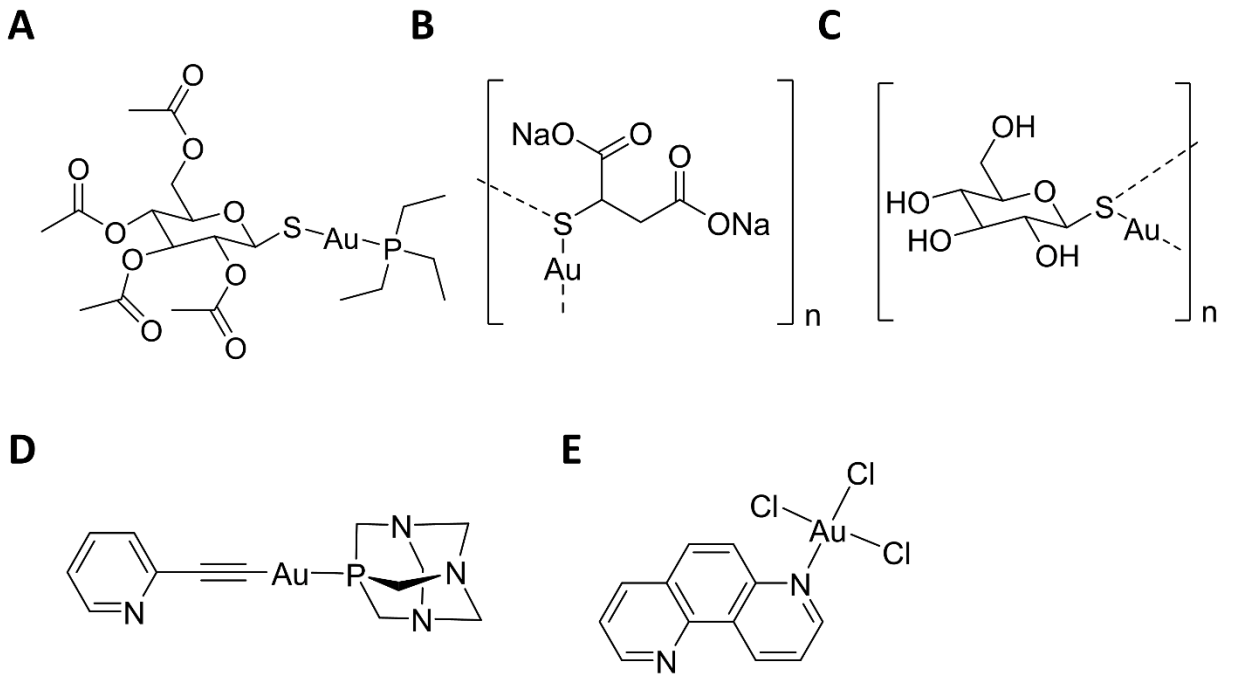

Figure 11. Chemical structures of (A) auranofin (clinically approved as antirheumatic agent) and (B-E) other lead anticancer gold complexes and those with published immune-regulatory activities. (B) aurothiomalate, (C) aurothioglucose, (D) alkynyl(I) gold complex, (E) [$AuCl_3$(1,7-phen-κN^7)] complex. For details see text.

However, the situation needs to be addressed more critically, as for several immune-based anticancer effects, pro-inflammatory signals might be essential, including immunocyte recruitment to the TME, NLRP3 inflammasome activation, and TLR-mediated signaling e.g. in frame of ICD induction. In line with this, one study by Marmol et al. investigating alkynyl Au(I) complexes (Figure 11D) in colorectal carcinoma demonstrated cell death induction via ROS-mediated necroptosis, involving TNF-α production and NF-κB signaling. However, the role of immune-related components is not further discussed by the authors.[332] Hence, the interplay between the mode of action in inflammation and cancer by Au compounds is still unclear and - resembling the data described for arsenicals - may vary between different cell types and conditions.

So, which of the above described observations might be relevant for anticancer effects of Au compounds established *in vitro* and *in vivo*?[665,668-671] In cancer cells, Au compounds have been shown to primarily act via disturbance of redox balance (e.g. thioredoxin (Trx) reductase (TrxR)), inhibition of anti-apoptotic molecules (e.g. bcl-2), as well as targeting of proteolytic enzymes and the proteasome. In addition, Au drugs inhibit, like already described for immune cell compartments, important proliferation and viability pathways including p38 MAPK, STAT3, and NF-κB.[692,700-702] Considering redox deregulation, Au compounds can induce apoptosis by targeting specifically the TrxR system in the mitochondria of cancer cells.[663,665,703] The Trx - TrxR system protects cells against ROS, and is central for maintenance of the intracellular redox balance.[704] Auranofin, although described to act as ROS scavenger in activated phagocytes via the

Au(I)/Au(III) redox system, can enhance ROS production and induce apoptosis in cancer cells,[705] as well as inhibit selenium metabolism and selenoprotein synthesis via interference with TrxR.[673,706] Several groups have shown high sensitivity of ovarian cancer cells to auranofin, via enhanced ROS production in a BRCA1-deficient background,[707] inhibition of the Trx-TrxR-system,[708] as well as interference with PKC iota.[709] The anticancer activity of auranofin was further investigated in several clinical trials and is currently tested against recurrent ovarian cancer (www.clinicaltrials.gov). Together, these data suggest that Au complexes selectively kill malignant cells based on enhanced ROS pressure but might at the same time protect phagocytic cells like DC in the TME. As outlined above, ICD induction by metal drugs is crucially involving ROS-mediated ER stress and UPR and subsequent engulfment of dying cancer cells by DC. This hypothetical ideal scenario for ICD induction by Au compounds should be urgently validated in appropriate models for anticancer immune response evaluation.

Nevertheless, modulation of cytokine profiles and pathways by Au compounds implicated in immune regulation has also been described in cancer cells. Such, auranofin inhibited phosphorylation of JAK1 and STAT3 and blocked IL-6 signaling in human hepatoma cells (HepG2), and in human breast cancer cells.[689] Another study by the same authors showed downregulation of telomerase via inhibition of STAT3 phosphorylation.[701,702] As discussed above for Pt drugs, this is interesting for immunotherapeutic approaches considering that STAT signaling is key for activation of immune checkpoint molecules like PD-L1 on cancer cells.[418] In glioblastoma, auranofin inhibited cathepsin B, a cysteine protease highly expressed in areas of invasion and neovascularization,[710] which for sure would also interfere with immunocyte homing into the TME. A recent study ascribed the anticancer activity of auranofin in human ovarian cancer cells (SKOV3) to inhibition of the IκB/NF-κB signaling cascade[711] as also observed in multiple immune cell compartments. However, this pathway might play an essential role in the HMGB1-mediated immunogenic effects of dying cancer cells (compare chapter 2.2.6).

In addition to direct action on tumor and/or immune cells, Au(I) compounds can affect expression of cell adhesion molecules on endothelial cells.[660,712,713] Auranofin was described to suppress angiogenesis and lymphangiogenesis by inhibiting VEGF and phosphorylation of VEGFR2,[714] downregulation of VEGFR3,[715] and by interfering with TLR3 signaling.[673,716] Antiangiogenic effects via interference with VEGFR2, MMP-2, MMP-9, and TrxR were also recently published for a newly synthesized Au(III)-phenanthroline complex (Figure 11E).[201] However, with the exception of this study,[201] no data about specific effects of Au(III) compounds on immune-related parameters are available.

Concerning adverse effects of Au compounds, a heterogenous picture arises with regard to the immune system. On the one hand, several adverse effects can be connected to immunosuppression like impairment of macrophages, T and B cells, Ig deficiency, aplastic anemia[717], as well as bone marrow suppression.[718] On the other hand, also immune-stimulating reactions can occur. The most frequent adverse effects of Au(I) compounds involving immune stimulation comprise diverse types of skin reactions including dermatitis,[662]

but also cases of severe organ damage due to glomerulonephritis,[662] enterocolitis,[719] and hepatitis[720] have been reported. Considering the allergic/autoimmune reactions observed in Au-treated patients, an unspecific TLR-, or even an adaptive T cell-mediated immune response to specific Au-modified peptides might be postulated.[678,721,722] However, the relevance of such a hypothesis on cancer cell adjuvanticity and antigenicity during anticancer therapy with Au complexes is yet to be established. Apart from reactions occurring upon treatment with Au drugs, Au-induced contact dermatitis is another condition pointing toward immune-stimulating properties of Au. Interestingly, patients with dermatitis have been reported to have a high frequency of Au allergy.[661,678] Au-related contact dermatitis is probably induced by slow ionization of Au upon contact with the skin and subsequent absorption and haptenization (modification of otherwise non-immunogenic cellular (protein) structures by antigenic compounds, such as metal ions, leading to an immune response).[661] The issue of long-term toxicity and adverse effects is especially interesting with regards to application of Au NP used for anticancer therapy (compare chapter 4.3.1).

In summary, although the direct impact of Au compounds on an anticancer immune response has not been comprehensively investigated so far, the massive immunosuppressive effects both *in vitro* and *in vivo* in frame of acute and chronic inflammation call for caution, especially when applying Au compounds in the context of immunotherapeutic approaches. However, it needs to be considered that in selected cases chronic inflammation might also act as a strong tumor promoter presumably based on the activity of regulatory immune cell compartments (e.g. tumor-infiltrating neutrophils, TAM, T_{reg}, and MDSC). The sensitivity of inflammation-associated neutrophils in non-malignant, inflammatory diseases against Au compounds suggests that these drugs might exert similar effects on cancer-promoting neutrophils. Unfortunately, with regard to the regulatory immune cell compartments mentioned before, all important targets for clinically successful Pt and As compounds (compare above), have not been worked out so far in case of Au-compounds. However, such information seems absolutely critical for development of anticancer Au compounds especially in frame of combination therapy approaches.

3.5. Cobalt (Co)

Co is usually present in two oxidation states, Co(II) and Co(III).[194] In the human body, Co(III) plays an essential role as metal constituent of cobalamin (vitamin B12), which acts as coenzyme in multiple metabolic processes (in these enzymes also Co(I) is formed during the redox cycles).[723] Systemic toxic effects of excessive Co levels are thought to be mainly mediated by free ionic Co^{2+} (reviewed by Paustenbach et al.[724]) and affect the function of multiple organs.[725] At least in rodents, Co^{2+} has carcinogenic effects.[726] This is based predominantly on genotoxicity by ROS-mediated oxidative DNA damage[727] as well as by interference with the nucleotide excision repair machinery, the latter mechanism being based on substitution of Zn ions from zinc finger-containing DNA-binding proteins such as xeroderma pigmentosum

A (XPA).[728] Co-induced systemic toxicity has been predominantly associated with metal-on-metal hip implants, an effect termed “arthroprosthetic cobaltism”, which is believed to be derived from elevated serum levels of free ionic Co^{2+} concentrations.[729] In addition, in the wear process of hip implants, Co NP may result in osteolytic inflammatory fluid formation. It is not entirely clear, whether such immune responses are caused by a “metal-reactive” innate immune response mounted against metal debris, or whether the adaptive immune system plays a role in frame of a “metal allergy”, which is also typically associated with contact dermatitis.[729,730] The nature of these local reactions is further complicated by the fact that Co-containing products are often mixed with other metals such as Ni.[729,731] Hints for an involvement also of cells of the adaptive arm of the immune system are derived from a study showing that Co^{2+} ions stimulated migration of T cells, but not B cells, *in vitro*.[732]

Concerning cancer therapy research, resistance to traditional Pt-based anticancer drugs has driven investigations on alternative transition metal-based compounds. Over the last decades, the anticancer potential of Co complexes as well as their modes of action have been extensively studied. The development of antiproliferative Co complexes has been reviewed in depth by Munteanu et al.[733] These include primarily Co coordination complexes such as hexaamminecobalt(III) chloride (Figure 12A), Co(III)-acetylacetonate complexes (Figure 12B) Schiff base complexes (Figure 12C) and Co-carbonyl clusters (Figure 12D).[194,733] Furthermore, Co-containing cobalamin has been suggested as a cancer-specific delivery agent for cytotoxins including cisplatin.[734] Some of these complexes have been designed as redox-active Co(III) prodrugs with bioactive ligand(s) attached.[735] In a hypoxic microenvironment, these Co(III) compounds are activated by reduction with release of all (bioactive) ligands and formation of Co(I).

The majority of reports regarding the impact of Co on the immune system is available only for Co(II) salts, which, however, are generated at least in case of hypoxia-activated prodrugs. In 2015, a study by Wang et al. reported Co chloride ($CoCl_2$) to induce ROS and necroptosis in a human colon cancer cell model.[331] Accordingly, Co-induced necroptosis was accompanied by increased IL-1α and IL-6 expression, suggesting an immune-stimulatory component in the mode of action of $CoCl_2$, potentially leading to an inflammatory response.[331] Importantly, $CoCl_2$ has also been identified as a hypoxia-mimetic based on stabilization of hypoxia-inducible factor-1α (HIF1α).[736,737] This is reflected by the fact that increased Co exposure has marked effects on the hematological system.[729] Such, a reversible increase in hematocrit and hemoglobin levels as well as erythrocyte count (polycythemia) has been documented,[738] earning $CoCl_2$ also a shady reputation as doping agent to stimulate erythropoiesis.[739] The exact molecular mechanism by which $CoCl_2$ mediates HIF1α and HIF2α stabilization is not entirely resolved, but includes the prevention of HIF interaction with the von Hippel-Lindau protein (pVHL) by occupying the VHL-binding domain and thereby preventing HIF degradation.[740] Additionally, and speculatively, in the *in vivo* situation, $CoCl_2$ may interfere with hemoglobin synthesis/homeostasis by replacing Fe as coordination center of protoporphyrin IX component of hemoglobin (analogous to the synthetic molecule “coboglobin”). Importantly, Co

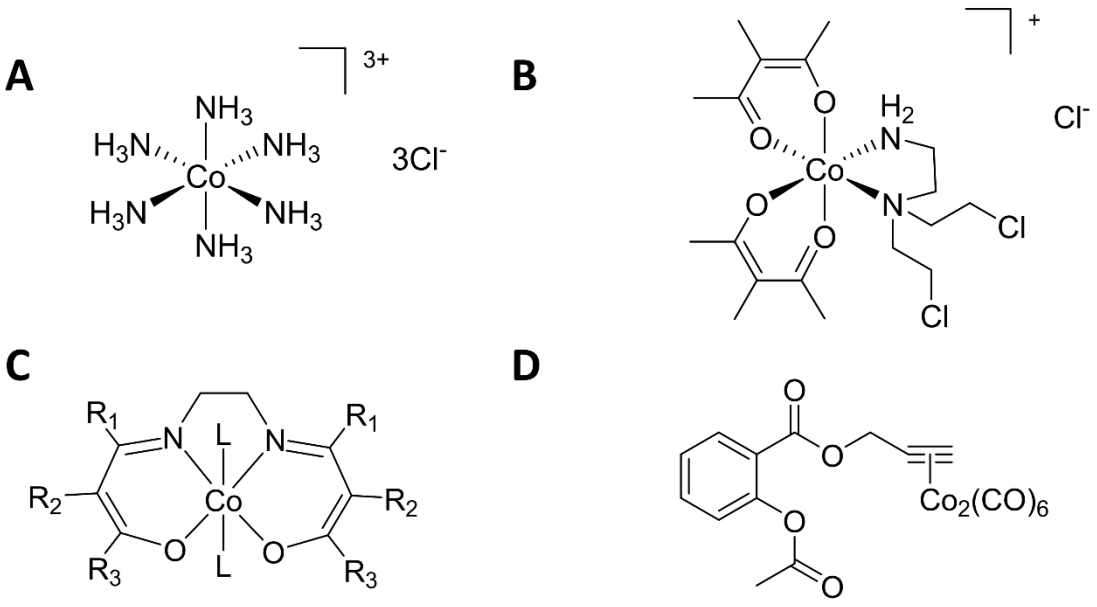

Figure 12. Chemical structures of lead anticancer cobalt-based compounds and those with published immune-regulatory activities. (A) [Hexaamminecobalt(III)] chloride, (B) Co(III)-acetylacetonate complex (C) Schiff base-derived Co(III) complex, R_1, R_2, R_3 = H or CH_3 or CF_3, L = NH_3 or imidazole or nicotinamide; (D) [{2-acetoxy(2-propynyl)benzoate}-hexacarbonyldicobalt] (Co-ASS). For details see text.

protoporphyrin IX - besides its hypothetic role in hypoxia induction - induces expression of cytoprotective HO-1 e.g. in MDSC (compare chapter 3.3), protecting tissues from oxidative stress upon reoxygenation under ischemic conditions.[741-743] Given the down-modulatory effects of HO-1 on immune cells such as macrophages,[744,745] as well as the stimulatory effects on regulatory compartments such as MDSC, it will be interesting whether Co may decrease pro-inflammatory processes and, thus, reduce leukocyte activation in the context of anticancer immune responses. Corroboratively, protoporphyrin exerted immunosuppressive effects also by T_{reg} induction in rat splenic lymphocytes and led to a decrease in IFN-γ and IL-2 secretion from T_H1 cells as well as an increase in IL-10 and TGF-ß secretion from T_H2 cells.[746] In contrast, based on the observation that HIF1α may also enhance innate immune cell functions, one study performed in 2016 demonstrated that HIF1α induction by $CoCl_2$ induces a NKT cell response against primary human cancer cells.[747] This effect was mediated by enhanced 5'-AMP-activated protein kinase (AMPK)-dependent, CD1d-mediated NKT cell activation and the production of IL-2.[747] This suggests that $CoCl_2$, mimicking AMPK activation, might alter the DAMP repertoire of malignant cells presented to CD1d, serving as danger signal for NKT recognition.[747] Furthermore, there is data suggesting enhanced responsiveness of immune cells to cancer-derived DAMP in response to hypoxia. Illustratively, $CoCl_2$ induced expression of TLR4 in RAW264.7 macrophages in a HIF-dependent manner, leading to increased expression of cyclooxygenase (COX)-2, CCL5 and IL-6.[408] Correspondingly, TLR4 neutralization abrogated Co ion-induced pro-inflammatory cytokine and chemokine production by macrophages *in vitro.*[748] However, Co ions abrogated IL-1ß production in titanium-exposed macrophages.[749] Additionally, immunosuppressive cell compartments

like T_{reg} might be directly targeted by Co compounds. Hence, $CoCl_2$ was demonstrated to block T_{reg} differentiation by downregulating FOXP3 expression (as already known for As compounds, see above), even in a HIF-independent manner.[750] Furthermore, $CoCl_2$ induced the production of inflammatory cytokines (including TNF-α and IL-1ß) of macrophages *in vitro*, but reduced their MHC class II expression and capacity to activate $CD4^+$ cells,[751] and may also induce macrophage death at higher doses.[752]

Little data is available on the interactions of anticancer organometallic Co complexes with immune mechanisms. Co alkyne complexes such as the acetylsalicylic acid-derivative [2-acetoxy-(2-propynyl)benzoate]hexacarbonyldicobalt (Co-ASS) (Figure 12D) have been shown to exert anticancer activity via their non-steroidal anti-inflammatory drug (NSAID)-mimetic inhibitory activity on COX enzymes.[753] For instance breast cancer cell lines have been shown to be strongly hypersensitive towards COX inhibition.[753] Accordingly, a chlorinated derivative of Co-ASS showed no cytotoxicity towards non-tumorigenic human bone marrow stromal cells,[754] indicating a cancer-selective mode of action of this group of Co compounds. It has to be kept in mind, however, that experiments with these compounds have so far only been conducted *in vitro*. In the *in vivo* situation, COX inhibition - blocking the production of e.g. prostaglandins and eicosanoids - might clearly interfere with inflammatory responses in the TME. Thus, regarding anticancer immunity, it will be important to investigate, whether the COX-inhibiting activity of NSAID-mimetic Co complexes might impair immune homeostasis by downregulating pro-inflammatory signals and conditioning the TME towards a tolerogenic, immunosuppressive state.

In summary, the current data demonstrate that extensive research efforts will be necessary to understand the impact of Co complexes on inflammatory processes as well as on immune cells, and the implications this might have with respect to the anticancer immune responses.

3.6. Nickel (Ni)

Ni is the first transition metal element of the 10^{th} group of the periodic table. The most common oxidation state is Ni(II).[755] Several Ni complexes have been synthesized as anticancer agents and show promising anti-proliferative activities. These include, amongst others, N-heterocyclic carbene complexes,[756] Ni pyrithione,[329] Ni diacetyl monoxime-2-pyridyl hydrazine,[757] Ni thiosemicarbazones,[758,759] Schiff base Ni chelates,[760,761] a Ni-dithiocarbamate phenanthroline complex,[762] and Ni diacetyl monoxime-2-pyridyl hydrazine.[757]

Ni is the most frequent cause of metal allergy (reviewed by Saito et al.[763] and Schmidt et al.[764]). Accordingly, the vast majority of data about Ni effects on the immune system is derived from reports concerned with Ni-induced hypersensitivity reactions.[406,765] Nevertheless, these studies may have implications also with respect to anticancer immune responses, as Ni salts and complexes interfere with multiple immunologic parameters such as DAMP responsiveness of phagocytes, inflammation, as well as T cell activation (see below).

Independently of its application as antineoplastic agent, Ni-containing NP utilized for instance in pharmaceuticals, clothes or cosmetics have been shown to exhibit inflammogenic properties.[766] These hypersensitivity reactions are constituted by both innate and adaptive immune components and include binding of Ni to MHC-associated self-peptides at the surface of APC such as DC.[764] Ni allergy was shown to be initiated by the release of pro-inflammatory mediators (including IL-1β and TNF-α) by epidermal keratinocytes.[763] Also, exposure of human bronchial epithelial cells to Ni compounds led to pro-inflammatory signaling activation predominantly via the MAPK pathway, involving nuclear factor of activated T cells (NFAT), NF-κB, and AP-1, in turn inducing expression of TNF-α.[767] Furthermore, haptenization of originally non-immunogenic cellular structures by Ni engages innate immune receptors such as TLR4 via distinct histidine residues and induces NF-κB-dependent production of pro-inflammatory cytokines including TNF-α and IL-8.[406,768] The resulting activation of DC leads to presentation of Ni-modified epitopes and priming of T cells, which then migrate to areas of Ni exposure and - upon hapten encounter - cause pruritic lesions characteristic of contact dermatitis.[769,770] In the context of cancer, this implies that Ni exposure might render also malignant cells more visible to innate immune recognition via PRR engagement. Furthermore, it can be hypothesized that Ni modification of endogenous peptide structures might alter the cancer cellular antigen repertoire, facilitating adaptive anticancer immune activation. It, thus, appears tempting to develop strategies for tumor-specific delivery of Ni compounds with the aim to achieve cancer cell-specific antigen haptenization in order to boost adaptive, T cell-mediated anticancer immunity.

Furthermore, direct stimulatory effects on immune cells have been reported. A recent study by Bechara et al. reported Ni sulfate ($NiSO_4$) to promote T_H17 cell induction by TLR4-activated DC via p38 MAPK-, JAK/STAT- and NF-κB-induced secretion of IL-23.[407] Moreover, Ni-stimulated $CD8^+$ T effector cells were found to produce IFN-γ, and $CD4^+$ T cell polarization into T_H1 and T_H17 phenotypes is implicated in Ni allergy.[771] In human THP-1 monocytic cells, Ni exposure has been linked to activation of NF-κB and secretion of IL-8.[772] Furthermore, a transcriptome-wide analysis of gene expression alterations induced by Ni^{2+} in primary human monocytes was conducted. Besides already known target genes (e.g. CCL20, CCL21), Ni induced deregulation of a multitude of transcripts, many of which corresponded to immunological and inflammatory processes related also to hypersensitivity and cancer.[773] In contrast, a negative-regulatory effect in Ni hypersensitivity has been observed for T_H2 cells via secretion of IL-4, IL-5 and IL-10.[774] Another study demonstrated that neutrophil-like-derived ectosomes in response to $NiSO_4$ reduced hypersensitivity reactions.[775] This was mediated by down-modulation of DC maturation, characterized by decreased levels of, amongst others, IL- 1ß, IL-6 and TNF-α, followed by $CD4^+$ polarization into a T_H2, IL-13-expressing phenotype. Interestingly, it has been shown that T_{reg} cells - induced by engineered microparticles releasing TGF-β, rapamycin, and IL-2 - were able to promote tolerance towards Ni contact dermatitis.[776]

Other studies suggest toxic effects of Ni on various immune cell types. A toxicological analysis investigated Ni^{2+}-dependent biological responses of human monocytes by proteomic profiling, revealing functional annotation clusters such as metal ion binding, cytoskeletal remodeling and cell death.[777] Consequently, the authors confirmed monocyte apoptosis induction upon exposure to Ni^{2+}. Interestingly, for cancer immunosurveillance, T cell viability was not affected at equal concentrations. A recent study reported that cytotoxicity of Ni chloride ($NiCl_2$) against human lymphocytes was associated with increased ROS formation, mitochondrial membrane potential collapse, glutathione depletion, lysosomal membrane damage, cellular proteolysis, and activation of caspase 3.[778] Long-term pro-inflammatory allergic reactions towards Ni might even be directly linked to hematopoietic cancer development (compare also chapter 7).[779] This carcinogenic property of Ni has been linked to oxidative stress and ROS production.[779] In a clinical case report, chronic allergic contact dermatitis upon Ni exposure has been connected with the occurrence of cutaneous T cell lymphoma.[765]

Studies on the anticancer potential of the mentioned compounds were predominantly performed to dissect the respective modes of action with a focus on hematological malignancies.[329,780,781] Accordingly, interactions of Ni-based anticancer compounds with cancer immunosurveillance - e.g. via the potential of Ni-modified tumor neoantigens or DAMP to boost antitumor immune responses by mimicking allergy reactions - are widely unknown. As mentioned earlier for Co (compare chapter 3.5), Ni has been reported to induce expression of HIF1.[747,782] With regard to the TME, this might trigger pleiotropic effects, on the one hand, by jeopardizing the ability of immune cells to induce tumor cell cytotoxicity by down-modulation of inflammatory processes, and, on the other hand, by acting as metastasis-promoting factor via induction of angiogenesis. Regarding anticancer Ni complexes, Ni(II) 2,3-dihydroxybenzaldehyde thiosemicarbazone (Figure 13A) showed anti-inflammatory effects in murine leukemic macrophages as well as cervix carcinoma models by inhibiting nuclear NF-κB translocation.[783] This resulted in downregulation of the pro-inflammatory cytokines TNF-α, IFN-Iβ and IL-6. Conversely, a Ni(II)-dithiocarbamate phenanthroline complex (Figure 13B) was reported to induce necroptosis in breast cancer stem cells without causing ROS production and PARP activation.[762] In addition, the complex Ni(II) diacetyl monoxime-2-pyridyl hydrazine was synthesized with the aim to generate a compound with a favorable toxicity profile as compared to cisplatin, including reduced bone marrow depression.[757] This compound exerted antitumor activity against Ehrlich ascites carcinoma allografts comparable to cisplatin, and elevated the levels of anti-oxidative mediators (SOD, glutathione, catalase) while decreasing the amounts of the oxidative stress marker malondialdehyde. However, although erythrocyte counts were increased, total leukocyte numbers of tumor-bearing mice decreased dose-dependently upon drug treatment to levels comparable to cisplatin-treated animals. It is currently unclear to which extent the anti-oxidative and (potentially) anti-inflammatory activity in combination with the leukocyte-depleting effects of this Ni complex contribute to or even counteract its anticancer efficacy. It might be suggested that decreased ROS levels in the TME suppress cancer cell DAMP

exposure and antigenicity, thus hampering antitumor immune responses. In addition, decreased leukocyte counts in treated animals implicate general immune suppression. Conversely, it might also be possible that regulatory immune cell compartments are selectively eradicated by this drug treatment. However, considering the higher susceptibility of certain regulatory immune compartments such as T_{reg} to redox stress-induced eradication (as described for ATO, compare chapter 3.2), it appears unlikely that the mentioned Ni complex exerts such effects due to is anti-oxidative activities. Regarding effects of anticancer Ni compounds on the fitness of immune cells *per se*, one *in vitro* study reported similar cytotoxic activity of a guanosine-selective benzimidazole-derived di-Ni complex (Figure 13C) towards the transformed macrophage cell line RAW 264.7 as compared to a cervical cancer model.[784] In this case, it will be important to investigate whether also healthy macrophage counterparts are affected in the *in vivo* situation, which would indicate unspecific myelotoxicity.

Thus, the interaction between Ni-based anticancer compounds and the immune system is virtually unexplored and clearly calls for more comprehensive investigations of the effects of Ni compounds on cancer immunogenicity, the impact on the immune cells, and whether these compounds may serve as immunogenic adjuvants alone or in combination with immunotherapy.

3.7. Vanadium (V)

The transition metal V exists in several oxidation states, of which V(IV) and V(V) (and, to a lesser extent, V(II) and V(III)) are biologically most relevant.[785] In aqueous solution V is either present in the oxidation state V(IV) as vanadyl (VO^{2+}) or V(V) as vanadate (VO_4^{3-} and VO_3^-), whereas also different polymeric species can be formed depending on the pH and concentration. V compounds have been demonstrated to exhibit therapeutic potential for various disease types including diabetes type 2, tropical diseases such as trypanosomiasis, bacterial or viral infections but also malignant diseases (reviewed by Crans et al.[786,787]). In contrast, environmental and occupational V exposure has even been linked to lung cancer development.[788]

Despite a broad array of V-based complexes designed as pharmacological agents, none of them have found their way into clinical application so far.[789,790] Several V species exert inhibitory functions, targeting a variety of intracellular enzymes crucial in metabolic and signaling processes. These include glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glucose-6-phosphate dehydrogenase, glycogen synthase, adenylate cyclase, cytochrome oxidase, as well as inhibition of protein tyrosine phosphatases.[791,792] In contrast, V has been found to stimulate the catalytic function of the Na/K-ATPase, dynein, myosin ATPase or the adenylate kinase.[787,793] Consequently, V compounds exert multiple pharmacological effects on physiological processes, including, e.g. insulin sensitivity or regulation of hypertension and hyperlipidemia.[794] These interactions with intracellular proteins also suggested V compounds to be active against deregulated signaling and metabolic processes in cancer cells. Indeed, V compounds have been demonstrated to induce

cancer cell apoptosis as well as inhibit proliferation and metastasis,[785] based, on the one hand, on disruption of oncogenic signaling circuitries[795] and, on the other hand, on ROS-induced DNA damage.[785,796,797] V compounds with promising anticancer potential in preclinical studies include e.g. ammonium metavanadate(V) (polymeric NH_4VO_3, Figure 13D), sodium orthovanadate(V) (Na_3VO_4, Figure 13E), V(III)-L-cysteine (Figure 13F), as well as organometallic vanadocenes and V coordination complexes.[798] Regarding the influence of V compounds on anticancer immune responses, available information is sparse. There is a considerable body of literature describing multifaceted interactions of V species with immune cells (reviewed by[787]). Both pro- and anti-inflammatory effects have been documented for V-containing compounds, however, these effects depend on the physicochemical characteristics of specific formulations such as nature of the ligands (peroxide, oxido, organic chelators), oxidation state, or the hydrophilicity of metal-ligand complexes.[787,799] Furthermore, strong cell-, tissue-, dose- and time-dependent variations have been observed.[787]

On the one hand, V has been implicated in immune suppression and downregulation of inflammation.[798-800] Illustratively, there are hints that V species negatively influence the function of T cells. For instance, NH_4VO_3 has been shown to reduce splenic T cell proliferation[801] as well as peripheral blood T cells, together with diminished IL-2 and IL-6 serum levels.[802] The molecular mechanisms underlying these effects are elusive, however, there is speculation about an interference of V with the selection process of developing $CD4^+$ and $CD8^+$ T cells in the thymus.[787] This hypothesis is supported by a recently reported mouse model, in which V pentoxide (V_2O_5) inhalation resulted in altered thymic cytoarchitecture with decrease in DC and a distorted cortex-medulla distribution of thymic epithelial cells, potentially leading to impaired negative selection of autoreactive T lymphocytes.[803] In addition, V_2O_5 inhalation decreased surface expression of CD11c and MHC class II on thymic DC.[804] As thymic DC - besides mTEC - are critical for self-antigen presentation in frame of negative selection of self-reactive T cells (compare chapter 2.2.4),[805] these observations point to a disruptive role of V in key thymocyte developmental processes. It would be important to investigate whether anticancer V compounds may exert similar MHC class II down-modulatory effects on DC as this might hamper T cell mediated adaptive anticancer immune responses. With respect to cellular signaling pathways, Na_3VO_4, was reported to alter T_H cell activity by modulating cAMP-responsive element-binding protein (CREB), NF-κB and AP-1 as well as NFAT signaling, probably contributing to various immunosuppressive effects induced by V.[806]

Furthermore, V affects immunocyte activation via dysregulation of the cytokine profile. Such, V_2O_5 as well as NH_4VO_3 were shown to prevent T cell activation[807] by downregulating expression of proinflammatory cytokines including IL-2, IL-6, TNF-α and IFN-γ. In case of V_2O_5, similar inhibitory effects were observed for NK cell proliferation.[808] This potentially explains innate immune suppression upon airborne V_2O_5 inhalation. In addition, V-containing alloy as implant material was reported to decrease pro-inflammatory gene expression (including IL-1β, IL-6 and IL-8) in human mesenchymal stem cells. Interestingly, further

downregulated factors included TLR4 and CD40L.[809] One might hypothesize that, in case similar effects also apply to V-exposed immunocytes, this may result in downregulation of several crucial innate and adaptive immune-physiological processes including DAMP-PRR-mediated APC activation as well as priming and activation of T and B cells. The presumed anti-inflammatory action of V has important implications with respect to the effects of other anticancer drugs on the immune system. Such, an important aspect of the therapeutic application of V compounds is illustrated by their chemo-preventive potential, based on antioxidant activity, induction of detoxifying enzymes, as well as reduction of carcinogen-derived toxic intermediates.[796] For instance, an oxovanadium(IV)[810] complex and V(III)-L-cysteine[811] have been analyzed for their chemo-protective capacity in cisplatin-induced nephrotoxicity (compare chapter 6.2). V(III)-L-cysteine prevented cisplatin-induced ROS generation and lipid peroxidation and reverted the depletion of antioxidant enzymes such as SOD, catalase, or glutathione in renal tissue.[811] This went hand in hand with decreased levels of pro-inflammatory mediators such as NF-κB, COX-2 and IL-6. With respect to the effects of V compounds as anticancer agents *per se*, it will be crucial to carefully evaluate whether these anti-inflammatory mechanisms might hamper efficient immune activation in the course of cancer cell death and DAMP release.

In contrast, also immune-stimulatory effects of V compounds have been described.[787] These include B and T cell activity, regulation of inflammation and cytokine expression, and NF-κB- and TLR-signaling.[787] V(IV) complex N,N'-bis(salicylidene)-*o*-phenylenediamine V(IV) oxide,[812] bis(peroxide)V(V),[813] as well as pervanadate(V)[814] induced NF-κB activation in *in vitro* models. However, it has to be mentioned that these compounds were not primarily designed as anticancer compounds and that only the latter compound was tested in (transformed) T cells, while for the former two, NF-κB induction was determined in non-immune cell types. Nevertheless, the fact that NF-κB is a central player in immune cells and the TME, these data suggest distinct regulatory functions of the mentioned V compounds within neoplastic tissues. A further study in human bronchial epithelial cells showed that V_2O_5 exposure induced COX-2 expression in a NFAT-dependent manner, leading to anti-apoptotic signaling.[788] Although in this context described to promote malignant transformation, this signaling might in turn activate immune cells for an antitumor attack. Furthermore, immune-stimulatory effects of sodium metavanadate(V) ($NaVO_3$) have been demonstrated in B cells of γ-irradiated mouse splenocyte populations, which included B cell expansion, IFN-γ and antibody production.[815] In macrophages, $NaVO_3$ has been found to induce NF-κB signaling via IKK and JNK activation.[816] In contrast, investigation of the immune-modulatory impact of anticancer oxovanadium(IV) complexes revealed no ROS induction in human phagocytes, but strong inhibitory activity on T cell proliferation *in vitro*.[817] Regarding anticancer immune responses, a recent study demonstrated that V-based phosphatase inhibitors including, besides orthovanadate and metavanadate, also V(V) oxytriethoxide (Figure 13G), V(IV)oxide sulphate (Figure 13H) and bis-maltolato oxovanadium(IV) (Figure 13I) increased

the anticancer activity of oncolytic viruses *in vitro* and *in vivo*.[791] This potentiation had direct implications with respect to anticancer immune responses, as it was characterized by a switch from an antiviral type I IFN response towards a pro-inflammatory type II IFN response, enhancing antitumor immune activity.

Together, these data demonstrate a strong dependency of the coordination chemistry of V drugs on immune system modulation. It will therefore be crucial to integrate the currently ambiguous understanding and multifaceted modes of action of different V compounds on the immune system to generate anticancer V-based drug formulation with modulatory effects eliciting favorable anticancer immune responses.

3.8. Rhodium (Rh)

Rh is widely applied as catalyzer in industrial and pharmaceutical processes.[818] Regarding therapy of cancer, Rh complexes with the oxidation states Rh(I), Rh(II) and Rh(III) have been predominantly described. DNA binding, reminiscent of cisplatin, has often been proposed as important anti-proliferative mode of action.[819] Several Rh complexes have also been designed to specifically interfere with oncogenic signaling[820] and cancer-promoting/epigenetic regulatory processes.[821,822] For instance, cyclometalated Rh(III) complexes (Figure 13J) inhibited JAK2 phosphorylation[820] as well as the oncogenic and pro-inflammatory autotaxin-lysophosphatidic acid signaling axis.[821,823] Also, another cyclometalated Rh(III) complex exerted anticancer activity *in vitro* via inhibition of lysine-specific histone demethylase 1.[822] Rh(III)-based complexes have originally been considered poor candidates as metal cores of anticancer agents due to their kinetic inertness. In contrast to Ru(III) compounds, which become activated by conversion to a Ru(II) species (compare Ru, chapter 3.3), reduction of (even structurally related) Rh(III) compounds is considered improbable.[819,824] Thus, deregulation of the cellular redox balance has not been observed to contribute to the pharmacological activity of Rh(III) complexes *in vivo*.[825] Nevertheless, several octahedral Rh(III) complexes containing polypyridyl and other aromatic chelates as ancillary ligands have been demonstrated to yield high cytotoxicity in cancer cells (reviewed in[826-828]). Furthermore, several Rh(I) and Rh(II) compounds have shown promising anticancer activity.[829,830]

However, with regard to cancer immunosurveillance, the picture emerging from the literature is fragmentary. In addition, it needs to be stated that data on immune-related mechanisms have been published virtually exclusively for Rh compounds not primarily developed as anticancer agents. Only few reports on the (predominantly immunosuppressive) influence of Rh on the immune system exist. Hence, exposure of female Wistar rats to Rh(III) chloride ($RhCl_3$) via drinking water revealed a generalized decrease of cytokine levels in the serum, with the exception of IL-1α and IL-2,[831] suggesting an anti-inflammatory role of this Rh salt. Similar inhibitory effects on cytokine production (including IFN-γ, TNF-α and IL-5) have been observed in PBMC, using the complex ammonium hexachloridorhodate ($(NH_4)_3[RhCl_6]$) or the salt $RhCl_3$,[832] again indicating these compounds to exert rather inhibitory effects on immune cells. In addition,

an early study dating back to the 1980s found abrogating effects of Rh(I) complexes on macrophage chemotactic behavior.[833] Liu et al. reported on an anti-angiogenic cyclometalated Rh(III) complex (Figure 13J), and suggested an immune-impeding role via inhibiting NO production in macrophages in response to LPS by attenuation of NF-κB signaling.[834] Furthermore, in analogy to Ru-based CORM (see chapter 3.3), CO-releasing binuclear Rh complexes have been analyzed as anti-inflammatory agents by inhibition of NO generation in macrophages.[835] Multiple Rh(III) complexes have been analyzed for their anti-*Leishmania* activities.[836] Importantly, some of these agents exerted cytotoxicity towards J-774 macrophages, indicating that the anti-parasitic activity of these compounds observed in Wistar rats may be accompanied by myelosuppression.

In contrast to these data indicating immune down-modulation, exposure to Rh compounds can also induce allergic reactions including asthma and contact dermatitis.[837,838] Accordingly, pro-inflammatory effects of sodium hexachlororhodate(III) $Na_3[RhCl_6]$ have been observed under *in vitro* conditions, mimicking allergic reactions. Such, exposure of DC to $Na_3[RhCl_6]$ stimulated expression of IL-4, IL-5 as well as IFN-γ in co-cultured T cells.[839] However, *ex vivo* exposure of PBMC isolated from Ni- and palladium-allergic patients to $Na_3[RhCl_6]$ resulted in upregulation of IL-10, while not affecting IFN-γ levels.[840] This demonstrates that the effects of Rh compounds on the immune system are not straight forward, and that immune-inhibitory or -stimulatory activities are the result of the pleiotropic effects of these compounds on different immunocyte compartments.

With regard to effects of Rh compounds on anticancer immune surveillance, the field is virtually unexplored so far, and the picture emerging from available data on Rh-immune interactions is rather vague. However, the apparently predominant anti-inflammatory activity of Rh salts and organometallic complexes, together with comparably low propensity for redox activity-mediated ROS induction, argue against the involvement of immunogenic mechanisms in the anticancer activity of Rh compounds.

3.9. Zinc (Zn)

Zn is after Fe the second most abundant transition metal in the human body and essential for manifold enzymes and transcription factors.[841] Zn is exclusively present in the oxidation state +2 and is consequently (in contrast to e.g. Fe, copper and Co) not considered to be redox active and to induce formation of ROS. Important in the biological activity of Zn are its antioxidant and anti-inflammatory functions as well as its impact on immunity.[842] Zn deficiency can lead to immune dysfunctions.[843,844] For instance, Zn^{2+} is directly involved in TLR3/4 signaling, where it serves as negative regulator of Toll/IL-1R domain-containing adapter inducing IFN-β (TRIF) activity via inhibiting IFN regulatory factor (IRF) 3.[845] This regulatory function was demonstrated to down-modulate TLR-dependent induction of IFN-β and iNOS. In general, data on Zn impacts on immune cell phenotypes reveal a not fully conclusive picture. For instance, in a recent

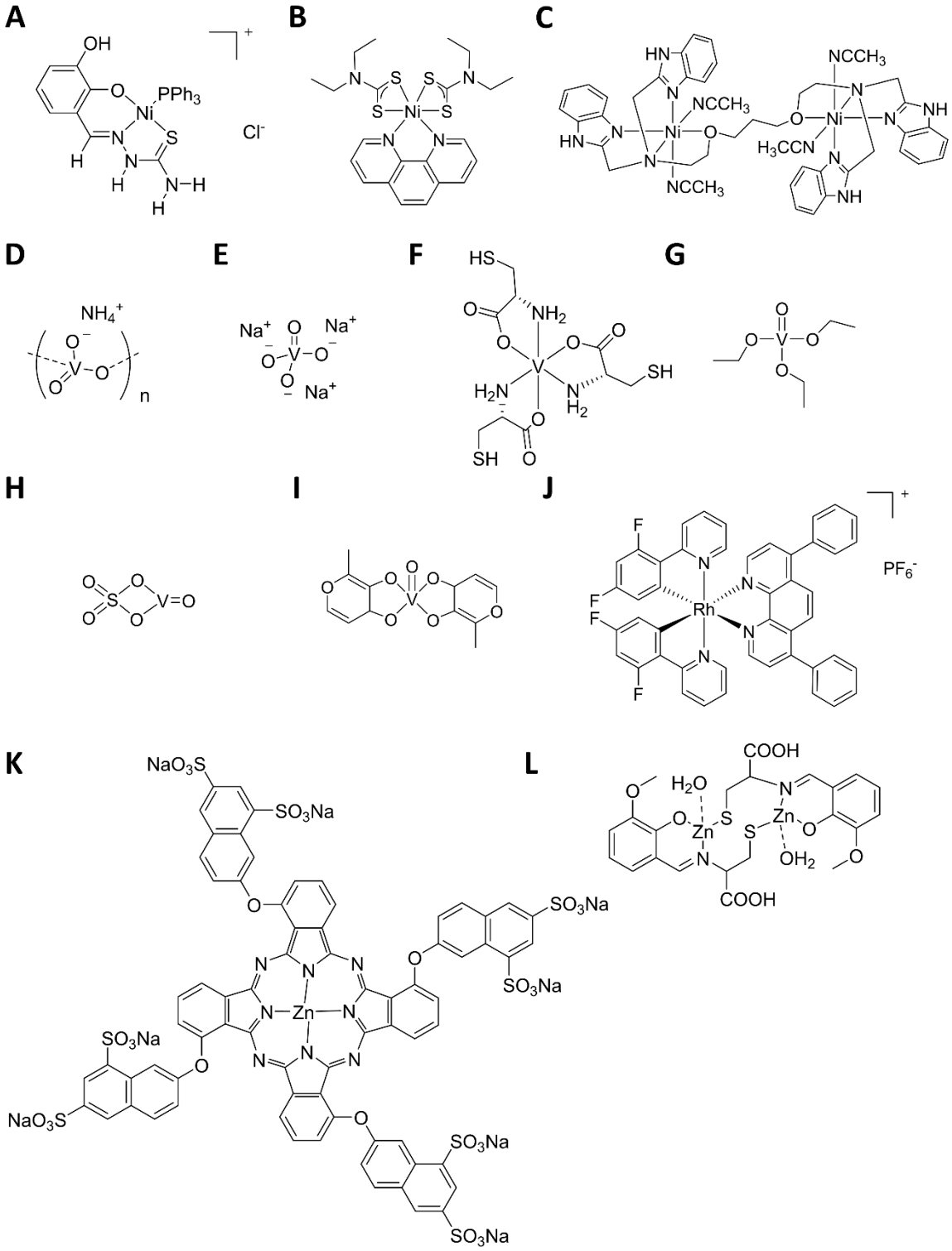

Figure 13. Chemical structures of lead anticancer nickel (A-C), vanadium (D-I), rhodium (J) and zinc (K-L) compounds with published immune-regulatory activities. (A) Ni(II) 2,3-dihydroxybenzaldehyde thiosemicarbazone, (B) Ni(II)-dithiocarbamate phenanthroline complex, (C) benzimidazole-derived di-Ni complex (di-Ni(II) ethylene glycol-bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetrakis(2-benzimidazoyl), (D) ammonium metavanadate, (E) sodium orthovanadate, (F) V(III)-L-cysteine, (G) V(V) oxytriethoxide, (H) V(IV)oxide sulphate, (I) bis-maltolato oxovanadium(IV), (J) cyclometalated Rh(III) complex (2-(2,4-(difluorophenyl)pyridine) C^N), (K) Zn(II) phthalocyanine-containing octa-sulphonate, (L) 2-[(2-hydroxy-3-methoxy-benzylidene)-amino]-3-mercapto-propionic acid Zn complex. For details see text.

study, effects of Zn homeostasis on macrophage polarization have been investigated.[846] This study revealed that both Zn supplementation and deficiency cause M1 macrophage polarization. Consequently, further in-depth analyses will be necessary to determine the dose- and time-dependency of Zn effects on immune cell phenotypes. Zn was demonstrated to induce a tolerogenic DC phenotype by diminishing MHC class II surface display, upregulating PD-L1, PD-L2, and IDO, and suppressing pro-inflammatory reactions in response to TLR ligand stimulation in models of antifungal immune responses. In addition, Zn shifted the ratio of T_{reg}/T_H17 in favor of the T_{reg} subtype.[847] Furthermore, FcεRI activation has been shown to trigger Zn release from the ER ("zinc wave") of mast cells, serving as intracellular signaling mediator to enhance NF-κB DNA-binding activity in delayed-type allergy reactions.[848] In addition, Zn-binding to metallothionein proteins was found to be essential for FcεRI-induced IL-4 production in basophils.[849] L-carnosine Zn chelate (ZnC) is an anti-inflammatory compound used clinically against peptic ulcer of the upper gastrointestinal (GI) tract.[850] Ooi et al. recently demonstrated that this complex induced elevated expression of HO-1 in RAW 264.7 macrophages. This resulted in attenuated induction of NF-κB, iNOS, as well as of NO production.[850]

With regard to cancer, Zn deficiency in cancer patients was associated with impaired NK cell activity and IL-2 production, leading to (presumably) impaired T_H1 function, enhanced oxidative stress and inflammation (assessed by an increase in IL-1β). Zn supplementation lowered oxidative stress-related parameters in healthy individuals with again a central role for attenuation of NF-κB signaling.[843] Hence, Zn may directly exert anticancer activity by interfering with oncogenic NF-κB signaling to prevent the expression of antiapoptotic, pro-proliferative and angiogenic regulators.[844] In the light of this, Zn should be discussed for its potential cancer-protective, immune-stimulatory role. Corroboratively, in glioblastoma models, Zn supplementation has been shown to enhance temozolomide cytotoxicity.[851] However, the role of potential anticancer immune responses in this model was not investigated.

Various Zn-containing compounds have been tested for their anticancer potential.[852-855] The anticancer effects of Zn-containing complexes have been demonstrated to include amongst others apoptotic and oxidative stress-inducing mechanisms, as exemplified for instance by a binuclear Zn(II) complex with the angiotension II receptor blocker azilsartan in human lung cancer cells.[856] However, the influence of these agents with respect to anticancer immune system activation is virtually unexplored. Scarce hints for beneficial effects on immune cells derive, for instance, from the study on a hierarchical Zn bud dressing γ-AlOOH mesostrand covered with the model cancer antigen ovalbumin. This compound has been investigated as adjuvant for cancer immunotherapy.[854] The formulation promoted intracellular uptake by macrophages and induced maturation and cytokine production of DC *in vitro*. In addition, the antigen-loaded particle enhanced $CD4^+$ and $CD8^+$ T cell populations as well as elevated IL-2 levels in the spleen, resulting in antitumor immunity in mice. In a study by Li et al., a Zn compound with affinity for macrophages was

designed with the rationale to induce intratumoral drug accumulation. This photoactive Zn(II) phthalocyanine-containing octa-sulphonate (Figure 13K) exhibited its affinity for macrophages via scavenger receptor-A binding.[853] Furthermore, an anticancer Zn Schiff base complex (Figure 13L) was found to be active against human multidrug-resistant and sensitive ALL cancer cells without showing toxicity towards peripheral blood monocytes (PBMC).[857]

Hence, also with regard to Zn compounds, the picture remains fragmentary. It is currently unclear in how far such drugs might contribute to anticancer therapy via influencing immune-regulatory functions both in cancer and immune cells.

3.10. Iron (Fe)

Fe is the most abundant transition metal in the human body with a total amount of ~ 5 g, of which about two thirds are used for the oxygen transport via hemoglobin. A crucial feature of the biological activity of Fe is the possibility to readily switch between Fe(II) and Fe(III), which is essential for enzymatic functions but can also result in formation of highly reactive hydroxyl radicals. Partly based on this redox activity, Fe plays vital roles as constituent of metalloproteins[858] in multiple biological processes, including oxygen transport (hemoglobin), the respiratory chain for mitochondrial ATP synthesis (NADH dehydrogenase, cytochrome c reductase, and cytochrome c oxidase),[859] metabolization of endogenous and exogenous chemicals (cytochrome P450),[860] redox balance (catalase),[861] or the production of deoxyribonucleotides (ribonucleotide reductase).[862] Fe deficiency has, amongst others, immunosuppressive effects, for instance by impairing NF-κB activation[863] and inducing HO-1 expression in response to oxidative stress (compare chapter 3.3).[864] In addition, there is evidence for an involvement of low Fe levels in impairing anticancer immune surveillance (reviewed in[865]).

Several Fe complexes have been tested for their anticancer potential. In fact, one of these, a ferrocene (bis-cyclopentadienyl Fe) (Figure 14A), is an organo-metallic compound for which already early anti-proliferative properties were demonstrated.[866] Anticancer Fe complexes include, besides ferrocene itself, several derivatives thereof, like ferrocene nucleoside analogs, iminosugar conjugates and ferrocene-tamoxifen (termed ferrocifens) as well as Fe carbonyl complexes (reviewed in detail by Gasser et al.[867]). Recently, chemical oxidation of ferrocifens[868] has yielded a heterocyclic, tetrahydrofuran-substituted quinone methide derivative with promising anti-proliferative behavior.[869] Data on the effects of Fe complexes with respect to anticancer immune responses are scarce. Such studies include those on ferrocenes, demonstrating their antitumor properties to be at least partially attributable to anticancer immune stimulation. This was elegantly exemplified in melanoma and lung carcinoma mouse models, where adoptive immune cell transfer of ferrocene-treated animals elicited antitumor responses in untreated animals.[870] Ferrocene treatment was furthermore accompanied by splenocyte proliferation, increased LPS-

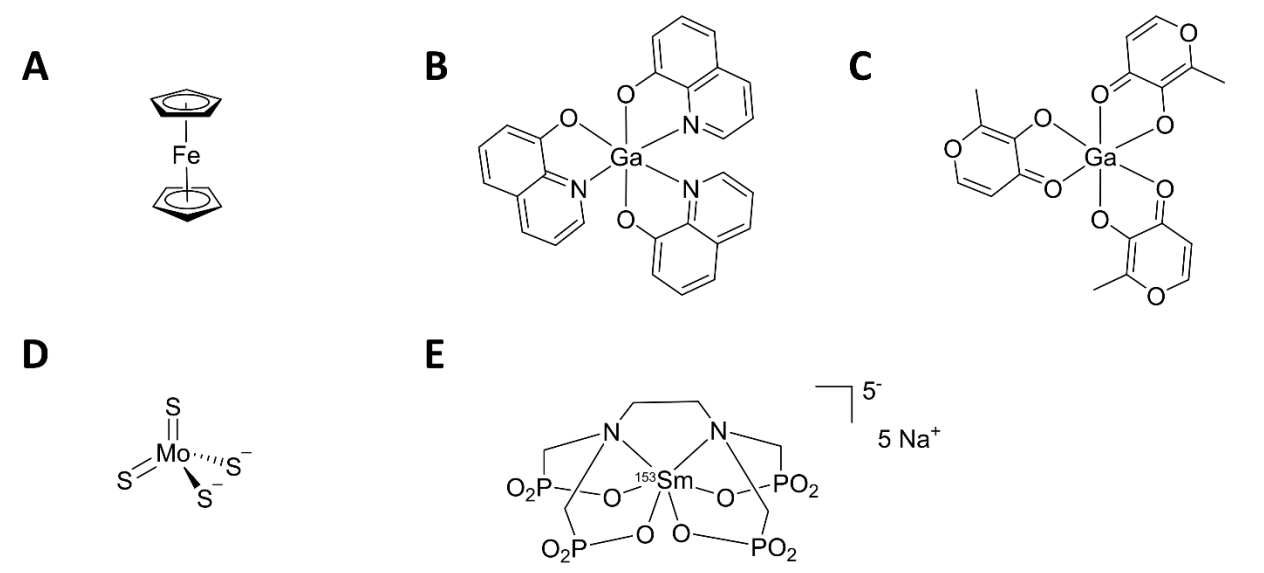

Figure 14. Chemical structures of lead anticancer iron (A), gallium (B-C), molybdenum (D) and samarium (E) compounds with published immune-regulatory activities. (A) Ferrocene (dicyclopentadienyliron), (B) KP46, (C) Ga maltolate, (D) tetrathiomolybdate, (E) samarium-153 ethylene diamine tetramethylene phosphonate (EDTMP). For details see text.

induced production of TNF-α and NO as well as oxygen burst in macrophages, and enhanced NF-κB activation in PBMC. Challenging these data, different ferrocifenes (assessed in that case as glucose biosensor components) decreased the rate of lymphocyte proliferation, especially of B cells and NK cells *in vitro*.[871] In another study by Zhang et al., the polyoxometalate Fe hepta-tungsten phosphate oxygen cluster complex $Na_{12}H[Fe(PW_7O_{28})_2]\cdot 44H_2O$ (IHTPO) was investigated for its antineoplastic potential in several human cancer cell models.[872] Despite low *in vitro* cytotoxicity, this compound showed significant tumor growth-inhibiting activity in a sarcoma allograft model. Furthermore, evaluation of a potential immunological involvement in this antitumor effect revealed that IHTPO promoted splenocyte proliferation, NK cell and CTL activation as well as antigen-specific IgG antibody responses. In addition, the authors found induction of T_H1-produced cytokines IFN-γ and IL-2. These results suggest a critical involvement of T cell-mediated as well as humoral immune activation in the anticancer activity of IHTPO.

Additionally, ferrocenyl complexes induced senescence in glioma and melanoma cell models.[873] Interestingly, this effect was accompanied by secretion of various cytokines, including IL-1α, IL-1β, IL-6, IL-8 as well as TNF-α, suggesting an immune-stimulatory role of these Fe compounds. Another study reported an anti-tumorigenic role of Fe-loaded TAM in hemorrhagic areas of the TME in patient samples of NSCLC. Mechanistic *ex vivo* as well as *in vivo* experiments performed by the group of da Silva and colleagues showed that exposure to hemolytic erythrocytes converted TAM into a pro-inflammatory phenotype, capable of direct tumor cell killing.[874] This effect was accompanied by elevated expression of chemokines (CXCL1/2, M-CSF, GM-CSF) as well as of iNOS, IL-6, and TNF-α. This suggests delivery of Fe to TAM as adjuvant therapeutic strategy, repolarizing immune cells in the TME from immunosuppressive

towards an immune-stimulatory anticancer phenotype. Together, these data are encouraging and warrant further investigation of the potential of Fe complexes to foster anticancer immunity.

3.11. Gallium (Ga)

Ga is a main-group element which exclusively occurs in the oxidation state +3. Ga(III) shares certain chemical characteristics with Fe(III), e.g. the octahedral and tetrahedral ionic radius and the ionization potential.[875] However, in contrast to Fe, Ga is not redox active. Consequently, incorporation of Ga into Fe-dependent proteins disrupts their proper function, including deoxyribonucleotide formation by ribonucleotide reductase, functioning of mitochondria (by e.g. ROS production), as well as the cellular Fe transport and storage.[875] Ga exerts antimicrobial effects but also exhibits antineoplastic activity against tumor cells.[187] In recent years, several reviews have comprehensively dealt with the modes of action and (pre)clinical development of first as well as later generation Ga-based pharmaceuticals as anticancer therapeutics.[187,876] These include the simple Ga salts Ga nitrate and Ga chloride, as well as Ga complexes including tris(8-quinolinolato)Ga(III) (KP46) (Figure 14B) or Ga maltolate (Figure 14C), all of which are being or have been assessed for their anticancer potential in clinical trials for solid tumors (CNS tumors, neuroblastoma, urothelial carcinoma, lung cancer and others) and hematological malignancies (including non-Hodgkin's lymphoma, multiple myeloma, diffuse large B cell lymphoma)[877,878] (www.clinicaltrials.gov). Furthermore, Ga compounds in preclinical testing include Ga-pyridines, -phenolates and -thiosemicarbazones.[187,879-881]

Several articles have been published on immune-modulatory effects of Ga compounds. On the one hand, over a decade ago, immunosuppressive effects of Ga nitrate in Cyprinus carpio (carp) were reported and comprised decreased blood leukocyte numbers, Ig production and phagocyte activity.[882] Additional animal studies in mice revealed suppressive effects of Ga nitrate on inflammation-related diseases such as inflammatory arthritis and systemic lupus erythematodes.[187,883,884] On the other hand, and with respect to therapy of cancer, Wu and coworkers found Ga maltolate to increase expression levels of immune-activating CXCL10, CXCL11 and IL-13, as well as a decrease in immunosuppressive IL-10 expression in human cutaneous T cell lymphoma xenograft models.[885] However, it remains to be determined whether these effects may also apply to other, non-immune cell-related malignancies, and whether immune activation plays a role in the antitumor activity of Ga nitrate. To date, no information is available as to whether Ga salts or complexes may interfere with the immunogenicity of malignant cells. Given that several Ga-based drugs are being evaluated in clinical trials, elucidation of the impact of these compounds on anticancer immunity will be of special importance. Furthermore, the question remains whether the activity of Ga compounds in hematological malignancies might also interfere with the fitness of healthy immune cell compartments.

3.12. Molybdenum (Mo)

Mo is the only essential trace element for humans of the second row transition metals. In enzymes, Mo is always bound to an organic co-factor (molybdopterin) and cycles between the oxidation states +4 and +6. An extensive overview of Mo compounds has been given recently by Jurowska and colleagues.[886] As for Co, knowledge of Mo interactions with the immune system majorily arises form prosthetics. Wear debris of Mo-based alloy material for hip replacement has been investigated for its effects on T cell phenotype and cytokine profile induction in human blood samples.[887] In this analysis, results were inconclusive, with only a subset of samples showing pro-inflammatory responses (induction of IL-17 and IFN-γ). Furthermore, the investigators found gender- and age-dependent effects, i.e. an increase in IL-10-producing T cells in females and a tendency towards decreased IL-6 expression in blood from older donors.

Mo has been assessed for its anticancer potential mainly in the form of tetrathiomolybdate (Figure 14D), a copper chelator.[888] This compound is currently extensively tested in preclinical phase but is also registered in several clinical trials.[889,890]

Knowledge on the interplay of therapeutic Mo compounds with the immune compartment is fragmentary. In an *in vivo* mouse study concerned with vascular inflammation, oral gavage of tetrathiomolybdate prevented LPS-induced inflammatory responses, illustrated by reduced serum levels of soluble ICAM-1, MCP-1, and TNF-α.[891] Furthermore, this compound inhibited NLRP3 inflammasome activation via copper chelation in a SOD1-dependent manner.[892] In accordance with these observations, tetrathiomolybdate decreased SOD1 activity, increasing intracellular oxidant levels in B cell leukemia cells.[893] Furthermore, tetrathiomolybdate inhibited vascular inflammation and development of atherosclerotic lesions in apolipoprotein E-deficient mice by downregulating VCAM-1, ICAM-1, MCP-1 as well as pro-inflammatory cytokines.[894] In a co-culture study investigating the role of infiltrating M2 macrophages in breast cancer angiogenesis, tetrathiomolybdate decreased the angiogenic potential of the conditioned supernatant on chick chorioallantoic membranes.[895] It needs to be mentioned that the Mo compound did not modify the M2 phenotype or cytokine secretion profile. Thus, one might hypothesize that tetrathiomolybdate might primarily downmodulate cytokine-induced endothelial cell signaling. Accordingly, this compound reduced endothelial cell proliferation and ICAM expression in a mesothelioma model.[896] Interestingly, this was accompanied by $CD4^+$ T cell infiltration. In conclusion, the available data point rather to an anti-inflammatory role of tetrathiomolybdate especially in the vasculature. Further investigations will be necessary to determine to which extent the potential anti-angiogenic activities of this compound might be harnessed for improved anticancer responses, especially in combination schemes.

3.13. Other metals

In addition to the above described metal complexes and free metal ions, for which at least rudimentary data on the impact on anticancer immunity are available, there are numerous further compounds containing other metal cores for which effects on immunological mechanisms in frame of anticancer therapy are not described. This is in many cases due to their early stage of preclinical development. Such, osmium (Os) compounds exhibit diverse cancer cell-killing modes of action, depending on the choice of ancillary ligand, including redox activity, DNA targeting, or protein kinase inhibition.[897] Os complexes as anticancer agents have been reviewed in detail by Hanif et al.[897] These compounds have shown comparable or even superior activity as compared to clinically tested Pt and Ru drugs. Concerning effects on immune compartments, one study showed anti-inflammatory activity of the two half-sandwich Os compounds [Os(η^6-p-cymene)(1,2-dicarba-closo-dodecarborane-1,2-dithiolato) and [Os(η^6-p-cymene)(benzene-1,2-dithiolato)] in RAW 264.7 macrophages.[898] This was illustrated by decreased NO production of LPS-stimulated, Os complex-treated cells. However, these compounds were developed as non-cytotoxic, anti-inflammatory drug candidates. No data on immune interactions of Os-based anticancer compounds are currently available.

There are few reports on the lanthanide gadolinium (Gd) as metal-component of anticancer agents. Evidence for the potential of Gd as anticancer metal agent arises for instance from a report on Gd(III) complexes of coumarin-3-carboxylic acid, which have been tested for their antiproliferative activity in CML cells *in vitro*.[899,900] Information on immunological aspects of compounds containing Gd are lacking, owing to the yet limited existence of *in vivo* experiments or appropriate *ex vivo* co-cultures aimed at characterizing cancer-immune cell interactions.

Another aspect of metal-containing remedies are radiopharamceuticals of which some are even clinically approved. For this reason, it is surprising that very little data exist on immune interactions for these therapeutic agents, especially as for some compounds, strong indications for an involvement of the immune system as one component of their modes of action have been reported.[901,902] One illustrative example is a radionuclide of radium (Ra), namely ^{223}Ra, a calcium-mimetic, that was the first α-particle-emitting radiopharmaceutical prolonging overall survival, ameliorating symptomatic skeletal events and improving quality of life in bone metastatic castration-resistant prostate cancer.[903] Several years ago, ^{223}Ra has been approved by US and European regulatory agencies for the treatment of this cancer type.[903-905] Mechanistic data on potential immune-stimulatory effects of this radionuclide are so far fragmentary. One study, investigating the survival benefit ^{223}Ra dichloride in mice bearing breast cancer bone metastasis, observed the presence of macrophages in necrotic metastasis foci.[901] Here, the authors additionally showed DNA double-strand break induction in neoplastic areas, which they attributed to the α-particle emission properties as compared to β- and γ-emitting radionuclides. These observations suggest immune-stimulatory properties of ^{223}Ra by tumor necrosis-associated DAMP release and activation of innate immune cells such as macrophages. It would be worth investigating whether such effects might also enhance DC-mediated priming of specific anticancer T cell responses. Corroboratively, encouraging reports have been published

for the radioactive isotope of the lanthanide samarium (Sm), ^{153}Sm as radiopharmaceutical.[906] Chakraborty and colleagues showed that the radionuclide ^{153}Sm (a β-particle emitter) in complex with ethylene diamine tetramethylene phosphonate (EDTMP, Sm lexidronam) (Figure 14E) alters the immunogenicity of tumor cells, rendering them more susceptible to T cell-mediated killing.[902] This laid the basis for investigation of this compound in combination with immune-stimulatory agents. This approach included a phase 2 clinical trial with the prostate-specific antigen (PSA) vaccine rilimogene galvacirepvec/rilimogene glafolivec (PSA-TRICOM) for the treatment of bone-metastatic, castration-resistant prostate cancer.[907] Although only moderate response rates were observed, this might encourage the combination of radionuclides with immunotherapy. Since prostate cancer is considered inherently immunogenic,[908] the potential of a number of immunotherapeutic strategies including, besides checkpoint inhibition, for instance DC- or cancer cell lysate-based vaccination is being assessed.[909] This suggests combined treatment regimens of immune-stimulating therapeutics not only with ^{153}Sm , but also with ^{223}Ra.

This should encourage more in depth evaluation of the immune-stimulatory potential of these agents, for instance in combination settings with immunotherapeutic approaches.

4. Nanoparticles (NP) of metal drugs and the immune response

4.1. General considerations

As stated above, metal complexes occupy a special place in the fight against cancer, with the three central Pt drugs - cisplatin, carboplatin, and oxaliplatin - remaining the backbone of oncologic treatment.[27] Nevertheless, their clinical effectiveness is hindered by several factors, most importantly by their poor tumor specificity. In order to overcome this issue, in the last 15 years researchers all around the world employed NP-based platforms to encapsulate these and other anticancer drugs and selectively deliver them into tumors.[414,910] NP display indeed unique properties for potential clinical application in the treatment of cancer: 1) they preferentially accumulate in tumor tissues due to the EPR effect; 2) their surface can be easily modified with active moieties (antibodies, peptides), which allows selective release of the cargo into the tumor tissue; 3) their specific physical-chemical properties protect the drugs from degradation processes in a physiological environment.[911-914]

Despite first successful applications of several drug nanoformulations (liposome-based, albumin NP, and polymeric micelles) in the clinic,[915] low delivery efficiency still represents a limiting factor. Furthermore, NP are known to interact with both the innate and adaptive immune system and can lead to hypersensitivity, immunogenicity and autoimmunity.[913] One very prominent example is the interaction of liposomes with the mononuclear phagocyte system (MPS) and its macrophage uptake. This main disadvantage is usually reduced by PEGylation of the NP, avoiding opsonisation and recognition by the MPS.[916] The future potential

of nanomedicine in oncology is undebatable. Indeed, the generated immune response, long considered as drawback, could even be the key to the discovery of new ways of fighting cancer cells.[917] In the last years there is an increasing interest in NP as immune adjuvants to create vaccines and/or to augment the anticancer effects of conventional chemotherapeutics.[918]

Concerning metals, we can distinguish between (organic and inorganic) NP that contain metal-based drugs (e.g. nanoformulations of cisplatin) and nanomaterials consisting of a metal core (e.g. Au NP). The revision of all strategies concerning anticancer metal drug nanoformulations is out of the scope of this review. Similarly, in this chapter we will omit the effects of non-cancer-related nanomaterials on the immune system. A number of respective reviews can be found elsewhere.[910,913,914,917,919,920] Here, we will focus specifically on immune-related data concerning either nanoformulations of metal-containing anticancer drugs or NP consisting of metals related to anticancer strategies.

4.2. Nanoformulations of metal-based anticancer drugs

Considering that, together with ATO, Pt chemotherapeutics are the only metal-based drugs used in the clinic for cancer treatment, it is not unexpected to find a high number of studies reporting on nanoformulations to improve pharmacokinetics and selectivity of these anticancer therapeutics. First data about immune implications of such delivery systems is relatively recent, dating back to 2009.[921] Eriksson et al. reported the ability of the nanosized protein α-helical right-handed coiled coil (RHCC) to carry cisplatin to cancer cells without interfering with its cytotoxic potential. With regard to immunogenicity, intravenous injection of the cisplatin-loaded protein construct in Balb/c mice induced only marginal activation of specific $CD8^+$ T cells in splenocytes. This was accompanied by only weak maturation of BMDC, characterized by a slight induction of CD40 expression and IL-12 production, whereas no increase of CD80, CD86 and MHC class II expression was observed as compared to unstimulated controls. Further developments of this delivery system have been hampered by its short half-life. In 2014, Li et al. used multi-walled carbon nanotubes (CNT) to encapsulate cisplatin and a Pt(IV) prodrug and intravenously injected them into mice to investigate their biodistribution.[922] While the localization of cisplatin was unaffected by CNT, Pt(IV)-coupled CNT was found increased in the lungs and, at the same time, reduced in kidneys and liver. The authors suggested, as a consequence, potential application in lung cancer therapy. Moreover, the effect of CNT on proinflammatory cytokine production (IL-1β, IL-6, and TNF-α levels in the serum) was evaluated. 4 hours post-injection measurements revealed a distinct enhancement of IL-1β levels when compared to control or the metal-based compounds alone. Nevertheless, IL-1β decreased to control levels after 24 hours. Additionally, no significant changes were observed in the production of IL-6 and TNF-α, indicating that these drug delivery systems did not induce any significant immune response or inflammation.[922]

A more comprehensive study on the immunological impact of nanomedicine including Pt drugs was conducted by Nie's group in 2016.[923,924] The authors encapsulated oxaliplatin into monomethoxy-poly(ethylene glycol)-poly(D,L-lactide-co-glycolide) (mPEG-PLGA) polymeric NP with the purpose to evaluate the effect of NP on ICD induction provoked by oxaliplatin (compare chapters 2.2.6 and 3.1.5). This is, indeed, the first study on the contribution of nanomedicine to ICD. Two nanoformulations were prepared, containing oxaliplatin or gemcitabine (a non-ICD inducer). Firstly, the induction of apoptosis by the different drug formulations on Panc-1 (human pancreatic carcinoma cells) and Pan02 (mouse pancreatic carcinoma cells) was evaluated. Both NP with oxaliplatin and gemcitabine induced significantly more apoptosis than the corresponding free compounds, while the naked NP had virtually no impact on cell viability. Next, the release of DAMP required for ICD induction (compare chapters 2.2.5 and 2.2.6) after treatment with different drug formulations was measured. NP with oxaliplatin resulted in a dramatic enhancement of HMGB1 release and ATP secretion and even a two-fold increase in CRT exposure as compared to the free drug, while the gemcitabine system had only a weak effect looking at the same parameters. Moreover, the nanoformulation induced a stronger immune response than oxaliplatin alone. Furthermore, oxaliplatin NP significantly induced DC maturation with expression of CD80 and CD83 in human primary immature DC and a DC cell line derived from C57BL/6 mice. In addition, IFN-γ secretion was significantly enhanced in human primary DC co-culture systems with autologous $CD3^+$ T lymphocytes. Finally, in a vaccination experiment, dead or dying Pan02 cells treated either with the oxaliplatin NP system or the free drug were subcutaneously injected into C57BL/6 mice, and after seven days, mice were re-challenged with untreated Pan02 cells. The protective effect against tumor formation was much more pronounced with oxaliplatin NP than with the free drug. Accordingly, oxaliplatin nanoformulation induced an increase of CTL in the tumor tissue, together with enhanced expression of IFN-γ. As expected, no such effects were seen in case of gemcitabine encapsulation. These results overall demonstrate the ability of the nanocarriers to further improve the ICD-inducing potential of Pt drugs.[923]

One year later, in 2017, Ishida's group reported on the immune-modulatory properties of oxaliplatin encapsulated in PEGylated liposomes,[212] for which enhanced antitumor efficacy had been previously demonstrated in C26 colorectal carcinoma-bearing Balb/c mice.[925,926] Strikingly, the authors found significant growth suppression of tumors implanted in immunocompetent mice, but not in their immunodeficient counterparts. This therapeutic effect decreased when the authors depleted $CD8^+$ T cells, confirming that the contribution of the host immune system was crucial. When immunosuppressive cell components were investigated in tumor tissue, the liposomal preparation induced reduction of T_{reg}, MDSC and TAM, the levels of last one being unaffected by free oxaliplatin. Furthermore, when looking at $CD8^+$ T cell populations in the spleen and tumor tissue, free oxaliplatin reduced the amount of splenic and tumor-infiltrating $CD8^+$ T cells, whereas the liposomal oxaliplatin preserved the levels of both cell populations. Corroboratingly, $CD8^+$ T cell activation, assessed by IFN-γ expression, followed the same pattern. MHC

class I expression was increased by both free and liposomal oxaliplatin, with the liposomal formulation showing slightly minor effects as compared to the free drug.[212]

Quite recently, the group of Wang investigated the possibility to combine chemotherapeutics with immune-modulating agents within the same nanocarrier.[927] In particular, they developed TME-sensitive cluster NP (SCN) loaded with BLZ-945, a highly selective inhibitor of colony stimulating factor 1 receptor (CSF-1R) and a Pt(IV) prodrug. CSF1, also termed M-CSF, and its receptor are known to be the primary signaling pathway for the function TAM (compare chapter 3.1.2).[928,929] The difficulty of this strategy is that two areas have to be targeted: BLZ-945 should target TAM which are enriched in perivascular regions, while the Pt drug should reach the tumor cells spread throughout the bulk tumor mass. To solve this dilemma, the authors prepared a bi-loaded nanosystem called $^{BLZ\text{-}945}$SCNs/Pt, where BLZ-945 was encapsulated in the hydrophobic domain, while the Pt prodrug was covalently conjugated to the particle. Since it was demonstrated that SCN have pH-sensitive hydrophobic−hydrophilic transitions leading to cluster disassembly, BLZ-945 is released once the system is deposited at the acidic tumor site,[930] while the small particles with the attached Pt-prodrug can further penetrate into deeper layers of tumor tissue and release active Pt drugs intracellularly (Figure 15). Shen et al. demonstrated a promising *in vitro* activity and then proceeded with *in vivo* experiments using a 4T1 murine breast cancer model. Here, they tested the activity of $^{BLZ\text{-}945}$SCNs/Pt as compared to the formulation without the Pt prodrug ($^{BLZ\text{-}945}$SCNs), without BLZ-945 (SCNs/Pt), as well as to PBS and cisplatin as controls. Both $^{BLZ\text{-}945}$SCNs and SCNs/Pt only moderately inhibited tumor growth, while $^{BLZ\text{-}945}$SCNs/Pt showed 88.7% tumor suppression under the same conditions, demonstrating a clear synergistic antitumor effect of Pt combined with CSF-1R inhibition. When compared with cisplatin alone, $^{BLZ\text{-}945}$SCNs/Pt exhibited a comparable tumor suppression but with fairly reduced side effects (no weight loss). Finally, the effect of these nanoconstructs on the immune system was evaluated. SCNs/Pt treatment slightly increased while $^{BLZ\text{-}945}$SCNs distinctly reduced the TAM proportion in the tumor tissue. Surprisingly, treatment with the $^{BLZ\text{-}945}$SCNs/Pt system further reduced TAM numbers even below the level observed for $^{BLZ\text{-}945}$SCNs. In addition, tumors receiving $^{BLZ\text{-}945}$SCNs/Pt treatment showed a pronounced increase of $CD8^+$ and $CD4^+$ T cells as well as a reduced fraction of $CD4^+$ T_{reg} cells.[927]

A similar strategy was adopted by Lu et al., who described a nanocarrier which simultaneously incorporates the IDO inhibitor indoximod (compare chapter 1.2.5 and 3.1.10) and the ICD inducer oxaliplatin.[553] First, synergistic activity of an indoximod-containing nanovesicle with oxaliplatin was demonstrated in pancreatic carcinoma animal models.[553] This was accompanied by an increase of $CD91^+$/$CD11b^+$/$CD11c^+$ cells (corresponding to DC) in the tumors, release of IFN-γ and decrease of IL-10 levels. Eventually, a mesoporous silica NP containing both indoximod and oxaliplatin was designed, which significantly prolonged overall survival upon orthotopical administration of KPC cells (derived from pancreatic ductal adenocarcinoma (PDAC) developed in a transgenic $Kras^{LSL\text{-}G12D/+}$/$Trp^{53LSL\text{-}R172H/+}$/Pdx-1-Cre (KPC)

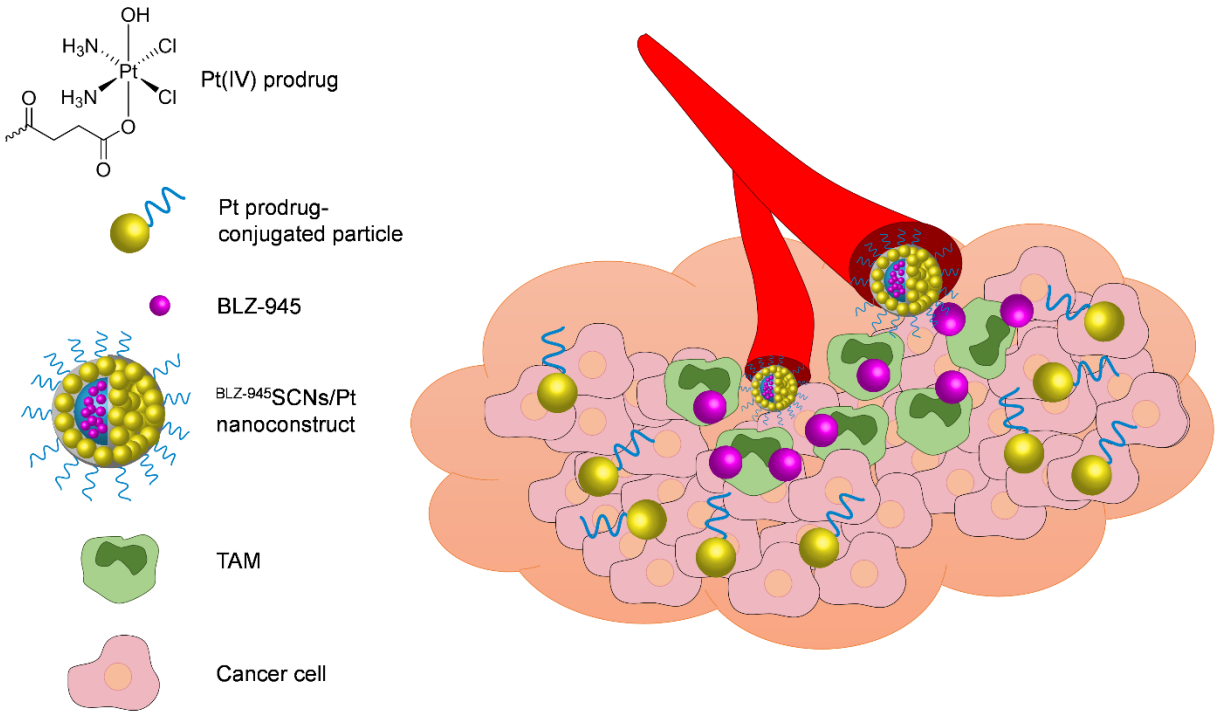

Figure 15. Schematic illustration showing drug delivery by a nanoconstruct loaded with the CSF-1R inhibitor BLZ-945 and a Pt(IV) prodrug, targeting TAM and cancer cells, respectively. $^{BLZ\text{-}945}$SCNs/Pt is transported via the blood vessels to the tumor tissue, where both components are released upon nanoconstruct collapse. While BLZ-945 blocks CSF-1R on TAM in the perivascular region, the Pt(IV) prodrug penetrates deeper into the tumor tissue, where it gets activated by acidic tumor pH and subsequently induces cancer cell death. TAM, tumor-associated macrophage. CSF-1R, colony stimulating factor-1 receptor. For details see text.

mouse).[553] Subsequent analysis proved upregulation of S6K phosphorylation and reduced IL-6 levels together with a significant increase of CTL in the tumor tissue after treatment with the nanoformulation. In line with this data, co-treatment with CD8- or TLR4-inhibitory antibodies significantly suppressed the *in vivo* activity of the new nanoformulation.[553]

Interestingly, a recently reported new nanoformulation loaded with a cisplatin prodrug and an IDO inhibitor exerted even stronger anticancer activity when compared to the one described by Lu et al., both *in vitro* and *in vivo* (Figure 16).[554] In detail, Wang and colleagues employed layered double hydroxide biocompatible NP to co-load the IDO inhibitor 4-[[2-(4-bromophenyl)hydrazinyl]sulfonyl]benzoic acid with the Pt(IV) prodrug c,c,t-[Pt- $(NH_3)_2Cl_2(OC(=O)CH_2CH_2COOH)_2$] (disuccinatocisplatin, DSCP). The idea behind this strategy was to build a nanohybrid which enters cells and releases the Pt(IV) prodrug. Subsequently, the prodrug is reduced to cisplatin and induces apoptosis. Simultaneously, the IDO inhibitor is released, boosting the immune system in the TME. The results suggested that this system enhanced T cell proliferation, cell cycle arrest in S phase and induced T cell-mediated apoptosis.[554]

4.3. Metal-based NP and cancer

The development of nanomaterials which involve metals for use in cancer treatment concerns not only delivery systems of metal-based drugs, but also inorganic NP that have been tailored as therapeutic or imaging agents. Inorganic NP include nano-semiconductors (quantum dots), carbon nanotubes, oxides (Fe oxides) and those derived from metals. Regardless of their composition, inorganic and organic NP (e.g. liposomes, micelles, polymers, dendrimers, nucleic acids, lipids, viruses) interactions with biological relevant entities mostly depend on size, shape and post-synthetical modifications. The impact and implications of these aspects on the therapeutic applications of NP has been extensively reviewed elsewhere.[911-914] Metal-based NP can be constructed to activate the immune system. This effect can be dependent on the intrinsic particle characteristics but also on the immune-modulatory cargo (e.g. cytokines).[931] This chapter will include various examples of metal-based NP which have significance in the field of cancer therapy and at the same time report immune system interactions.

4.3.1. Gold (Au)

Among metal-based NP in cancer therapy, those made of Au (GNP) are certainly the most thoroughly investigated. GNP have indeed been employed in multiple applications including drug carriers, photothermal agents, contrast agents and radiosensitizers.[932,933] In addition, the effects of GNP on the immune system have been well characterized.[934] Constructing GNP for cancer treatment taking advantage of immune-related strategies has hence attracted strong interest in the last years. Cruz et al. developed 13 nm GNP conjugated to prostate cancer-associated antigen peptides for cancer immunotherapy.[935] By various techniques they demonstrated that antigen-conjugated GNP were internalized in DC and produced a significant immune response that was not observed for the native antigen alone. The authors suggested that this approach could pave the way for further development of antitumor vaccines. In 2016, Saha and co-workers investigated whether unmodified 20 nm GNP could be employed to disrupt the crosstalk between pancreatic cancer cells (PCC) and pancreatic cancer-associated fibroblasts, also known as pancreatic stellate cells (PSC), which alters the TME in PDAC in favor of malignant progression.[936] Various cytokines like TGF-β, fibroblast growth factor 2, connective tissue growth factor, IL-1β, and VEGF play major roles in this crosstalk and the activation of PSC. The authors demonstrated that their GNP disrupted the bidirectional communication between PCC and PSC via alteration of the cell secretome, and proposed ER-stress as probable mechanism causing growth inhibition in cancer cells. Importantly, cytokines like IL-8 and GM-CSF, which are important in immuno-suppressive processes, were downregulated by GNP, suggesting a possible improvement of therapeutic outcome at least in this type of cancer.[936]

Webb et al. developed GNP combining diagnostic and therapeutic properties by taking advantage of an antibody targeting PD-L1. In particular, they studied multi-branched Au nanoantennas (MGN), demonstrating that coupling of these MGN to PD-L1-targeting antibodies can be exploited - on the one hand

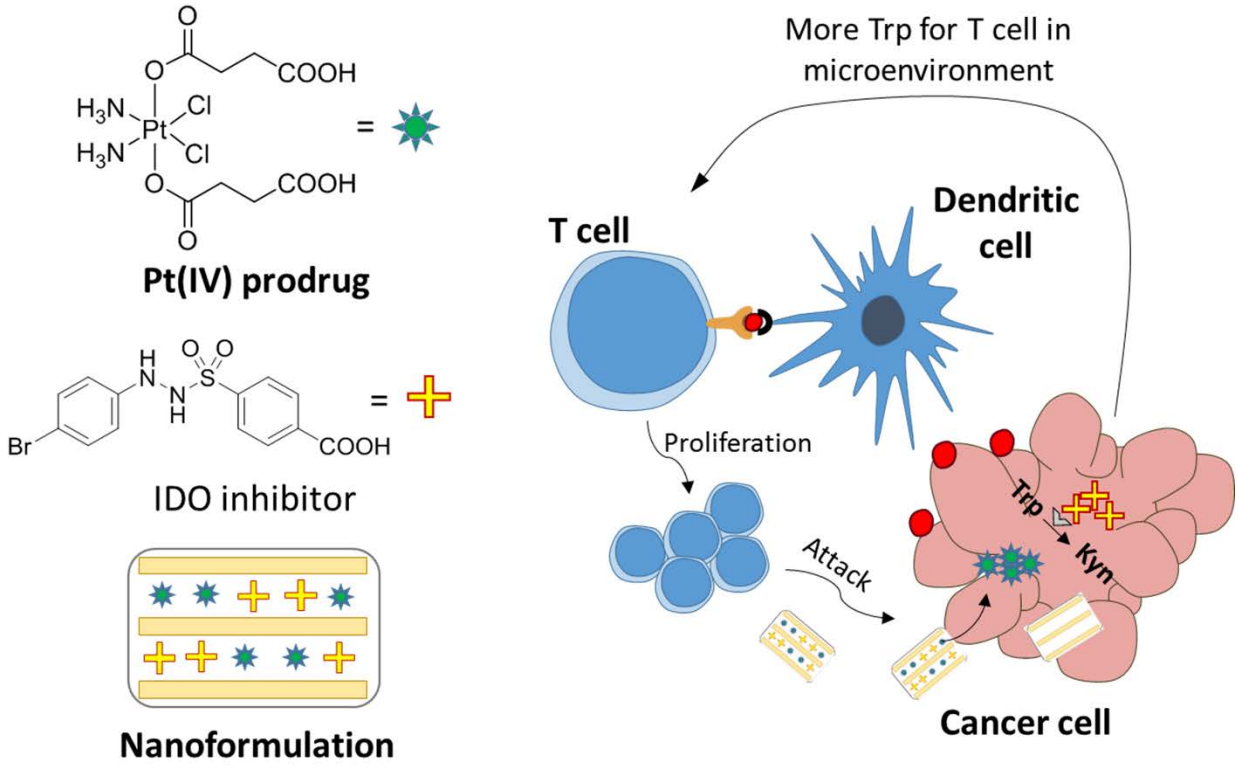

Figure 16. Pt prodrug nanopreparation with immune-related activities. Nanoformulation of cisplatin combined with the IDO inhibitor 4-[[2-(4-bromophenyl)hydrazinyl]sulfonyl]benzoic acid exerts anticancer activity via direct cytotoxicity as well as T cell-mediated cell death induction. Trp, tryptophane; IDO, indoleamine-2, 3-dioxygenase; Kyn, kynurenine. For details see text.

- to track this immunomarker with spatiotemporal control using surface-enhanced Raman scattering (SERS) (visualizing membrane surface binding and receptor-mediated endocytosis), and - on the other hand - to provide a light-triggered therapeutic approach via the photothermal activity of this compound.[937] Chen et al. described GNP coupled to synthetic unmethylated cytosine-guanine (CpG) ODN, known for their immune-stimulatory activity via TLR9 (compare chapter 3.1.7.) and widely used as a therapeutic tool for cancer and other diseases. The authors demonstrated induction of immune responses in a TLR9-dependent manner *in vitro*, characterized by secretion of TNF-α, IL-6, and IL-12 of RAW264.7 cells. Furthermore, the same NP were administered to ICR mice. Elevated levels of IL-12 and MCP-1 were measured in the serum 3 hours after injection, implicating a potential for therapeutic application of these nanomaterials.[938] In a recent paper, Jeon and Lim described promising radionuclide-embedded GNP that provoked DC maturation and antitumor immunity to levels comparable or even higher to DC pulsed with tumor lysates. The authors prepared GNP modified with oligotyrosine able to incorporate radioisotopes with imaging capability. These NP were internalized by DC without inducing apoptosis. GNP treatment of immature DC induced a significant increase in proinflammatory cytokine production. Furthermore, up-regulation of CD80 and CD86 as well as production of TNF-α and IL-6 was even more pronounced as compared to TC-1 tumor lysate-pulsed DC. Also *in vivo*, these radiolabeled GNP produced strong antitumor immunization in cervical carcinoma-bearing mice. In addition, GNP-treated DC increased the amount of $CD8^+$ CTL precursors in

total splenocyte populations and exhibited enhanced killing potential as compared to animals treated with unpulsed or with tumor lysate-pulsed DC. This effect was accompanied by elevated IL-6 and TNF-α levels in the spleen and draining lymph nodes.[939]

4.3.2. Cobalt (Co)

Several years ago, the group of Chattopadhyay investigated a Co-based tumor vaccination approach.[940,941] Co oxide (CoO)-based NP were created as carriers of tumor antigens for delivery to macrophages. N-phosphonomethyl iminodiacetic acid (PMIDA) was used to modify CoO to overcome toxicity and to provide anchorage sites via COOH groups for tumor antigens. In this study, human oral carcinoma cell lysates were used as antigen source. The killing potential of primed macrophages on oral carcinoma cells was tested in co-culture experiments. CoO NP-mediated antigen exposure to macrophages elicited immune-stimulatory responses characterized by upregulation of IFN-γ and TNF-α. Co-incubation of primed macrophages also led to an increase of the $CD4^+$ T cell population and induced differentiation into immune-stimulatory T_H1 cells.[941] In parallel, besides the immune-stimulatory role of Co NP-tethered tumor antigens, Co ions themselves were found to interfere with immune-modulatory signaling. A follow-up study by the same group describing PMIDA-conjugated CoO NP reported liberation of Co^{2+} ions to induce stress-associated TNF-α/p38 MAPK/caspase 8/caspase 3 signaling in human leukemia *in vitro* (Jurkat, K562 and KG1A cells) as well as *in vivo*.[942] The authors attributed these responses to ROS generation by increased concentration of free Co^{2+} ions in the TME. As mentioned above, free, ionic Co^{2+} is believed to contribute most to Co's cytotoxic activity (compare 3.5). *In vitro*, at physiological pH, these CoO NP were expected to liberate only little amounts of Co^{2+} ions into the media, thereby exerting lower toxicity to normal cells. CoO NP caused DNA damage in leukemic cell lines, which was reflected by an increase in apoptosis of Jurkat, KG-1A and K562 cells as well as increased production of pro-inflammatory cytokines, primarily TNF-α. A follow-up study investigated the immune-stimulatory potential of the combination of the CoO-based NP with cancer antigens in the *in vivo* situation in Dalton's lymphoma ascites cells.[943] In this setting, peritoneal macrophages were isolated from mice, incubated with cancer cell lysate antigen-conjugated Co NP or, as a control, cancer cell lysate alone *ex vivo* and injected back into the animals. The cancer cell lysate antigen-conjugated NP activated macrophages as determined by increased serum IFN-γ and TNF-α levels. Immunization of mice with the NP further induced anticancer IgG responses together with increased ADCC response as compared to the control group. Furthermore, an enhanced anticancer $CD4^+$ T cell response was reported. The authors suggested that this NP-mediated antigen delivery may improve the anticancer immune response by enhanced macrophage activation, probably based on the combined pro-inflammatory activities of both the delivered cancer antigen as well as the liberated Co^{2+} ions. Hence, a possible antitumor response might be achieved by macrophage-mediated T cell activation via IFN-γ and TNF-α.[943]

4.3.3. Zinc (Zn)

A proteomic approach revealed toxic mechanisms of Zn oxide NP in macrophages.[852] Phagocytosis of these particles triggered responses in mitochondrial proteins, enhanced levels of MyD88 and impaired glucose catabolism by GAPDH inhibition. This led to elevated levels of the cytotoxic metabolite methylglyoxal, and, as a consequence, to methylglyoxal-mediated DNA base modification. Besides representing potentially cancer cell-selective modes of action, these effects may also negatively affect the systemic phagocyte status and impair anticancer immune responses. A study by Yun and colleagues tested the immune response of C57BL/6 mice to Fe oxide-Zn oxide core-shell NP for tumor-specific antigen delivery to DC. Upon subcutaneous injection, NP formed macroscopic depositions leading to granulomatous inflammation and macrophage infiltration at the injection site.[855]

4.3.4. Iron (Fe)

The potential of Fe oxide NP with magnetite (Fe_3O_4) cores for therapeutic application has been widely discussed and reviewed.[944,945] Such NP exhibiting a diameter of less than 20 nm are termed superparamagnetic Fe oxide NP and can be used as contrast agents in magnetic resonance imaging (MRI). Furthermore, they can be coated for conjugation with tumor-targeting moieties.[946]

In 2016, Zanganeh and co-workers discussed the therapeutic effect of the FDA-approved Fe oxide NP ferumoxytol (used for Fe deficiency treatments) on cancer, demonstrating that these NP inhibited cancer growth by inducing a pro-inflammatory immune response with M1 macrophage polarization.[947] The authors first tested ferumoxytol on MMTV PyMT-derived mammary carcinoma cells, proving no direct cytotoxic effects at clinically relevant doses. Interestingly, increased cancer cell cytotoxicity in co-cultures of cancer cells with macrophages was observed, demonstrating that - based on interaction with the macrophages - ferumoxytol induced hydrogen peroxide which then provoked cancer cell death. Next, the impact of ferumoxytol exposure on tumor growth was determined *in vivo*, implanting MMTV-PyMT-derived cancer cells into the bilateral mammary fat pads of female FVB/N mice. Interestingly, increased quantities of pro-inflammatory M1 macrophages were detected in cancers co-injected with ferumoxytol at day 7 after implantation. After further 7 days, tumors treated with ferumoxytol showed a higher quantity of monocytes and TAM as compared to the control group. The authors suggest that these results might have major implications for therapeutic applications of Fe oxide NP.[947] In 2016, Perica and collaborators developed biocompatible Fe-dextran paramagnetic particles (50-100 nm in diameter) with covalently attached dimeric MHC-Ig with an appropriate peptide and chimeric B7.1-Ig fusion protein (or anti-CD28 as alternative) as artificial antigen presenting cells (aAPC). It could be demonstrated that these platforms induced antigen-specific T cell proliferation and functional responses *in vitro* and *in vivo* in a mouse melanoma model. Moreover, these systems were able to induce T cell expansion from TCR transgenic mouse splenocytes with

CTL generation and inhibition of tumor growth *in vivo*.[948] In addition, Zhu et al. demonstrated that Fe-based NP induced the generation of exosomes in the alveolar region of BALB/c mice. These exosomes and their membrane-bound antigen induced APC maturation (DC and macrophages), characterized by elevated expression of MHC class I and II, CD80, as well as the production of the T_H1 cytokines IL-12 and TNF-α.[949]

4.3.5. Gadolinium (Gd)

Recently, Gd endohedral hydroxylated metallofullerene NP ($Gd@C_{82}(OH)_{22}$) have been associated with antineoplastic activity.[950,951] Interestingly, these NP - rather than via direct cytotoxicity - seem to exert their antitumor activity by targeting the microenvironment and promoting immune-activating and anti-angiogenic mechanisms.[952,953] As mentioned in earlier sections (chapter 1.2.2), inflammatory immune responses also act in favour of tumor proliferation and metastasis. In several *in vitro* and *in vivo* experiments, $Gd@C_{82}(OH)_{22}$ NP were demonstrated to trigger immune responses by activating lymphocytes and macrophages.[954,955] $Gd@C_{82}(OH)_{22}$ NP were shown to enhance immunity by induction of lymphopoiesis (new generation of lymphocytes), lymphocyte proliferation, and neutrophil infiltration into tumor tissues. Moreover, in NP-treated animals, lymphoid follicles (folliculus lymphaticus) were observed around implanted tumors. In addition, the authors reported immune-modulatory effects of $Gd@C_{82}(OH)_{22}$ NP on T cells and macrophages.[955] Gd NP were found to induce IL-6 production in human monocyte-derived DC, suggesting a DC maturation-inducing effect. This was confirmed by the observation that the identical NP induced increased production of IL-1β, IL-8, IL-10, IL-12p70, and TNF-α, as well as an enhanced expression of CD83, CD80, CD86, and HLA-class I (HLA-A,B,C) and II (HLA-DR) molecules. When ovalbumin-immunized mice were treated with $[Gd@C_{82}(OH)_{22}]_n$, an enhanced ovalbumin-specific T_H1-polarized immune response was detected. In particular, the Gd NP induced an increased production of IFN-γ, IL-1β, and IL-2.[956] These results demonstrate the immune-activating potential of Gd compounds and encourage in-depth characterization of the (so far elusive) molecular mechanisms of immune cell activation mediated by these compounds.

5. The gut microbiome and the response to anticancer metal drugs

The GI tract comprises various highly specialized biological cell systems that work together in a complex and tightly interconnected manner.[957] In the last years, the role of the gut microbiota (also called microbiome) has come into the focus of attention, demanding reconsideration of various concepts in several fields like pharmacology, immunology, neurology, and cancer biology, including metal drug-based treatment regimens.[86,958-962] The microbiome consists of myriad microorganism species (bacteria, fungi, archaea, protozoa as well as viruses) living on the intestinal epithelial barriers of the host. The intestinal

commensal flora in humans has vital implications with respect to health and survival. These implications include maintenance of barrier homeostasis, symbiotic metabolism, supplying the host with essential nutrients such as vitamins, and regulation of inflammation and immunity.[86,963,964] The microbiota are layered on top of the gut epithelium, which consists of various cell types with unique functions (reviewed in[957]). Cells of the gut epithelium are tighly interconnected with the mucosal immune system, a specialized part of the immune system that has evolved to tolerate presence of luminal microbiota. This immune cell compartment at the same time protects the body against harmful substances and stimuli.[48,957] Both the gut epithelium and the mucosal immune system interact with the enteric nervous system (ENS), the intrinsic nervous system of the gut often referred to as "the second brain".[965] Moreover, the ENS and the mucosal immune system interact with each other both directly and via cytokines and neuropeptides, and both cell types can produce inflammatory cytokines as well as neuropeptides.[197] From an oncological perspective, a major focus of research on the microbiome has been to elucidate the role of gut microorganisms in cancer risk and etiology.[966] This is because the gut microbiota take on central positions in a number of interplays imperative for immunosurveillance that include pro-/antineoplastic metabolism and immune-regulatory and inflammatory mechanisms both on local and systemic levels.[967] However, growing evidence suggests the microbiome to also modulate response to targeted cancer- and immune checkpoint therapies as well as to chemotherapy, affecting key pharmacological aspects: enhancement/abrogation of drug efficacy and modulation of toxicity.[960,968-971] Response to treatment with Pt compounds as well as therapy-associated co-morbidities or even mortalities are highly unpredictable.[972] It is becoming increasingly clear that this pharmacological variability is - at least partially - accounted for by the composition of microbiota. The current understanding of the intimate link between gut bacteria and the pharmacological effects of anticancer drugs has been summarized in several comprehensive reviews.[86,973] This interplay is mediated by highly personalized, but also dynamic shifts in ecological variation, drug metabolization and impairment of gut barrier function, all affecting local and systemic drug efficacy and toxicity. Thus, despite the microbiota-host-drug interactions (also referred to as pharmacomicrobiotics) being poorly understood to date, their intricate interplay is becoming increasingly implicated in anticancer therapy response. Generally, understanding of the influence of microorganisms on anticancer drug efficacy is rudimentary. *In vitro* studies as well as *in vivo* experiments have shown that bacterial drug metabolization may either result in inhibited or enhanced anticancer efficacy, depending on both compound and species of the involved microorganism.[974,975] *In vivo*, mechanistic insights into the influence of the gut microbiome on chemotherapeutic efficacy are so far only available for the DNA-damaging agent cyclophosphamide as well as Pt compounds.

Therapy-induced impairment of gut barrier integrity might lead to translocation of luminal commensal or pathogenic bacteria into the systemic milieu.[976] On the one hand, this is associated with infection-related co-morbidities, in extreme cases even leading to life-threatening sepsis (compare the last paragraph below). On

the other hand, microbe translocation might also impact on chemotherapeutic efficacy on a systemic level by modulation of antitumor immune responses. Compelling evidence for this comes, for instance, from mouse models using the non-metal compound cyclophosphamide, showing that translocation of a specific set of Gram-positive bacteria (e.g. *Enterococcus hilae*) into secondary lymphoid organs was necessary for the antitumor activity of cyclophosphamide against a subcutaneous mastocytoma allograft via the stimulation of antitumor T_H1 and T_H17 responses.[977] Strikingly, antibiotics eradicating Gram-positive bacteria blunted T_H17 responses, intratumoral T cell infiltration and reduced the antitumor activity of cyclophosphamide. A follow-up study conducted by the same group identified a specific Gram-negative microorganism, *Barnesiella intestinihominis*, as another key influencer of cyclophosphamide efficacy by increasing antitumor CTL and T_H1 responses as well as IFN-γ-expressing γδ T cells.[978] Interestingly, *Barnesiella intestinihominis* mediated this effect without translocating through the gut barrier to secondary lymphoid organs. These observations are noteworthy also from a clinical perspective, as presence of *Barnesiella intestinihominis*- as well as *Enterococcus hilae*-specific T_H1 lymphocytes correlates with favorable prognosis in chemoimmunotherapy-treated patients with lung and ovarian cancer.[978]

Interestingly, related findings have been published which concerned the microbiome-dependent anticancer activity of also Pt compounds (Figure 17A). In a work by Iida and colleagues, the anticancer activity of oxaliplatin but also cisplatin against subcutaneous lymphoma allografts in C57Bl/6 mice was impaired by co-administration of an antibiotic cocktail consisting of vancomycin, imipenem and neomycin.[979] Interestingly, the diminutive effect of antibiotics on oxaliplatin and cisplatin efficacy was detectable already two days after treatment. This suggested, besides inflammation-related immune activation following oxaliplatin-induced ICD (compare chapter 2.2.6), also a suppressive effect of microbiome ablation on immediate cytotoxicity. In case of cisplatin, which is generally not considered to trigger complete ICD, this observation suggests that commensals might modulate genotoxicity independently of the immune-stimulatory effects of ICD. In parallel, gene expression alterations in tumor tissue upon oxaliplatin treatment - associated e.g. with monocyte differentiation and activation as well as induction of pro-inflammatory markers - were not apparent in antibiotics-treated mice. Analogously, an independent group found downregulated inflammatory genes (e.g. IFN-γ) in $CD8^+$ T lymphocytes of abiotic mice having received cisplatin chemotherapy.[980] This immunosuppressive effect was reverted by *Lactobacillus acidophilus* inoculation, going hand in hand with enhanced antitumor efficacy. Furthermore, in the study conducted by Iida et al., microbiome ablation led to attenuation of oxaliplatin-induced NOX and ROS-responsive iNOS as well as SOD1 and SOD2 expression.[979] Consequently, the authors identified impaired ROS production in tumor-infiltrating neutrophils and macrophage-like cells of antibiotics-treated mice. As a consequence, the authors could show that myeloid cell depletion as well as ROS inhibition by N-acetyl cysteine (NAC) exerted abrogating effects comparable to that of antibiotics treatment with respect to oxaliplatin activity on tumor growth and animal survival. This might be reflected, despite comparable DNA platination levels, by

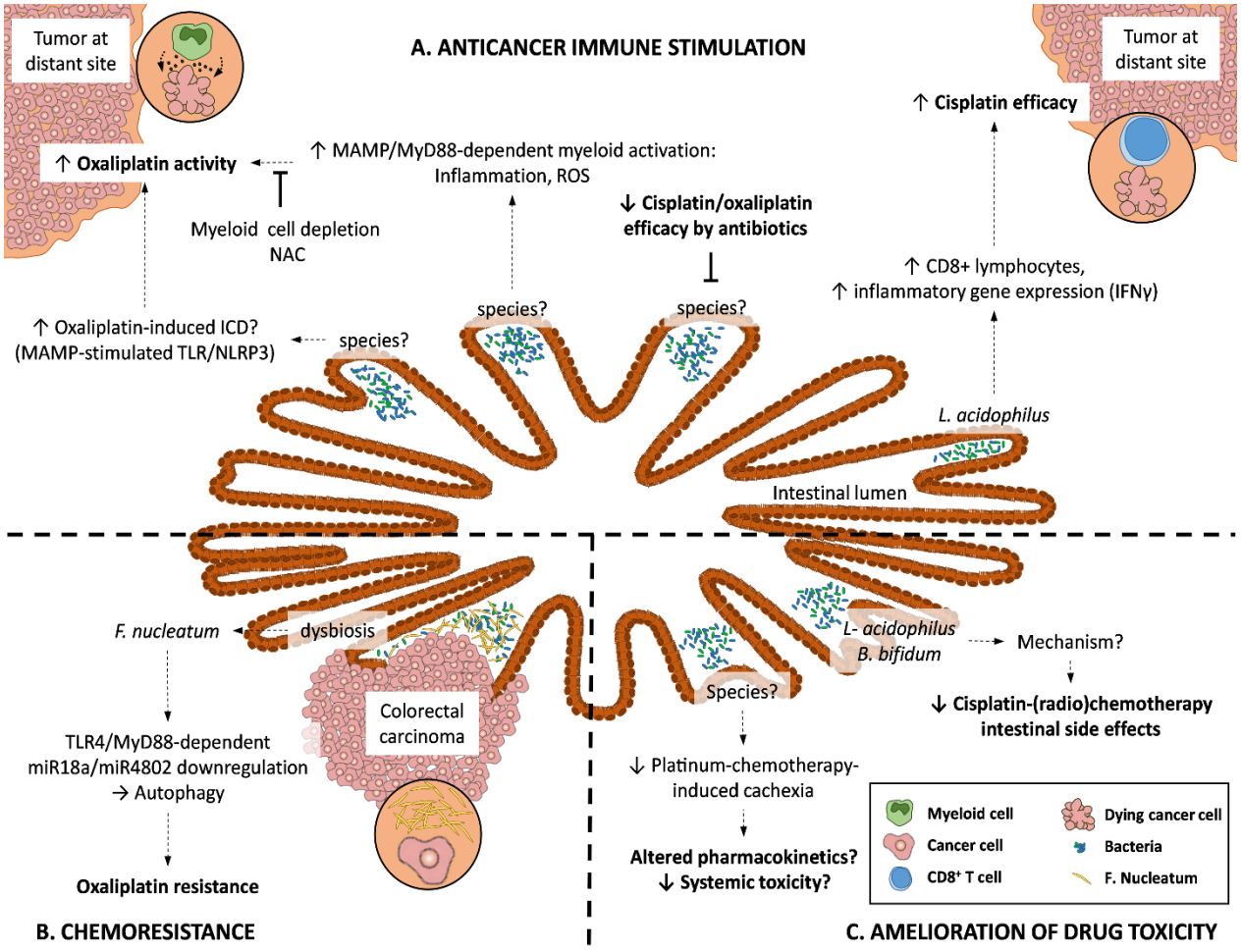

Figure 17. The gut microbiota contribute to various immunological aspects of Pt anticancer drug efficacy and toxicity. (A) Damage to the mucus layer of the intestinal epithelium by Pt drugs may enable commensal bacteria to penetrate through the lamina propria. Translocated bacteria elicit immune responses enhancing antineoplastic activities against cancer cells at sites in the body distant from the intestinal tract. Mechanistically, the mode of systemic immune stimulation by gut microbiota and involved MAMP/immune sensor interactions are poorly understood. Also, bacterial species relevant for this process are widely unidentified. Antibiotics (vancomycin, imipenem, neomycin) have been demonstrated to reduce the antitumor efficacy of cisplatin and oxaliplatin against subcutaneous lymphoma allografts. The gut commensal *Lactobacillus acidophilus* contributes to cisplatin anticancer activity by increasing inflammatory gene expression and CTL responses. Bacterial MAMP (of yet elusive identity) increase oxaliplatin antitumor efficacy in a MyD88-dependent manner by increasing myeloid cell activation and intratumoral ROS production. This effect was reverted by myeloid cell depletion as well as ROS scavenging by NAC. MAMP may amplify oxaliplatin-induced ICD via TLR/NLRP3 activation. (B) *Fusobacterium nucleatum* mediates resistance of colorectal carcinoma against oxaliplatin by TLR4/MyD88-dependent miR18a/miR4802 downregulation, resulting in de-repression of ULK1 and ATG7 and, consequently, autophagy induction. (C) Probiotics supplementation reduces cancer-related or therapy-induced cachexia, potentially altering drug pharmacokinetics and ameliorating systemic toxicity. Reduction of dysbiosis by *Lactobacillus acidophilus* and *Bifidobacterium bifidum* supplementation reduces intestinal side effects of cisplatin-based radiochemotherapy. MAMP, microbial-associated molecular pattern; CTL, cytotoxic T lymphocyte; ROS, reactive oxygen species; NAC, N-acetyl cysteine; ICD, immunogenic cell death; TLR, Toll-like receptor; L. *acidophilus, Lactobacillus acidophilus; F. nucleatum, Fusobacterium nucleatum; B. bifidum, Bifidobacterium bifidum;* For details and references see text.

reduced DNA damage in mice having received antibiotics. Furthermore, mice deficient in the TLR-downstream adapter MyD88 experienced hampered early antitumor responses of oxaliplatin, presumably by impaired myeloid cell stimulation by microorganism-associated molecular patterns (MAMP). As described in chapters 2.2.6 and 3.1.5-3.1.8, oxaliplatin exerts at least part of its anticancer effects via ICD,

involving DC activation via TLR4-binding to HMGB1 released from dying cancer cells as well as on formation of the intracellular NLRP3 inflammasome.[352] The observation that the gut microbiome increased the long-term anticancer effect of oxaliplatin suggests that immune stimulation by MAMP via TLR and NLRP3 engagement acts as amplifying switch for anticancer immune cell activation. Conversely, a study by Shen et al. demonstrated a crucial role for gut microbiota in oxaliplatin-induced neuropathic pain (see chapter 6.3) that was partly mediated by TLR4 signaling of hematopoietic cells.[981] Overall, these observations suggest a key role of microbiotics as primers of antitumor myeloid cell activation and ROS production as well as on the immunogenicity of Pt-induced cancer cell death.

In contrast to the enhancing effects of microbiota on chemotherapy efficacy, cancer-associated microbiome deregulation, leading to the specific loss of some microorganism species and to excessive amounts of others, may also contribute to chemoresistance (Figure 17B). For example, *Fusobacterium nucleatum* is a bacterial species commonly associated with periodontal disease, an inflammatory condition of the tooth gums.[982] Recently, it has been shown that the abundance of this microorganism in the colon shows a direct correlation with the stage of progression of colorectal carcinogenesis.[983,984] Furthermore, high levels of this bacterial species in colorectal cancer tissues have been associated with poor patient prognosis.[985] Importantly, in a comprehensive mechanistic study by Yu and colleagues, this microorganism was demonstrated to cause autophagy-mediated chemoresistance of colorectal cancer cells towards oxaliplatin.[986] Interestingly and in contrast to what might be expected, bacterial drug metabolization was not involved in this effect. Further investigation provided mechanistic insight into the mode of microorganism-mediated autophagy activation in cancer cells: *in vitro* co-culture experiments revealed a *Fusobacterium nucleatum*-dependent upregulation of the autophagy-related genes unc-51-like autophagy activating kinase 1 (ULK1) and autophagy related 7 (ATG7) as well as increased LC3 processing, critical hallmarks of autophagy induction. Consequently, the authors demonstrated a TLR4- and MyD88-dependent downregulation of miR-18a and miR-4802, which target the seed regions within the 3'-untranslated regions (UTR) of ULK1 and ATG7, respectively. Importantly, in clinical tissue samples of a colorectal cancer cohort, the authors additionally found that the amount of *Fusobacterium nucleatum* negatively and positively correlated with miR-18a and miR-4802 levels as well as with ULK1 and ATG7 expression, respectively.[986] Thus, it is feasible that PRR-induced autophagy via selective MAMP stimulation in some cases indirectly causes chemoresistance as a consequence of a cellular antimicrobial defense response. Bearing in mind that in other cases (see above[979]), MAMP-induced TLR/MyD88 stimulation of myeloid cells constitutes an essential contribution to efficient anticancer immune responses upon oxaliplatin treatment, these findings indicate a multifaceted role of microbial TLR agonists with respect to chemotherapy response and that MAMP may cause reciprocal anticancer responses depending on whether PRR stimulation occurs on the cancer cell itself or on cells of the immune compartment.

The gut microbiome plays also a central role in Pt drug-induced adverse effects (compare chapter 6). Chemotherapy-induced damage and increased permeability of the gut barrier, although essential for an effective antitumor immune answer (by facilitating translocation of microbiota into regional lymphoid tissues where they can stimulate an active anticancer immune response), can also lead to excessive translocation of gut bacteria into the blood stream, resulting in increase of cancer-related infections, systemic inflammation and, in the worst case, sepsis, especially in combination with chemotherapy-induced neutropenia.[987] Drug-induced dysbiosis might foster outgrowth of pathogenic bacteria, further exacerbating chemotherapy toxicity.[973] There is, however, some evidence that probiotics can ameliorate Pt-induced side effects (Figure 17C). Chitapanarux et al. showed that supplementation of cancer patients with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* reduced intestinal side effects upon radiotherapy and concomitant cisplatin.[988] This finding, together with the above-mentioned preclinical data that *Lactobacillus acidophilus* enhanced cisplatin-antitumor immunity,[980] suggests this bacterial species to mediate favorable effects on both chemotherapy efficacy and toxicity. Accordingly, Perales-Puchalt et al. showed that cisplatin-induced dysbiosis and mucosal damage could be restored by fecal pellet gavage.[989] Such, dysbiosis and excessive damage to the intestinal mucosa might be therapeutically targeted by supplementation with specific bacterial strains helping to restore the GI balance. According to a model about onset of mucositis by Sonis et al.,[990] relevant mechanisms might include modulation of inflammation and oxidative stress, attenuation of intestinal permeability, maintenance of the mucus layer, stimulation of epithelial repair, and regulation of immune effector molecules.[991]

Of note, the gut microbiota also regulate important physiological processes such as lipid uptake and inflammation of adipose tissue.[86] Energy metabolism and adipose tissue homeostasis are strongly affected in cancer patients, often resulting in cachexia.[992-995] This pathological process may be further aggravated by chemotherapy, for instance with Pt agents[996,997] inducing GI symptoms like nausea and vomiting (compare chapter 6.5). Loss of adipose tissue and muscle waste as a result of cancer progression might additionally negatively affect other, systemic chemotherapy-related side effects due to altered drug pharmacokinetics and increased drug exposure of other tissues. The close relation of the intestinal microbiome with energy metabolism raises the question whether the gut microbiota might play a role in the development of cancer cachexia.[995,998,999] Support for this hypothesis comes from studies demonstrating that supplementation with probiotics had beneficial effects on bodyweight in cachectic mice and human cancer patients.[1000,1001] Consequently, in the future it will be crucial to investigate, whether targeting/modulation of the microbiome homeostasis might depict a feasible approach to ameliorate cancer co-morbidities such as cachexia and therapy-related toxicity.

6. The role of the immune system in adverse effects associated with Pt therapy

6.1. General side effects of Pt compounds

Pt drugs not only target cancer cells but also affect healthy tissue, causing sometimes severe side effects. Nevertheless, it has to be considered that both antitumor and unwanted side effects might be mediated by identical mechanisms, with absence of side effects often indicating lack of antitumor efficacy.[981] The most frequent and dose-limiting toxic effects of Pt compounds comprise nephrotoxicity, peripheral neuropathy, and ototoxicity (damage to the inner ear). The impact of immune reactions on induction of these unwanted side effects is outlined in more detail below in chapters 6.2 and 6.4. Furthermore, hematologic toxicity (as frequently mentioned throughout this review), as well as hepatotoxicity and cardiotoxicity (including myo- and pericarditis), GI tract toxicity (compare chapter 5 and chapter 6.5), and hypersensitivity reactions (HSR), all involving immune reactions, can pose serious problems,[412,1002] however, a detailed description of all aspects is beyond the scope of this review. Generally, cisplatin is the most toxic compound (especially to kidney, nervous system, and inner ear), whereas carboplatin shows less severe adverse effects, with the main dose-limiting factor being myelosuppression.[1002,1003] For oxaliplatin, the toxicity pattern observed slightly differs from that of cis- and carboplatin. The most frequent severe adverse effect of oxaliplatin is sensory peripheral neuropathy, whereas ototoxicity is a comparably rare event, and nephrotoxicity, like in case of carboplatin, seems to be absent at conventionally applied doses.[412,1004]

Hematologic toxicity of Pt compounds affects the bone marrow and blood cell production. Myelosuppression, the main dose-limiting effect of carboplatin, mainly manifests clinically as thrombocytopenia and neutropenia.[1002,1005] Myelosuppression upon application of oxaliplatin as single agent is not as severe as reported for carbo- and cisplatin, however, in all cases, thrombocytopenia and neutropenia represent the most dangerous complications.[1005] Interestingly, it should be mentioned here that platelets, although not classically attributed to the immune system, have prominent roles in promoting tumor growth and metastasis (reviewed in[1006]). Moreover, platelets have been shown to suppress $CD4^+$ and $CD8^+$ T cells via secretion of TGF-β and, at the tumor site, via expression of TGF-β-docking receptor glycoprotein A repetitions predominant (GARP).[1007] A recent study demonstrated overexpression of CD97 in tumor cells that stimulated activation and ATP release of platelets, promoting disruption of endothelial junctions. Additionally, tumor cell migration was fostered by platelet-derived lysophosphatidic acid (LPA), via CD97-LPA receptor signaling.[1008]

GI tract toxicities frequently occur, including diarrhea, constipation, nausea and vomiting (the latter fortunately widely manageable by administration of antiemetic drugs). However, if severe and long-lasting, these reactions together with effects caused by damage to the microbiome can also contribute to cachexia (compare chapter 5).

HSR can be life-threatening, involving several types of allergic reactions as well as the cytokine release syndrome.[1009] A more detailed description of Pt-induced HSR is provided by[1009-1012]. It should be mentioned

that thrombocytopenia can also arise in frame of HSR, in some cases based on formation of autoantibodies.[1010] HSR occur only in a small percentage of patients upon first drug administration, however, the risk increases with repetitive Pt drug application and, hence, can raise problematic situations for patients with recurrent disease.[1012]

Finally, also in case of Pt drug-induced hepato- and cardiotoxicty, ROS generation and inflammation have been described as major contributing mechanisms.[1013-1015]

In the following, the contribution of immunological factors to the most frequent, dose-limiting adverse effects of the clinically approved Pt drugs will be shortly outlined, with a focus on effects on kidneys (chapter 6.2), the nervous system (chapter 6.3), the inner ear (chapter 6.4), as well as the GI tract (chapter 6.5, compare also chapter 5).

6.2 Nephrotoxicity

One of the major dose-limiting side effects of Pt agents, majorily of cisplatin, is nephrotoxicity.[411,412] Clinical manifestations include the dangerous acute kidney injury (AKI) in 20-30% of patients, hypomagnesemia (in 40-100%), as well as Fanconi-like syndrome, distal renal tubular acidosis, renal concentrating defect, thrombotic microangiopathy, glucose intolerance, and others.[411,1016] The toxic effects of cisplatin have recently been further comprehensively reviewed by Manohar and Leung.[1016] In mice, administration of cisplatin reliably results in kidney inflammation, recapitulating many features encountered in human AKI. As a consequence, this is being exploited as experimental model to study biological mechanisms underlying kidney damage. Cisplatin is not only freely filtered from the blood, but also actively secreted into the urine. Two transporters on the basolateral side of proximal and distal renal tubular cells are mainly involved in the transport of cisplatin for subsequent excretion into the tubular lumen. These are copper transport protein 1 (Ctr1) and organic cation transporter 2 (Oct2).[1017,1018]

Besides direct cytotoxic effects in renal cells, cisplatin has been found to exert inflammatory effects in the kidney.[1016] Renal cell damage is believed to result in DAMP release. TLR activation on immune cells may trigger the release of cytokines like TNF-α and chemokines, leading to the recruitment of inflammatory cells.[1019,1020] This model is substantiated by TLR4-deficient mice, showing lower cytokine (e.g. TNF-α) levels after cisplatin treatment in the kidneys as compared to wildtype mice, going hand in hand with decreased renal damage.[1021] This suggests the DAMP/TLR4/TNF-α axis to contribute to renal injury.[1021] In contrast, IL-10 may have a protective role, decreasing inflammatory responses following cisplatin treatment.[1022,1023]

Hints for an immune cell type-specific role of the TLR4 axis in promoting renal inflammation, also involving NF-κB, were found for M1 macrophages.[1024] In these cells, NF-κB binds to the promoter region of a PRR termed macrophage-inducible C-type lectin (Mincle). The pro-inflammatory propagation of M1

macrophages was demonstrated to be promoted by Mincle and its downstream signaling effector Syk. This cascade induced expression of IL-1ß, MCP-1 and iNOS to promote renal inflammation. Importantly, Mincle-expressing macrophages were found to be increased in a cisplatin-induced AKI model.[1025] In line with this, adoptive transfer of Mincle-positive M1 macrophages exacerbated renal inflammation. Furthermore, TLR- and inflammasome-dependent IL-17A expression, likely by neutrophils and NK cells, has also been demonstrated to mediate cisplatin-induced nephrotoxicity in mice.[362] Chan et al. have shown that concomitant depletion of neutrophils and NK cells by neutralizing antibodies (directed against Ly6G and NK1.1, respectively) had similar protective effects on renal tissue as IL-17A depletion. Investigation of other immune cell types potentially mediating IL-17A-dependent renal inflammation revealed no contribution of at least $CD4^+$ T and γδ T cells. Interestingly, the same group showed in a follow-up report that TLR9 on intrarenal T_{reg} cells downmodulated cisplatin-associated AKI.[1026] In addition, independent studies have confirmed the renoprotective effects exerted by T_{reg} cells.[1027-1029] Intriguingly, in 2010, also renal DC were found to ameliorate cisplatin toxicity.[1030] Illustratively, the authors demonstrated that depletion of DC induced renal dysfunction, tubular injury, and neutrophil infiltration, going hand in hand with increased mortality upon cisplatin treatment. This suggests that - besides "classical" T cell-stimulating activity based on DAMP release - renal DC exhibit yet another, immune-regulatory component necessary for the integrity of Pt-exposed tissue. Corroboratively, cisplatin decreased MHC class II (but not MHC class I) expression on renal DC, suggesting decreased DAMP presentation via this complex as immune-tolerogenic mechanism towards cisplatin-induced renal cell damage. Furthermore, the role of T cells in Pt-based kidney injury was investigated.[1031] In mouse models, depletion of T cells, especially of $CD4^+$ cells, attenuated cisplatin-induced elevation of renal TNF-α and IL-1ß levels, accompanied by reduced renal dysfunction, tubular injury and improved animal survival. This suggests a contribution of $CD4^+$ T cells to kidney inflammation following cisplatin treatment. Another kidney-specific toxic effect of cisplatin is renal interstitial fibrosis.[1032] Yamate et al. demonstrated a role of macrophages in the development of fibrotic lesions in nephrons, showing dilated ducts in the corticomedullary junction of the kidneys.[1033] The authors demonstrated enhanced macrophage infiltration in this region, together with increased MHC class II display and further suggested a role of TGF-β1 and TNF-α in the process of renal fibrosis.[1032,1033]

Consequently, various immune-inhibiting strategies have been devised to reduce inflammation-mediated nephrotoxicity. For instance, salicylate has led to reduced TNF-α serum levels in cisplatin-treated mice.[1034] However, the feasibility of this approach is complicated by the need for high doses as well as by the risk of renal dysfunction by this treatment *per se*. TNF-α induction by cisplatin in the kidneys has been shown to be dependent on NF-κB.[1035] NF-κB, in turn, acts via ICAM-1 to attract inflammatory cells. With the attempt to reduce immune cell infiltration, alpha lipoic acid reduced ICAM-1 expression on endothelial cells and NF-κB signaling.[1035] Also, inhibition of TNF-α, TLR4 and p38 MAPK signaling have been investigated to prevent the propagation of inflammation.[1021,1036,1037] Further strategies to prevent nephrotoxicity have been

attempted with JNK inhibitors to abrogate cisplatin-induced ROS-mediated cytokine production. Furthermore, PPAR-α ligands have been used to reduce cytokine expression in kidney cells via NF-κB inhibition.[1038,1039] Poly (ADP-ribose) polymerase (PARP) inhibitors reduced oxidative stress pathways (MAPK, TNF-α, NF-κB).[1040] With regard to the latter strategy, enalapril - an inhibitor of angiotensin-converting enzyme (ACE) - has been demonstrated to bear renoprotective potential by inhibition of PARP and subsequent MAPK/TNF-α/NF-κB-mediated inflammation and apoptosis.[1040] Analogously, amelioration of carboplatin-induced nephrotoxicity has been found by the angiotensin II receptor antagonist candesartan in combination with the anti-inflammmatory/antioxidant substance coenzyme Q10, accompanied by reduced levels of TNF-α and IL-6 in the kidneys.[1041] Regarding the protective role of T_{reg} on cisplatin-induced renal inflammation, Stremska and colleagues attributed this effect to IL-2 and IL-33.[1042-1044] Consequently, this group created a hybrid of IL-2 and IL-33 (termed IL-233), which increased T_{reg} numbers in blood, spleen and kidneys and suppressed $CD4^+$ T cell proliferation.[1042] Application of this hybrid cytokine protected mice from cisplatin-induced nephrotoxicity. In another experimental mouse model, the immune-modulatory effects of mesenchymal stem cells (MSC) with regard to renoprotection have been investigated.[1045] Interestingly, intraperitoneal application of MSC attenuated cisplatin nephrotoxicity, with decreased levels of TNF-α, IL-17 and increased levels of IL-10, IL-6, NO, and kynurenine in the blood. Intraperitoneal MSC transplantation decreased renal infiltration of macrophages, DC, neutrophils, $CD4^+$ T_H cells, and CTL, as well as attenuated the proinflammatory activity of DC, $CD4^+$ T_H cells and CTL in the kidneys. Interestingly, intraperitoneal injection of MSC-conditioned media exerted similar effects, suggesting soluble paracrine effector molecules, especially iNOS, to promote a systemic switch, tolerizing immune cells towards cisplatin-induced kidney damage and, thus, limiting renal inflammation.

In conclusion, the picture emerging from current data suggests the incorporation of adjuvant anti-inflammatory immune modulators into Pt-containing regimens to ameliorate Pt-induced kidney inflammation. However, it will also be crucial to more comprehensively investigate the immunological mechanisms underlying Pt-induced kidney injury in order to devise therapeutic strategies that reduce renal inflammation without compromising potent antitumor immune activation.

6.3. Peripheral neurotoxicity

Peripheral neuropathies represent the most common neurologic adverse effects of Pt compounds. In contrast, central neurotoxicity leading e.g. to encephalopathy, aphasia, or seizures is rare.[1002,1046]

Chemotherapy-induced peripheral neuropathy (CIPN) is a common side effect of several chemotherapeutics including Pt compounds. It is characterized by pain sensations and/or a highly increased sensitivity for stimuli that normally are not painful (like burning or electric shock-like pain; mechanical/thermal allodynia, hyperalgesia) and sensory symptoms (numbness, paresthesia, dysesthesia, altered touch sensations, in severe

cases loss of sensory perception), usually starting at the periphery and developing in a “glove and stocking” pattern.[1047,1048] Symptoms develop dose-dependently and increase with the total accumulating dose.[1049,1050] Especially oxaliplatin and cisplatin can induce CIPN,[1051,1052] whereas in case of carboplatin, these effects are only seen in a very low percentage of patients or upon administration of particularly high doses.[1052] Pt drug CIPN not always resolves post treatment and can become chronic or even worse (“coasting”). Only with oxaliplatin, about 90% of patients suffer acute neuropathy, starting during or shortly after chemotherapy administration and resolving again within a week, that is characterized by presence of cold-induced neuropathy (cold allodynia and hyperalgesia).[1047,1053]

While the exact interrelations are still unclear, evidence for a substantial contribution of inflammatory and neuroinflammatory processes to CIPN increases.[1048,1052,1054,1055] However, most data are derived from studies on tumor-free rodent models, with sometimes inconsistent outcome due to different animal models, drug dosages, and time point measurements. Other mechanisms contributing to CIPN comprise nuclear and mitochondrial DNA damage, oxidative stress,[1051] altered Na^+-ion channel activity and calcium homeostasis,[1048,1056] altered responsiveness of transient receptor potential ion channels,[1052] axon degeneration,[1057] as well as individual genetic variations[1058,1059].

Pt drugs preferentially target neurons of the dorsal root ganglia (DRG) and their long axons.[1053,1054,1057] These cells, located just outside the spinal chord (but inside the spinal column), are not shielded by the blood-brain-barrier and are vascularized by a fenestrated capillary network, making them easily accessible for Pt drugs.[1047,1054] Generally, proinflammatory signals arise upon peripheral or central nerve damage**,** changing nociceptive function and processing and leading to induction of pain.[1060,1061] In line with this, one study in human breast cancer patients found higher levels of IL-6 and soluble IL-6 receptor in patients with painful CIPN as compared to patients without CIPN.[1062] Pt drugs, in addition to their role in immune cell recruitment and activation, can interfere with and change the activity of glial and myeloid cells of the nervous system (microsatellite glial cells and Schwann cells in the peripheral nervous system (PNS); microglia, which are the macrophages of the central nervous system (CNS), and astrocytes in the CNS).[1054]These cells can then start to proliferate, undergo morphology changes, and release - like immune cells - proinflammatory signals (e.g. cytokines like IL-6, IL-1β, TNF-α), affecting the sensory neurons of the DRG and the dorsal horn of the spinal chord[1048,1054,1061,1063] as well as special areas in the thalamus and cortex involved in nociceptive processing (“pain matrix”).[1054] Glial and neural cells also release ATP, recruiting monocytes and microglia and inducing production of proinflammatory cytokines like IL-1β.[17,1054] Extracellular ATP, also released from damaged (cancer) cells (compare chapter 2.2.6), is a potent mediator of neuropathic pain.[1060] In mice with cisplatin-induced hyperalgesia, ablation of TLR3, TLR4, or MyD88 attenuated neuroinflammation and neuropathic pain.[1064] In addition, Das et al. reported induction of neuroinflammation and allodynia by HMGB1 binding to TLR5 and subsequent NFκB pathway activation that could be reduced upon inhibition of TLR5.[1065] Thus, various features like HMGB1 release and TLR signaling associated with Pt drug-induced

cancer cell damage (outlined in chapter 3.1.6 and 3.1.7) might also strongly contribute to neuroinflammation and neuropathy.

Reports on Pt-induced effects on the PNS include increased glial fibrillary acidic protein (GFAP) expression and GAP junction-mediated coupling in satellite glial cells (covering the neuron bodies of DRG neurons) upon treatment with oxaliplatin.[1054,1066] In the sensory ganglia of several cisplatin-treated patients (analysed post mortem) hypertrophy of satellite glial cells caused by neuronal damage led to formation of nodules of Nageotte .[1067] In the DRG, upon treatment with oxaliplatin, one study showed increased macrophage activity and IL-1β production,[1068] whereas, in contrast, no change in macrophage number was seen in another study[1069]. In addition, a role of mast cells in oxaliplatin CIPN has been reported.[1070]

No infiltration with adaptive immune cells (T or B cells) of the DRG (PNS) or spinal chord (CNS) could be detected in oxaliplatin-induced neuropathy, despite activation of glial cells of the spinal chord.[1069,1071] In the CNS, oxaliplatin CIPN was linked to activation of astrocytes and microglia and production of proinflammatory cytokines in the spinal chord.[1048,1052,1054,1055,1072,1073] Also in the brain, oxaliplatin induced activation of microglia and astrocytes.[1054] Data are less clear with regard to the exact role of these two cell types in oxaliplatin-induced mechano-hypersensitivity (allodynia and hyperalgesia). Di Cesare Manelli et al. showed initial microglia activation in the dorsal horn of the spinal chord following treatment of rats with oxaliplatin that was reversed at a later time point. In contrast, astrocyte activation was elevated both at early and late time points. Neuropathic pain was attenuated by application of minocycline (inhibiting microglia activity) as well as fluorocitrate (inhibiting selectively astrocytes), with a stronger effect observed for fluorocitrate. Astrocytes stayed activated even in minocycline-treated animals, suggesting a microglia-independent activation of astrocytes.[1074] The crucial role for astrocyte (but not microglia) activation in oxaliplatin CIPN together with increased production of pro-inflammatory cytokines (TNF, IL-1β) and reduction of anti-inflammatory and neuroprotective cytokines (IL-10, IL-4) in the spinal chord was corrobated by another study in rats.[1071] In contrast, another study reported no elevated levels or increased activation of astrocytes or microglia in the spinal chord of oxaliplatin-treated mice, except for a reduction of P2ry12^{+} homeostatic microglia.[1069] CIPN symptoms described in the above-mentioned reports could be attenuated by targeting several receptors interfering with inflammatory processes in the nervous system like e.g. A3 adenosine receptor (A3AR),[1071] or nicotinic receptors.[1074,1075] AR are G-protein-coupled receptors widely expressed on neural and glial as well as immune cells with strong, mostly cytoprotective function.[1076,1077] Also, boosting of T_{reg} cells via bee-venom-derived phospholipase A2 successfully attenuated oxaliplatin-induced neuropathic pain[1068] (this strategy also reduced cisplatin-induced nephrotoxicity), whereas total depletion of T_{reg} cells did not aggravate oxaliplatin CIPN symptoms, despite reduction of T_{regs} in the lymph nodes.[1074] In case of cisplatin CIPN, long lasting microglia but not astrocyte activation in the spinal chord was observed in a mouse model, together with elevated levels of IL-1β, IL-6, TNF-α, iNOS, and CD16 (FcγRIIIa and b).[1078]Cisplatin enhanced receptor expressed on myeloid cells 2

(TREM2)/DNAX-activating protein of 12 kDa (DAP12) signaling in the microglia of the spinal chord. Conversely, TREM2 blockade attenuated cisplatin-induced neuropathy, reduced spinal IL-6, TNF-α, iNOS, and CD16, and induced production of the anti-inflammtory cytokines IL-4 and IL-10 and the anti-inflammatory microglial CD206 (in humans: macrophage mannose receptor).[1078]

6.4. Ototoxicity

Ototoxicity is a frequently observed side effect of cisplatin, followed by carboplatin (but rarely seen with oxaliplatin).[1003,1079,1080] Pt-induced ototoxicity is mainly based on increased oxidative stress via mitochondrial dysregulation and ROS production together with depletion of the cellular antioxidant defense system as well as induction of pro-inflammatory signals.[1081,1082] As in case of peripheral neuropathy, the exact mechanisms and order of cellular signaling events are unclear, and few reports with comparable settings exist. Moreover, the vast majority of studies is performed in tumor-free animals, making evaluation of the clinical relevance of these findings necessary.[1082]

Generally, the inner ear was - like the CNS - long considered an “immune-privileged” site, lacking active immune responses.[1082,1083] Comparable to the blood-brain-barrier in the CNS, the inner ear compartments are shielded by the blood-labyrinth-barrier, providing homeostasis and integrity of the inner ear fluid system (endolymph and perilymph).[1084,1085] More recently, in addition to recruitment of blood-derived monocytes/macrophages upon damage to the inner ear cells, a special population of resident immune cells/macrophages in the cochlea was identified (reviewed in[1083]). The precise role of these resident immune cells is not clear yet. One of their suggested functions is a protective role against immune-mediated auditory cell damage by regulating infiltration of $CD45^+$ immune cells via CX3CR1-CX3CL1 signaling.[1082,1086]

Most affected by Pt-induced ototoxicity are the outer hair cells (OHC) in the organ of Corti, but also cells of the stria vascularis, spiral ligament tissue and the spiral ganglion.[1087] Hearing loss typically starts at high frequencies (targeting the OHC at the basal turn of the cochlea) and progresses into the lower frequencies.[1088] Additionally, tinnitus and vertigo can occur.[1003] Symptoms depend on dose, administration route, and treatment duration.[1003] Risk factors comprise impaired renal function, age (with children having a higher risk[1089]), simultaneous cranial irradiation, co-medication with other oto- or nephrotoxic drugs, prior hearing disability, and several genetic variations (e.g. LRP2 (megalin), gluthation S-transferase, SLC22A2 (OCT2)). Moreover, gender-related differences have been reported, with males being more likely to be affected than females.[1003,1088-1090] Furthermore, a high melanin content has been associated with increased risk for Pt-induced ototoxicity, based on interaction of Pt with melanin in the melanocytes of the inner ear that might cause retention of the drug.[1091] To date no clinically approved compounds for otoprotective strategies exist, mainly due to simultaneous reduction of antitumor efficacy of Pt drugs or requirement of invasive

administration procedures.[1088] Efforts include application of antioxidant and anti-inflammatory agents, as well as local inhibition of cisplatin uptake transporters CTR1 and OCT2.[1082,1088,1090]

Suggested mechanisms underlying cisplatin-induced inflammatory processes and ototoxicity are described by mainly two groups: So and Park discuss induction of pro-inflammatory cytokine release and ROS, as well as signaling via MAPK, NF-κB, STAT6, and TLR4.[1087,1092-1096] Ramkumar and colleagues focus on NOX3-mediated ROS production[1081,1088,1097-1099] and STAT1 activation,[1099-1102] as well as on central roles of several receptors found expressed in the cochlea like A1AR[1102-1104] and transient receptor potential vanilloid 1 channel (TRPV1).[1097,1098,1105] Furthermore, a role of the cannabinoid 2 receptor (CB2)[1092] is discussed.

With regard to the group of So and Park, several studies both *in vitro* using the House Ear Institute-Organ of Corti 1 (HEI-OC1) mouse auditory cell line and *in vivo* emphasize pro-inflammatory cytokine (TNF-α, IL-6, and IL-1β) and ROS production, as well as altered MAPK, NF-κB, and STAT6 signaling as most important mediators of cisplatin-induced ototoxicity.[1087,1093-1095] The authors suggest cisplatin-induced proinflammatory cytokine production and downstream pathway activation to induce ROS and subsequent cell death.[1093,1094] Conversely, inhibition of ERK/MEK or TNF-α,[1094] suppression of cytokine production via Nrf2/HO-1,[1093] or via knockdown of STAT6[1095] counteracted cisplatin-mediated inflammatory effects and protected against ototoxicity. Interestingly, pro-inflammatory cytokine production was suggested to be directly mediated by several cell types of the cochlea, like spiral ligament fibrocytes and/or the auditory cells of the Corti organ, rather than by infiltrating lymphocytes/macrophages.[1087,1094] Cisplatin treatment also stimulated TLR4 expression in HEI-OC1 cells and organ of Corti explants, and cisplatin-LPS interaction induced production of TNF-α, IL-6, and IL-1β via NF-κB pathway activation. Moreover, combination of cisplatin and LPS induced severe hearing loss, enhanced expression not only of TLR4 and proinflammatory cytokines, but also of HMGB1, RAGE, advanced gycation end-product (AGE), COX-2, iNOS, and NOX in the cochlear tissues of mice. In turn, hearing loss was reduced in TLR4 mutant or knockout mice, arguing for a prominent role of TLR4 in cisplatin-induced inner ear damage that might even become drastically enhanced in the presence of infections frequently occurring in immunocompromised patients.[1087]

With regard to STAT signaling, Schmitt et al. demonstrated upregulation of STAT1 in a mouse model upon cisplatin administration, and hair cells of STAT1-deficient mice were resistant against cisplatin-induced damage.[1100] Moreover, the STAT1 inhibitor and anti-oxidant epigallocatechin-3-gallate (EGCG) protected against cisplatin-induced inner ear damage[1100] and attenuated hearing loss by downregulation of ERK1/2 and STAT1 signaling, leading to reduced ROS formation.[1101] Interestingly, anticancer effects of cisplatin were not affected by EGCG both in human cancer cells *in vitro* and in a mouse xenograft model, based on cisplatin-mediated downregulation of STAT3 that was not counteracted by EGCG.[1101] NOX3, a member of the NADPH oxidases expressed predominantly in the inner ear and readily stimulated by cisplatin, promotes ROS formation and might represent a further central player in cisplatin-induced ototoxicity.[1088] Several studies from the group of Ramkumar demonstrate that NOX3-mediated STAT1 activation, via ROS

production, induces expression of proinflammatory signals in the cochlea.[1081,1097-1099,1102,1106] Cisplatin induced STAT1 activation and transcription of downstream genes TNF-α, iNOS, and COX2 both in mouse immortalized organ of Corti cells (UB/OC-1) and *in vivo*, mediated via activation of NOX3 and ROS production. Accordingly, STAT1 siRNA suppressed inflammation and apoptosis, and STAT1 siRNA or the TNF-α inhibitor etanercept reduced hearing loss *in vivo*.[1099] Immunohistochemistry of the inner ear following cisplatin treatment showed increased immunostaining for TNF-α and CD14 (a monocytic marker), which seemed to be co-localizing. Comparable to the observations made by the group of So and Park[1087,1094], the authors did not observe strongly localized or discrete labeling for immune cell infiltrates but rather distribution in a diffuse manner throughout the cells of the cochlea. This and the observation that UB/OC-1 cells can be stimulated to express CD14 and CD45 *in vitro* suggest that pro-inflammatory markers are at least partly produced by cochlear cells (including resident macrophages) themselves.[1099] Furthermore, cisplatin also stimulated TRPV1 (a non-selective cation channel involved in nociceptive signaling and processing in the nervous system[1107] also expressed in the cochlea[1108]) and NOX3 in a ROS-dependent manner. Activation of TRPV1 by cisplatin induced (whereas blockade of TRPV1 or NOX3 inhibited) apoptosis and showed otoprotective effects in UB/OC-1 cells as well as *in vivo*.[1097,1098] Another receptor, the A1AR, is expressed on the inner hair cells of the cochlea that - in contrast to the OHC - are more resistant to cisplatin-induced cell damage.[1102,1106] A1AR was found upregulated upon cisplatin treatment, and adenosine agonists were able to protect against oxidative stress by increasing endogenous antioxidants.[1103] A1AR agonists reduced cisplatin-induced hair cell damage and hearing loss by decreasing the malondialdehyde levels (indicative for lipid peroxidation) in the cochlea.[1104] Application of an A1AR agonist suppressed ROS-induced inflammation via NOX3 and inhibition of (MAPK-mediated) STAT1 signaling and led to reduced levels of TNF-α, iNOS, and COX-2.[1102] Finally, also stimulation of CB2 was shown to inhibit cisplatin-induced apoptosis (assessed by caspase 3, 8, and 9 activation, PARP cleavage, and cytochrome c release), modulate MAPK pathway, reduce ROS production, as well as levels of TNF-α in HEI-OC1 cells.[1092]

As mentioned in chapter 1.2.2, caspase 1 is necessary for formation of the NLRP3 inflammasome. Accordingly, inhibition of caspase 3 and caspase 1 in rat organ of Corti explants rescued a high perecentage (80%) of hair cells from cisplatin-induced cell death. In addition, inhibition of NF-κB reduced caspase 3 activation and apoptosis in cisplatin-treated HEI-OC1 cells.[1081,1096]

6.5. Gastrointestinal tract toxicity

Pt compounds all exert GI tract toxicity, manifesting as nausea, vomiting, and dysmobility (diarrhea and constipation). As described in chapter 5, the GI tract is a highly specialized and complex system whose function depends on the interplay between the gut microbiota, intestinal epithelial cells, the gut-associated

mucosal immune system as well as the ENS. Nausea and vomiting, occurring most frequently and severe upon treatment with cisplatin, can be caused by damage to enterochromaffin cells, leading to release of serotonin and binding to 5-hydroxytryptamine type 3 (5--HT_3) receptors. Consequently, blockade by application of 5-hydroxytryptamine type 3 (5-HT_3) receptor or neurokinin-1 receptor antagonists effectively can suppress these symptoms.[412,1002] GI tract dysmobility leading to diarrhea and/or constipation can generally be caused by multiple reasons including tissue damage due to drugs or inflammation, secretory and osmotic dysfunction, and malabsorption.[1109] With regard to inflammatory reactions, the role of the gut microbiota seems to be of central importance. As already described in chapter 5, damage to the microbiota and breaching of the intestinal barrier promotes cancer-related infections and in severe cases leads to systemic inflammation and sepsis.[987] Microbiota additionally can mediate changes in metabolism, which in combination with nausea and GI motoric problems can promote cachexia (compare chapter 5).

Another axis important for Pt-induced GI tract toxicity might involve damage to the ENS.[197] This assumption is based on the observation that GI motor dysfunctions can persist up to 10 years post treatment with Pt drugs,[1110] as well as on several reports demonstrating enteric neurotoxicity induced by cis- and oxaliplatin.[1111-1113] Damage to the ENS might be either exerted directly by Pt drugs or by inflammation, inducing hypertrophy of neurons and neurodegeneration.[1114] As both ENS and gut-associated immune system are tightly interconnected, also the ENS may play a role in modulating GI immunity. Both systems have been shown to interact with each other directly and via cytokines and neuropeptides. Moreover, enteric neurons and gut-associated immune cells can both reciprocally produce and detect cytokines and neuropeptides so that each system can regulate and modulate the function of each other (reviewed in[197]). However, the exact role of Pt drugs in this interplay is still widely unexplored and needs to be specifically addressed.

7. Secondary immune-related cancers following anticancer metal drug therapy

For potentially curable patients who have received genotoxic regimens, late toxicity and particularly secondary cancers represent an important topic. Several publications in recent years have reported on secondary, treatment-associated hematological malignancies following therapy of various primary cancer types with metal-based anticancer agents.[1115-1117] Among these, a number of case reports were documented for Pt-based chemotherapy and ATO, but also for the radiotherapeutic compounds yttrium-90 (^{90}Y), strontium-89 (^{89}Sr) and ^{223}Ra. As cancer patients are frequently treated with combination therapies, the occurrence of hematopoietic malignancies such as leukemias or lymphomas raises the question, whether these secondary diseases are caused by just one, or rather more cytotoxic compounds.[1118,1119] In general, 10-20% of myelodysplastic syndromes (MDS) and acute leukemias are estimated as consequence of genotoxic anticancer therapy.[1120,1121]

Most cases of secondary MDS arise following chemotherapy of primary cancers with alkylating agents or Pt compounds.[1122,1123] The typical latency period for secondary MDS after exposure to alkylating agents or Pt drugs is 5 to 7 years with the relative risk depending on the dose of initial therapy. Long-term prognosis of MDS is poor.[1124,1125] As increasing numbers of malignancies become more successfully treated with Pt-based chemotherapy, the rate of secondary, Pt-related MDS is anticipated to increase. Two studies have explored the rates of secondary MDS and acute myelogenous leukemia (AML) among patients with non-Hodgkin's lymphoma who have undergone autologous bone marrow transplantation.[1126,1127] In both series, the rates of secondary MDS/AML approached 7% at 10 years, or 20% at 20 years of follow-up. All of these patients had received, amongst others, Pt drugs. Case reports also describe MDS development after treatment of other cancer types, such as breast cancer.[1128-1130]

Regarding secondary leukemias, the risk for developing such disease is highest between 24 and 72 months following cytotoxic therapy, with a steady decline in incidence thereafter.[1131] Of those patients developing secondary leukemia, approximately 6% do so within the first year, whereas in 15%, the latency period exceeds 7 years.[1131] In 2016, a study assessed the risk of secondary (not further specified) leukemia after treatment of pediatric solid tumors with chemo-radiation, including, amongst others, Pt agents.[1132] This report demonstrated that Pt compounds (administered at doses $\geq 2mg/m^2$) are associated with a 5.6-fold increased risk for secondary leukemia. Here, the highest risk appeared to occur for carboplatin, with a relative risk of 29.1.[1132] Similarly, another case-control study reported an increased risk of secondary AML and MDS following Pt-based treatment of childhood tumors.[1133] Cisplatin and carboplatin have been widely associated with therapy-related leukemia.[1134,1135] In contrast, for oxaliplatin, an etiological role in secondary hematological diseases has been suggested only in several case reports.[1120] In 2002, two cases of cisplatin- and carboplatin therapy-associated APL, a subtype of AML, have been observed.[1136] Carboplatin might also be implicated in the development of secondary acute erythroid leukemia.[1137] A further case report described AML in a metastatic colon adenocarcinoma patient following a FOLFOX (leucovorin, 5-FU, oxaliplatin) treatment regimen.[1138] In 2006, an independent case of secondary APL following irinotecan and oxaliplatin therapy for advanced colon cancer was observed. However, it remained unclear which of the two drugs contributed to the development of this secondary malignancy.[1139] It needs to be mentioned that the karyotype (the pattern of chromosomal aberrations) was distinct from that reported by Carneiro et al., arguing, in that case, for a causal role of irinotecan in APL development. In 2015, Zhang reported a patient with therapy-associated APL following oxaliplatin/capecitabine chemotherapy for gastric cancer.[1140] Furthermore, treatment of esophageal cancer has been associated with secondary AML after oxaliplatin-[1141] as well as with several hematological malignancies (including MDS, AML and Burkitt leukemia) following nedaplatin-based chemo-radiation.[1142] Another case of AML in a patient with cervical carcinoma developed 63 months following Pt-based chemo-radiation treatment.[1117] Although there are few reports that link oxaliplatin to therapy-related leukemia (TRL), when considering the widespread use in GI malignancies

these incidences cannot be neglected. However, further understanding the mechanisms involved in oxaliplatin-mediated hematological malignant transformation will be imperative to develop measures to minimize this severe health risk.

In contrast to the relatively frequently occurring chemotherapy-related AML, acute lymphoblastic leukaemia (ALL) is rare.[1143,1144] Although some cases of secondary ALL have been reported in the literature,[1145] the clinical-biological features of these leukemias are poorly defined.[1120,1146,1147] In 2008, a secondary ALL has been identified following adjuvant oxaliplatin treatment in colon cancer.[1120] Also CML might be considered as late adverse effect following treatment with Pt compounds. Philadelphia (Ph) chromosome-positive CML was diagnosed in a patient 3 years after treatment for rectal adenocarcinoma.[1148] The patient had received several chemotherapeutic agents including oxaliplatin. However, also in this case, the frequently applied multimodal treatment strategy complicates the identification of specific Pt compounds as key etiologic factors of secondary malignancies.

Furthermore, ATO is clinically approved for the treatment of APL (compare chapter 3.2). Besides the prevalent and severe complication caused by ATO and ATRA, termed differentiation syndrome (believed to be mediated by the release of pro-inflammatory cytokines from malignant promyelocytes),[1149] the development of secondary hematological malignancies cannot be excluded. A recent study on long-term survival of APL patients treated with ATRA and ATO revealed an estimated 5-year cumulative incidence risk of 1.0% to develop therapy-related myeloid neoplasms.[1150] In contrast, an independent report demonstrated no increased risk of secondary cancers following ATRA- and ATO-based therapy of APL.[1151]

In addition to the clinically approved Pt and As compounds, the application of several radioactive metal isotopes is also implicated in the occurrence of secondary malignancies. For instance, ^{90}Y is the radioactive, β-radiation emitting isotope of ^{89}Y. ^{90}Y-ibritumomab (IgG1)-tiuxetan is a radioimmunotherapeutic agent, in which the metal core is bound to antibody via modified diethylenetriaminepentaacetic acid (DTPA) containing an additional isothiocyanatobenzyl linker. This agent is applied in advanced follicular lymphoma, a common form of B cell non-Hogkin's lymphoma, binding to CD20 on B cells.[1152] Recently, the progression of follicular lymphoma to aggressive lymphoma after radioimmunoherapy with ^{90}Y-ibritumomab tiuxetan has been documented.[1152] In this study, the risk of follicular lymphoma progression after treatment was assessed in 115 patients treated during 1987-2012 either in progressive state or as first-line therapy. 28% of the patients experienced progression to an aggressive lymphoma, occurring at a median of 60 months from diagnosis or 20 months after therapy. 8% of patients developed therapy-related MDS or AML at a median of 41 months post-treatment. The estimated 10-year risk of secondary MDS/AML development was 13%. However, it is currently not clear whether ^{90}Y-ibritumomab tiuxetan treatment increases the overall risk of aggressive follicular lymphoma progression at 10 year as compared to patients not receiving radioimmunotherapy. In 2016, an independent group published a long-term follow-up study, reporting 7.3% secondary AML as "late hematological side effect" with a median occurrence of 42 months

after initial ^{90}Y therapy.[1153] However, the same group conducted another study with this radionuclide, which did not result in secondary malignancies within 2 years follow-up.[1154,1155] Another radioisotope, ^{89}Sr, a ß-emitting analogue of calcium, has proven beneficial in palliative setting of pain due to bone metastasis from prostate adenocarcinoma.[1156] Frequently described toxicity of ^{89}Sr includes reversible myelosuppression, above all thrombocytopenia. Two patients were reported with AML after ^{89}Sr treatment 17 and 26 months after treatment.[1156] Interestingly, as documented recently by Jacene et al.,[1157] follow-up of patients treated with radium-223 (^{223}Ra) shows little evidence for secondary malignancies. For this isotope, only two patients with metastatic castration-resistant prostate carcinoma developed AML[1158] or APL[1159] after ^{223}Ra-containing therapy. The overall low evidence of secondary (hematopoietic) malignancies upon treatment with metal radioisotopes may, at least partly, be due to the predominantly late-stage application of these compounds[1160] as well as the comparably limited patient numbers amenable to follow-up as compared to those receiving Pt-based anticancer agents.

In conclusion, close follow-up of patients after the completion of metal-based chemotherapy is necessary to monitor for relapse and development of long-term complications such as MDS or leukemia.[1161] As novel metal-based therapeutics are being introduced in the clinical practice, their late complications are only beginning to be understood.

8. Clinical perspective and outlook

8.1. Metal drugs and anticancer immunotherapy

As already mentioned before, the situation concerning the use of anticancer metal compounds in oncological routine is paradox. On the one hand, only a handful of substances are clinically approved (compare chapter 2.1, Figure 3), despite massive chemical synthesis and preclinical evaluation approaches, generating virtually thousands of metal complexes with anticancer activity.[23-27,605,663,665,1162] On the other hand, although regularly sentenced to death, these drugs are still a central hub of systemic cancer therapy not only in rare, but also in common and often deadly cancer types including NSCLC, colon cancer, ovarian cancer and urothelial carcinoma.[173] With regard to solid tumors, lung and colorectal carcinomas are the major causes for cancer mortality, estimated to account for around 37.5% of cancer deaths in the U.S. in 2017.[1163] The majority of these patients is treated at least in one line of therapy with metal drug-containing regimens. This situation demonstrates both, the high activity and quality of metal drug-based systemic cancer therapy *per se*, and the urgent need for guiding anticancer metal drug development by multidisciplinary approaches into the currently exploding field of personalized, multi-targeted chemoimmunotherapy of cancer. This challenging task can only be successful, if underpinned by enhanced knowledge on the complex effects of metal-based chemotherapy on the host's immune system. Recent discoveries regarding a major impact of the type of (cancer) cell death during (DNA-damaging) chemotherapy in general[11,1164] and metallodrugs in

particular[20,21,197] on anticancer immune responses have amplified the interest in this field (compare chapters 2-4).[71] Accordingly, the multiple interactions of chemotherapeutic metal complexes with different steps of the anticancer immune cycle and both innate and adaptive immune responses against malignant tissues are starting to be explored (compare Figure 5). With regard to the clinical situation, the question is burning and widely unresolved whether the nowadays used anticancer metal drugs still should be considered as pure DNA-damaging cytotoxic compounds[216] or have to be additionally seen at least as immune-modulating agents.[20-22] The situation gets even more complicated, as closely related substances like cisplatin and oxaliplatin might exert strongly differing effects on anticancer immune-responses, as outlined in chapter 3.1. The molecular mechanisms underlying these differences in the execution phase of cell death have been elucidated, but it is still unclear why and by interaction with which (obviously non-DNA[345,355]) targets, only oxaliplatin - but not cisplatin and carboplatin - is able to induce ICD by exposing CRT to the tumor cell surface.[337,509] At the same time, however, it has to be critically considered that in many cancer indications, cisplatin or carboplatin were superior to oxaliplatin, suggesting that ICD induction might not always be a major driver of clinical response. Indeed, diverse immune-modulatory functions have to be integrated with general immune parameters of the treated tumor/patient and other pharmacological differences of these compounds. To give a simple example: if no TAA exist in a given tumor, ICD-mediated hyperactivation of local immune responses will for sure not end up in a major tumor-specific CTL response.

Such, in the clinical situation, currently two main issues need to be addressed. First, it has to be determined more precisely in how far the immune system contributes to the anticancer activity of clinically used metal drugs when given as single agents or in combination schemes with other chemotherapeutics or targeted compounds. Second, and at the moment even more burning is the question, what happens if anticancer metal drugs are combined with immunotherapeutic approaches like checkpoint inhibitors or vaccination strategies including chimeric antigen receptor (CAR)-T cells. Are such approaches inherently antagonistic, based on the cytotoxic activity against activated immune effector cells, or might even strongly synergistic activities be achievable?

8.2. Immune parameters predict clinical metal drug response

Regarding the first issue stated above, several indications for an important role of immune effectors in the anticancer activity of metal drugs in the *in vivo* situation exist both in mouse models, but also based on clinical observations. One of the most conclusive facts concerning animal models is for sure that, as already mentioned, cisplatin and carboplatin work less and oxaliplatin not efficiently in mouse models lacking fully functional adaptive immunity (compare Figure 4).[211-213,508,509] Comparable experimental settings are of course not possible in human patients. However, multiple clinical studies with the few approved metal drugs have demonstrated that tumor-resident and/or systemic immune parameters including CTL infiltration or the level of regulatory immune cells like T_{reg} and MDSC are predicting either response to metal-drug

containing chemotherapy or at least prognosis of patients treated with such regimens. Already the hallmark report of Galon et al., published in Science 2009[1165] and leading to introduction of the term "immunoscore" (mainly $CD3^+$ and $CD8^+$ T cell infiltration in the tumor and its margins[1166]) as prognostic parameter, was based on a colon cancer patient cohort treated with a 5-FU-based chemotherapy certainly including oxaliplatin in many cases. Hence, the strong positive prognostic power of high lymphocyte infiltration might not only reflect better tumor control by the immune system *per se*, but also treatment-enhanced antitumor immune responses. Accordingly, lymphocyte infiltration was prognostic for stage III colorectal cancer patients treated in an adjuvant setting with the oxaliplatin-based FOLFOX regimen.[1167] In metastatic colorectal cancer, enhanced pretreatment levels of granulocytic MDSC correlated with poor prognosis, and FOLFOX therapy reduced both T_{reg} and granulocytic MDSC levels while enhancing T_H17 frequency.[432]

Comparable observations concern not only patients with colorectal cancer, but more or less all cancer types treated with Pt compounds. Convincing observations have been made e.g. in thoracic malignancies including lung cancer. While the amount of $CD3^+$, $CD4^+$, $CD8^+$ or $FOXP3^+$ TIL subsets or their ratios did not have prognostic power, the ratio $FOXP3^+$ to $CD8^+$ independently predicted poor response to Pt-based therapy in NSCLC patients.[1168] Accordingly, McCoy et al. demonstrated that Pt-based therapy induced massive T cell depletion especially concerning T_{reg} cells, and an enhanced ratio of CTL/T_{reg} at recovery correlated with improved lung cancer and mesothelioma patient survival.[431] This drop of T_{reg} levels also predicted response to neoadjuvant chemotherapy in NSCLC patients treated with a combination of cetuximab with cisplatin and docetaxel.[1169] Interestingly, in this study, T_{reg} cells were demonstrated to also inhibit cetuximab-mediated NK cell killing of cancer cells via ADCC. The number of T_{reg} within the $CD4^+$ cell compartment was significantly reduced in peripheral blood of lung cancer patients treated with a cisplatin-containing regimen within an adjuvant setting.[1170] These data again suggest that depletion of regulatory immune cell subsets might be a major axis of the anticancer activity of metal drugs also in the clinical situation. Concerning lung cancer, this assumption is supported impressively by a preclinical study demonstrating high activity of combining carboplatin with a CD25 antibody targeting T_{reg} cells in a lung cancer transgenic mouse model.[1171]

With regard to other tumors routinely treated with Pt drugs, e.g. response of muscle-invasive bladder cancer patients to neoadjuvant cisplatin-based chemotherapy correlated with the CTL/T_{reg} ratio in pretreatment biopsies but not with absolute amounts of these cell types or PD-L1 expression.[1172] Likewise, in patients with osteosarcoma, routinely treated with cisplatin-containing chemotherapy, an enhanced CTL/T_{reg} ratio in pretreatment biopsies clearly separated long-term from short-term survivors and none of the patients with a ratio above the third quartile died within the observation period of more than 5 years. This prognostic power was even independent of known prognostic factors like presence of metastases at diagnosis.[1173] In patients with ovarian carcinoma, the amount of $CD8^+$ lymphocytes within the tumor stroma significantly correlated with response to carboplatin combined with paclitaxel.[1174] Likewise, an elevated ratio of intraepithelial $CD8^+$

to $CD4^+$ T cells was correlated with better overall survival after Pt-based therapy in this cancer type, based on a high proportion of activated T_{reg} within $CD4^+$ cells.[1175] In the study by Mariya et al.[502] lack of $CD8^+$ T cells in the tumor tissue significantly correlated with Pt resistance. A higher ovarian tumor grade correlated with an enhanced ascites/blood ratio of T_{reg} ($CD4^+/CD25^+$) cells while reduced levels of NKT-like cells ($CD3^+/CD56^+$) in ascites predicted higher tumor grade and Pt resistance.[1176] Wu et al. reported that carboplatin/paclitaxel treatment of ovarian cancer patients resulted in "temporary immune reconstitution" at about 12-14 days after chemotherapy, with enhanced levels of several immune parameters including CTL activity, T_H1, memory T and NKT cells.[1177] These authors suggested that this time window of elevated anticancer immunity following chemotherapy opens the ideal period for combination with immunotherapeutic approaches. A strong indication for a high relevance of immune parameters for treatment response to metal drugs comes also from a clinical study in Her-2-positive and triple-negative breast cancer, testing addition of carboplatin to the paclitaxel+liposomal doxorubicin regimen. Especially in so-called lymphocyte-predominant tumors, multiple immunological factors (like PD-L1 or CCL5 expression) were distinctly predictive for pathological complete remission especially in the carboplatin-containing arm.[504] Deep TCR sequencing in colorectal carcinoma patients treated with a combination of a five-peptide vaccine and oxaliplatin-based chemotherapy suggested a massive difference between tumor-resident and blood T cells together with a treatment-induced distinct decrease in TCR diversity in patients benefiting from the therapy.[1178]

However, not only the local immune condition in the TME or technically challenging, immune-related on-treatment hematological parameters are able to predict response to anticancer metal drugs. The neutrophil-to-lymphocyte ratio (NLR) in the peripheral blood, for example, is a well-established, easy-to-perform marker of systemic and chronic inflammation in cancer patients often predicting poor prognosis but also treatment outcome. Several studies have indicated that an enhanced NLR or "derived-NLR" (absolute neutrophil count divided by white blood cell count minus absolute neutrophil count, dNLR) was associated with poor prognosis in advanced colorectal cancer patients treated with oxaliplatin-based regimens.[1179-1181] Likewise, an elevated pretreatment NLR independently predicted worse response to first-line Pt-based chemotherapy and survival of NSCLC patients.[1182] The identical parameter also indicated dismal prognosis of bladder cancer patients treated with a nedaplatin-containing regimen[1183] and was also associated with shorter survival in upper tract urothelial carcinoma. However, an association with the patients' treatment background was not given in this study.[1184] Interestingly, a rich lymphocytic tissue response and a low platelet-to-lymphocyte ratio (PLR) predicted response to induction chemotherapy (cisplatin/docetaxel/5-FU) while a low NLR correlated with improved survival of patients with SCCHN, suggesting a combination of treatment-dependent and -independent impacts of systemic immune conditions.[1185] In contrast, both parameters were clearly associated with improved survival and treatment response of gastric cancer patients to combined chemo/radiotherapy with cisplatin.[1186] Pretreatment dNLR was evaluated in data from two

clinical studies comparing gemcitabine versus gemcitabine/cisplatin in advanced biliary carcinoma. Interestingly, a lower dNLR predicted benefit of metal drug addition to systemic chemotherapy, obviously indicating a strong impact of immune parameters on cisplatin activity.[1187] In contrast, addition of oxaliplatin to gemcitabine in the treatment of pancreatic cancer patients was only beneficial in the highly aggressive subgroup with high NLR levels.[1188]

These clinical observations together with preclinical animal data clearly support the hypothesis that the conditions of systemic and local immune responses strongly impact on the efficacy of anticancer metal drugs and *vice versa*. Hence, these compounds might be ideal partners for immunotherapeutic approaches by supporting better visibility of the tumor by immune cells, but also enhanced killing of tumor cells by metal drugs based on ideal immune conditions in the TME.

8.3. Metal drugs as partners for immunotherapy of cancer

Several preclinical *in vitro* and *in vivo* effects of metal drugs on cancer cells but also immunocytes support the hypothesis that these drugs might serve as ideal combination partners for immunotherapeutic interventions. However, the clinical proof of this concept is currently only starting to emerge. Immunotherapeutic interventions and especially immune checkpoint inhibitor antibodies are very efficient anticancer strategies and have sustainably altered the world of systemic cancer therapy recently.[16,112,1189] In contrast to many other therapeutic options, immunotherapies may allow long-term remission or even cure of disseminated, treatment-resistant solid tumors. This concerns devastative malignancies like melanoma,[1190,1191] NSCLC,[1192,1193] urothelial cancer,[1194] renal cancer,[1195] and head and neck tumors[1196]. Nevertheless, it has to be considered that even in the most successful cases, only a patient minority profits from these therapeutic interventions. So, one central challenge for modern systemic cancer therapy research is to identify and develop therapeutic combination strategies and algorithms to enhance the response rates to modern immunotherapies. Accordingly, an avalanche of preclinical evaluations and clinical studies has been initiated (compare www.clinicaltrials.gov), combining checkpoint inhibitors with other immunotherapies, targeted anticancer compounds, antiangiogenic agents, and chemotherapeutic interventions with variable success. Unfortunately, not in all cases, clear-cut hypotheses and strategies are followed, bearing the danger that beneficial combinations might be missed due to disadvantageous doses, sequences or application regimens.

Literature in the field of combination approaches involving checkpoint inhibitors is currently emerging at a high rate. Multiple studies are under way (compare www.clinicaltrials.gov) and will deliver data soon. Their summary is, however, far beyond the scope of this review. Here, we will only give a short overview on data available for those cancers where metal drug-based therapy is used routinely. However, it should be mentioned that - based on the promising observations regarding combination of immune checkpoint inhibitors with Pt drugs in NSCLC (see below) - even a re-evaluation of metal compounds in cancer types

insensitive to this therapy, like metastatic melanoma, is currently considered. Some key clinical (primarily randomized phase 3 or approval-related) studies affecting the field of immunotherapies in comparison to or in combination with Pt-based chemotherapy are summarized in Table 1.

8.3.1. Lung cancer and mesothelioma

Regarding NSCLC, comprising adenocarcinoma (AC) and squamous cell carcinoma (SCC), as well as small cell lung cancer (SCLC), Pt-containing regimens are still standard first-line therapy in the majority of cases. Exceptions were - for quite some time - only smaller patient subgroups harboring EGFR mutations or ALK translocations receiving respective kinase inhibitors as first-line therapy.[1197] Many characteristics of NSCLC, like the high mutational burden especially in smoking-associated lung cancer, suggested this devastative disease especially suitable for immune checkpoint inhibitor therapy.[1198] Hence, such checkpoint inhibitor antibodies were first tested in second-line therapy after progression of NSCLC under Pt-containing chemotherapy. In all these studies, close attention was paid whether the expression levels of the target PD-L1 in tumor cells or immunocytes within the TME (TC and IC, respectively, in Table 1) would serve as biomarker for responsive patient subgroups. Following several phase 1 and 2 studies demonstrating good tolerability and clear-cut signs of activity of both CTLA-4 (ipilimumab) and PD-L1/PD-1 inhibitors (nivolumab, pembrolizumab, atezolizumab) (summarized recently in[1197,1199,1200]), various randomized phase 3 studies were performed either in adjuvant or palliative settings (Table 1). These studies demonstrated that application of PD-1/PD-L1 inhibitors, especially following disease progression under Pt-based chemotherapy, is highly effective, outstripping in almost all studies second- or later-line chemotherapy (e.g. CheckMate 017[1193], CheckMate 057[1192], Keynote 010[1201]). This led to the approval of several PD-1 and PD-L1 antibodies (nivolumab, pembrolizumab, atezolizumab) for treatment of advanced NSCLC progressed on Pt-containing chemotherapy. The question arising from these studies, if the prior chemotherapy has supported cancer immune recognition by a CTL response, remains open.

Next, the field started to explore whether checkpoint inhibitor immunotherapy might even move to first-line setting in chemotherapy-naïve patients. Concerning the PD-1/PD-L1 axis, e.g. the Keynote-024 study[1202] tested application of the PD-1 immune checkpoint antibody pembrolizumab in first-line therapy compared to Pt-doublet chemotherapy in those EGFR and ALK mutation-negative patients expressing PD-L1 in ≥50% of malignant cells. The distinctly higher response rate (with 45%, almost half of the patients responded) and lower hazard ratio for overall survival (Table 1) led to approval of pembrolizumab in this PD-L1-high patient subgroup in October 2016.[1203] Unexpectedly, another study (CheckMate-026), using in a comparable setting the PD-1 antibody nivolumab, did not indicate a significant difference between immunotherapy and Pt-based chemotherapy neither in the patient subgroup with ≥5%, nor in the group with ≥50% PD-L1-positive tumor cells.[1201] These observations suggest that immunotherapy might be superior to chemotherapy in first-

line treatment of patients with highly PD-L1-positive NSCLC. Nevertheless, it remains unclear whether also in these patients, addition of Pt-based chemotherapy to immunotherapy might induce further benefit.

To refine the picture more precisely concerning TMB, Checkmate-227 tested the ipilimumab/nivolumab combination versus nivolumab versus Pt-doublet chemotherapy in stage IV or recurrent chemotherapy-naïve NSCLC. In patients with PD-L1 expression in less than 1% of cells, a combination arm with nivolumab/chemotherapy replaced nivolumab monotherapy. Independently of PD-L1 expression, a high TMB (compare chapter 2.2.4) was associated with improved PFS of the immune-combination as compared to chemotherapy, while this difference was absent in patients with low TMB.[1200] Unfortunately, also this study does not allow direct addressing of a potential synergism between metal drugs and the checkpoint inhibitors, as the combination arm in the low PD-L1 expressing patients is not further discussed.

After these positive results in second- and first-line therapy, the question remained, whether also patients with unfavourable marker profiles like lower TMB and lack of PD-L1 expression might be sensitized to checkpoint inhibitors by combination with metal-based chemotherapy. Concerning such a combined application, data are accumulating rapidly. With regard to CTLA-4 inhibition in stage IV or recurrent NSCLC/SCC, addition of ipilimumab to Pt-based chemotherapy, despite promising early phase data, failed to improve PFS in first-line setting in the NCT01285609 trial.[1204] This indicates that CTLA-4 blockade might not be sufficient to fully exploit the immunogenicity of Pt-based NSCLC therapy. In contrast, several phase 2 and 3 studies delivered evidence that addition of chemotherapy with Pt drugs to PD-L1/PD-1 checkpoint inhibitor therapy is a highly active combination (Table 1). Thus, in the Keynote-189 trial, survival at 12 months was 69.2% in the combination group versus 49.4% in the Pt chemotherapy group.[9] The response rate in the combination group was across all PD-L1 expression levels almost approaching 50%, hence exceeding the one for the patient subgroup with PD-L1 expression in ≥50% cells for first-line monotherapy with pembrolizumab in the Keynote-024 trial.[1202] These findings suggest distinct synergism of PD-1 inhibition with Pt-based chemotherapy. As also in the Keynote-189 study, patients with high PD-L1 expression benefitted most from combination with chemotherapy,[9] it needs to be re-evaluated whether not also in this patient subgroup a combination with metal-based chemotherapy would be more efficient than the currently approved monotherapy with PD-1 antibodies. Accordingly, recent data from the IMpower150 study demonstrated superiority of a combination of the PD-L1 antibody atezolizumab with Pt-based chemotherapy and the VEGF inhibitor bevacizumab as compared to the arm without checkpoint inhibitor.[1205] Here, the response rate in the triple combination arm marks for the first time a response in distinctly more than half of the patients (63.5%). Interestingly, this benefit was regardless of PD-L1 expression and concerned patients with EGFR and ALK mutations/translocations as well.

Besides late stage NSCLC, also data regarding the use of immune checkpoint inhibitors in earlier tumor stages in adjuvant and neoadjuvant settings and their interaction with Pt-based chemotherapy are starting to emerge. Such, durvalumab, another PD-L1 antibody, strongly reduced the rate of disease progression (PFS

of 16.8 months versus 5.6 months in the placebo group) in locally advanced stage III NSCLC when given as consolidation therapy after two or more cycles of Pt-based radiochemotherapy (PACIFIC trial).[1206] Unexpectedly, also data are accumulating that Pt-based chemotherapy might be especially active in several tumor types including NSCLC and melanoma after prior immunotherapy.[1207,1208] The underlying mechanisms especially regarding an altered immune TME need to be explored in more detail. Altogether, these data suggest that metal drugs may represent ideal partners for therapy of NSCLC patients with immune checkpoint inhibitors in different schedules and lines of treatment.

In how far this holds true also for other immunotherapeutic interventions is by far less explored.[1209] Examples entering clinical evaluation comprise whole cell and target vaccination approaches against e.g. MAGE-A3, EGFR and hTERT.[1210] Randomized clinical studies using such immunotherapeutic strategies in combination with metal-based regimens are sparse. However, two observations are highly interesting regarding immunogenic effects of Pt-based chemotherapy. First, an allogeneic whole tumor cell vaccine (termed belagenpumatucel-L) based on NSCLC cell lines transfected with a TGF-β2-antisense vector failed to prevent recurrence of stage III/IV NSCLC patients in the entire study cohort but was active in patients receiving the vaccine rapidly (randomization within 12 weeks) after Pt-based chemotherapy.[1211] This suggests that, comparable to preclinical observations, metal-drug chemotherapy is followed by a window of enhanced immunogenicity (compare chapter 8.2). The second observation concerns tecemotide, a synthetic adjuvant-containing lipopeptide of MUC-1. Although it failed to improve survival of unresectable, stage III NSCLC patients as maintenance therapy after chemoradiation in a randomized phase 3 study, the results were positive in the subgroup receiving concomitant (but not sequential) Pt-containing chemoradiation.[1212] This again clearly indicates a major effect of metal-based chemotherapy on the immunological situation of the NSCLC patients and the response to immunotherapeutic interventions.

With regard to mesothelioma, also treated as standard with Pt-based chemotherapy and regularly overexpressing PD-L1, the respective studies with checkpoint inhibitors in second-line[1213] and combined with Pt-based chemotherapy[1214] are currently recruiting and the results are eagerly awaited.

Table 1: Selected clinical studies (either randomized phase 3 or earlier phase when connected to clinical approval) concerning a comparison or interaction of checkpoint inhibitor immunotherapy with metal drug-based chemotherapy

Histology, Line	**Study, Phase**	**Immuno-therapy**	**Chemo-therapy**	**ORR[1] %**	**Hazard ratio survial[2]**	**Ref.**
NSCLC						
Advanced	Keynote-010	Pembrolizumab	Docetaxel	30/29[4]	0.71 (ITT[5])	[1201]

2nd line, poPt[3]	Phase 3			vs 8 TC[5] ≥ 50%	0.58-0.88 0.54 (TC ≥ 50%[5]) 0.38-0.77	
SCC advanced 2nd line, poPt	CheckMate-017 Phase 3	Nivolumab	Docetaxel	20 vs 9	0.59 0.44-0.79	1193
non-SCC advanced 2nd line, poPt	Checkmate-57 Phase 3	Nivolumab	Docetaxel	19 vs 12	0.73 0.59.0.89	1192
advanced 2nd line, poPt	POPLAR Phase 2	Atezolizumab	Docetaxel	15 vs 15	0.73 0.53 vs 0.99	1215
advanced 2nd line, poPt	OAK Phase 3	Atezolizumab	Docetaxel	14 vs 13	0.73 0.62-0.87	1216
Stage IV/rec PD-L1 high 1st line	Keynote-024 Phase 3	Pembrolizumab	Pt based	45 vs 28	0.6 (TC ≥ 50%[5]) 0.41-0.89	1202
Stage IV/rec 1st line	CheckMate-026 Phase 3	Nivolumab	Pt-based	34 vs 39	0.9 (TC ≥ 50%[5]) 0.63-1.29	1217
Stage IV/rec high TMB[6] 1st line	CheckMate-227 Phase 3	Ipilimumab Nivolumab	Pt-based	45.3 vs 26.9	0.58 0.41-0.81 (PFS)	1218
SCC advanced 1st line	NCT01285609 Phase 3	Ipilimumab Pt-based	Pt-based	44 vs 47	0.91 0.77-1.07	1204
non-SCC advanced 1st line	Keynote-189 Phase 3	Pembrolizumab Pt-based	Pt-based	47.6 vs 18.9	0.49 0.38-0.64	9
non-SCC advanced +EGFR/ALK 1st line	IMpower150 Phase 3	Atezolizumab Bevacizumab Pt-based	Bevacizumab Pt-based	63.5 vs 48.0	0.78 0.64-0.96	1205
Urothelial carcinoma						
advanced 2nd line, poPt	Keynote-045 Phase 3	Pembrolizumab	Paclitaxel or Docetaxel or	21.1 vs 11.4	0.73 0.59-0.91	1219

			Vinflunine			
advanced 2nd line, poPt	IMvigor211 Phase 3	Atezolizumab	Paclitaxel or Docetaxel or Vinflunine	23 vs 22	0.87 (IC5≥5%) 0.63-1.21	1220
advanced 2nd line, poPt	CheckMate-275 Phase 2	Nivolumab	Single arm	19.6	-	1221
advanced 2nd line, poPt	NCT01693562 Phase 1/2	Durvalumab	Single arm	31.0	-	1222
advanced 2nd line, poPt	JAVELIN Phase 1b	Avelumab	Single arm	18.2	-	1223
Pt-ineligible 1st line	Keynote-052 Phase 2	Pembrolizumab	Single arm	29	-	1224
Pt-ineligible 1st line	IMvigor210	Atezolizumab	Single arm	23	-	1225
SCCHN						
advanced 2nd line, poPt	CheckMate-141 Phase 3	Nivolumab	Methotrexate Docetaxel Cetuximab	13.3 vs 5.8	0.7 0.51-0.96	1226
advanced 2nd line, poPt	Keynote-012 Phase 1b	Pembrolizumab	Single arm	16	-	1227
Gastric and gastro-esophageal junction cancer						
Advanced 2nd line, poPt	Keynote-061 Phase 3	Pembrolizumab	Paclitaxel	16 vs 14	0.82 0.66-1.03	1228
Advanced 2nd line or higher	Keynote-059 Arm 1, Phase 2	Pembrolizumab	-	11.6 PL-L1+:15.5 PL-L1-:6	-	1229
Advanced 1st line	Keynote-059 Arm 2, Phase 2	Pembrolizumab Cisplatin-based	-	60 PL-L1+:69 PL-L1-:38	-	1230

[1]ORR, overall response rate; [2]hazard ratio is always given for overall survival, if not stated extra as progression-free survival (PFS); [3]poPt, progressed on Pt-based chemotherapy; [4]pembrolizumab at 2mg/kg and 10mg/kg versus docetaxel; [5] subgroups analyzed either ITT (intended-to-treat population) or concerning PD-L1 expression in tumor cells (TC) or immune cells (IC); [6]high TMB, ≥10 mutations per megabase.

8.3.2. Urothelial carcinoma

Since about 30 years, metastatic urothelial carcinoma has been treated primarily with Pt-based chemotherapy. However, besides melanoma and lung cancer, also urothelial carcinoma therapy was recently revolutionized by introduction of immune checkpoint inhibitors. Comparable to lung cancer, immune checkpoint inhibitors were evaluated as second-line treatment following chemotherapy failure which led to approval of five antibodies so far, namely atezolizumab[1220], pembrolizumab[1225], nivolumab[1221], durvalumab[1222] and avelumab[1223] (the respective clinical studies are listed in Table 1). While pembrolizumab was clearly superior to second-line chemotherapy with regard to overall survival in this setting (Keynote-045),[1219] atezolizumab was only active in a smaller subgroup of patients and failed to extend overall survival (IMvigor211).[1220] For the other three antibodies, approval was based on single-arm phase 2 studies not allowing comparison to second-line chemotherapy.[1221-1223]

As Pt-containing chemotherapy is highly active in this tumor type with response rates of about 50%, no single drug checkpoint inhibitor study has been published so far. However, a substantial percentage (up to around half) of the mainly elderly patients are ineligible to this treatment based on comorbidities and poor prognostic parameters. In this Pt-ineligible patient subgroup, first-line treatment with checkpoint inhibitors was recently demonstrated to be beneficial with response rates of 24% and 23% for pembrolizumab and atezolizumab in phase 2 settings, respectively, leading to clinical approval.[1224,1225] Combination studies with checkpoint inhibitor antibodies and Pt-based chemotherapy, allowing to estimate a possible synergistic effect comparable to NSCLC, are under way (summarized in[1231]) but data have not been published so far.

8.3.3. Head and neck squamous cell carcinoma (SCCHN)

SCCHN is a quite common malignancy accounting for a high percentage of cancer deaths. Besides smoking and alcohol, HPV infection has been recognized as a major cause for SCCHN. Accordingly, this tumor is strongly dependent on immune evasion, and multiple immunological parameters like CTL infiltration and the amount of regulatory immune cells have strong prognostic power (reviewed recently by[1232]). With regard to immune-related interventions, for sure preventive vaccination of HPV-mediated SCCHN is the most impressive success story. In addition, numerous immune-related approaches have been tested with therapeutic intention including HPV-based vaccination approaches on the basis of peptides, nucleic acids, viral vectors, DC, and adoptive cell transfer including CAR-T cells.[1232,1233] However, despite the application with Pt-based therapy in some cases, no conclusive data on any interaction have been published so far.

With regard to checkpoint inhibitors, also in case of SCCHN impressive benefits were achieved, changing the landscape of systemic therapy at advanced disease. Such, nivolumab and pembrolizumab have been approved in second-line treatment following progression on Pt-containing chemotherapy based on a randomized phase 3 (CheckMate-141)[1226] and a multi-cancer phase 1b study (Keynote 012)[1227], respectively (Table 1). Again, it would be interesting to know how much the nature of first-line chemotherapy impacts on the immunotherapeutic response. Currently, several trials are testing the quality of checkpoint inhibitors

in the first-line setting. Unfortunately, only Keynote-048 tests a combination of immunotherapy (pembrolizumab) with metal-based chemotherapy (EXTREME protocol with Pt, 5-FU, and the EGFR antibody cetuximab) in addition to the monotherapy arms. The Kestrel (durvalumab) and the Checkmate-651 (nivolumab+ipilimumab) study only comprise separated immunotherapy and Pt-based chemotherapy arms. Considering the impressive data in lung cancer[9], the study design missing the combination arms might be somewhat shortsighted.

8.3.4. Gynecological malignancies

Pt-containing regimens are widely used in standard therapy of advanced gynecological malignancies including ovarian, cervical and endometrial carcinoma. Several of these diseases are strongly influenced by immunological parameters and diverse immune markers correlate with prognosis. Additionally, molecular subgroups prone to be especially responsive to immune checkpoint inhibitor blockade exist, including a hypermutation background in BRCA1- and BRCA2-associated ovarian cancer as well as exonuclease domain of polymerase ε (POLE)-ultramutated and MMR-deficient, hypermutated endometrial cancer.[1234] Moreover, cervical carcinoma is, due to its etiology based on HPV infection, generally characterized by a distinct immune TME. Although checkpoint inhibitor antibodies have not been approved for gynecological malignancies so far, several clinical studies are under way both in second-line therapy or in first-line combination with Pt-based chemotherapy or, e.g. in BRCA-related cancer types, with PARP inhibitors (www.clinicaltrial.gov).[1235] A phase 2 study in Pt-pretreated cervical cancer with the CTLA-4 antibody ipilimumab did not show single-agent activity, and the observed immunological changes did not correlate with clinical response.[1236] In contrast, for PD-L1 inhibitors some single cases with impressive responses have been reported, and a phase 1b pembrolizumab study (Keynote-028) found a response rate of 17% in patients pretreated with Pt-chemotherapy and in most cases with bevacizumab. Accordingly, 15% of cisplatin-resistant ovarian cancer patients responded to nivolumab with 2/20 durable, complete remissions.[1237] However, data for the respective randomized higher-phase trials are not published at the time of this review. Accordingly, combination studies with Pt-based chemotherapy are under way and the results are eagerly awaited.[1235]

8.3.5. Gastric and gastro-esophageal junction cancer

Despite declining in the Western hemisphere, gastric cancer is still characterized by a very high incidence especially in Eastern Asia. Consequently, it remains the third leading cause of cancer-related death worldwide.[1230] Pt-based chemotherapy is the mainstay of systemic therapy in advanced stages combined with other chemotherapeutics in HER2-negative or trastuzumab in HER2-positive cases.[1238] Regarding immunotherapy with checkpoint inhibitors, gastric cancer and gastro-esophageal junction cancer got into the focus of interest early, as these tumors express several checkpoint molecules and exert parameters of T

cell exhaustion including PD-L1 overexpression in up to 65% of cancer and immune cells.[1239] Earlier phase studies with several PD-1/PD-L1 antibodies (pembrolizumab, nivolumab, avelumab, durvalumab) and the CTLA-4 antibody tremelimumab in second- or later-line after chemotherapy delivered response rates from 6% in unselected up to about 22% in PD-L1-high tumors (reviewed in[1230]). However, pembrolizumab was not superior to paclitaxel in prolonging survival of patients with advanced gastric/gastro-esophageal junction cancer in second-line treatment after progression on Pt-based therapy (Keynote-061[1228]; Table 1). Nevertheless, pembrolizumab was approved by the FDA in September 2017 for patients expressing PD-L1 in second-line after Pt- or HER2-related therapy. The decision was based on data from a monotherapy arm of the phase 2 Keynote-059 trial[1229], indicating a response rate of 13.3% in PD-L1-high and MMR-deficient tumors in second or higher therapy line. These notable but still moderate responses might completely change with regard to a combination of pembrolizumab with Pt-based chemotherapy, indicating an impressive overall response rate of 60% in the entire cohort and even 69% in PD-L1-positive cases. These data are only available as abstract so far (discussed in[1230]) but would, comparable to NSCLC, indicate an excellent response level for this devastative disease and again suggest synergism of immunotherapy with Pt-based chemotherapeutic approaches.

8.4. Outlook

Over the last years, the picture regarding the impact of metal-based cancer therapy on immunological aspects of malignant diseases has changed fundamentally. It has increasingly been recognized that the cytotoxic effect against immune cells causing transient immune depletion might even offer the chance to overcome or revert major steps of cancer immune evasion, leading to a phase of renewed anticancer immune response. We propose, based on preclinical and clinical data, that these effects provide an ideal window for the combination of anticancer metal drugs and immunotherapeutic interventions. For the preclinical development of novel anticancer metal drugs, this implies that the complex interaction network with the anticancer immune response needs to be addressed in detail, if such drugs should indeed cross the border to clinical application. To be successful in that endeavor, for sure interdisciplinary research networks need to be established comprising, besides synthetic and analytical chemists, also biologists, immunologists and clinical oncologists. This implicates that the use of metal drugs in systemic anticancer therapy is not at an end. In contrast, a new area for these fascinating remedies in the fight against cancer has just begun.

Acknowledgments

This manuscript was supported by the Medical University of Vienna and the University of Vienna in frame of the interuniversity research cluster „Translational Cancer Research“ established in 2017.

Author information

Corresponding author

Walter Berger, Institute of Cancer Research, Medical University Vienna, Borschkegasse 8a, A-1090 Vienna, Austria, Phone: +43(0)140160-57555, Fax: +43(0)140160-957555, walter.berger@meduniwien.ac.at

Notes

The authors declare no competing financial interest.

Biographies

Bernhard Englinger obtained his Master's degree in Molecular Biology (2013) at University of Vienna. He completed his doctoral thesis (2018) at the Institute of Cancer Research of Medical University of Vienna under the supervision of Prof. Walter Berger. In his PhD thesis, he mainly focused on molecular resistance mechanisms of fibroblast growth factor receptor (FGFR) inhibition in lung cancer, as well as on intracellular compartmentalization of small molecule tyrosine kinase inhibitors into distinct organelles as critical determinant of drug efficacy. Currently still working in the lab of Prof Berger, he has been offered a postdoctoral fellowship in the lab of Dr. Mariella Filbin at Dana Farber Cancer Institute of Harvard Medical School, where he will work on the molecular characterization of pediatric brain tumors.

Christine Pirker (born in 1969) studied zoology and anthropology at University of Vienna, where she pursued her Master's thesis in the lab of Prof. Simon Panzer working on anti-platelet antibodies (1996). In 1999, she joined the group of Prof. Michael Micksche at the Institute of Cancer Research Vienna, where she received her PhD in 2003. In her thesis, she worked on the characterization of quantitative genomic aberrations in malignant melanoma. In 2003, she joined the lab of Prof. Walter Berger at the Institute of Cancer Research at Medical University of Vienna in frame of several postdoctoral fellowships and cooperative projects, mainly in the field of molecular characterization of melanoma, glioma and mesothelioma. Since 2013, she holds a faculty position as senior PostDoc in the lab of Prof. Walter Berger. Her current interest focuses on the field of cancer immunology, where she investigates molecular mechanisms promoting synergism between systemic cancer therapy and immune checkpoint inhibition in frame of a project financed by the Initiative Krebsforschung.

Petra Heffeter (aged 38) is an Associate Professor at the Institute of Cancer Research at the Medical University of Vienna. In addition, she is a long-term member of the research cluster “Translational Cancer Therapy Research”. With regard to her education, she is biologist and toxicologist and her research focuses on the development of new therapeutic strategies to overcome limitations of current cancer therapy. She is project leader of several third party-funded projects focusing on novel prodrug systems as well as mode of action studies on anticancer (metal) drugs. She has published more than 100 manuscripts, collecting ~2700 citations (h-index 28). Additionally, she is cofounder of three patent applications. For her scientific discoveries, P. Heffeter has was honored with several awards including the Otto-Kraupp Habilitation Award in 2016 and the two innovation awards of INiTS and RIZ GENIUS in 2014.

Alessio Terenzi was born in Palermo (1981), Italy. He obtained his master’s degree in chemistry at the University of Palermo (2007), where he also completed his Ph.D (2011), under the supervision of Prof. G. Barone. During his PhD, he worked in Prof. J. M. Quintela’s group at the University of La Coruña, Spain (2009), and as a Marie Curie Early Stage Researcher in Prof. M. J. Hannon’s group at the University of Birmingham, UK (2010). After completing his first postdoc at the University of Palermo (2014), he moved to the University of Vienna, Austria, with a Marie Curie Cofund Fellowship (INDICAR). In Vienna, he developed his project on the in-cell interaction of metal complexes with G-quadruplex DNA motifs, between the Institute of Inorganic Chemistry (Prof. B. Keppler) and the Research Platform of Translational Cancer Therapy Research (Prof. W. Berger). In 2018, thanks to a Marie Skłodowska-Curie Individual Postdoctoral Fellowship, he moved to San Sebastian, Spain, in the group of Prof. L. Salassa, where he is currently working on the development of photoactivatable anticancer prodrugs, which simultaneously incorporate nuclear imaging capability.

Christian Kowol is currently Assistant Professor (tenure track position) at the University of Vienna. He studied chemistry at the University of Vienna and finished his PhD in the area of synthetic inorganic chemistry of novel anticancer drugs in 2009. The research of Christian Kowol focuses on interdisciplinary studies in the area of anticancer drug design: mainly (metal-based) prodrug development, drug delivery systems and peptide targeting strategies to increase activity and tolerability of cancer drugs. He is active member of the research cluster “Translational Cancer Therapy Research” and the Comprehensive Cancer Center of the Medical University of Vienna and was honored in the last years with several awards including the “Research Award of the Vienna Fund for Innovative Interdisciplinary Cancer Research”, the 1st place of the “INITS Award” in the

area of Life Science and the RIZ GENIUS Award. He is author of about 55 publications with an H index of 21.

Bernhard Keppler is Chair of Inorganic Chemistry, Head of the Institute of Inorganic Chemistry and Dean of the Faculty of Chemistry at the University of Vienna, Austria. Together with Prof. Walter Berger, he is founder of the inter-university research platform, since 2017 cluster-project, *Translational Cancer Therapy Research*. He completed his PhD in Chemistry at the University of Heidelberg (Germany) in 1981 and, after having received his licence to practice medicine and a period of clinical experience, completed his PhD in Medicine at the German Cancer Research Centre Heidelberg in 1986. In 1995 he became Chair of Inorganic Chemistry at the University of Vienna. His research areas include Bioinorganic Chemistry, Coordination Chemistry, and Environmental Chemistry. He developed tumor-inhibiting metal complexes from basic synthesis via activity, mode of action and toxicology studies to clinical development. So far, three first-in-class compounds based on titanium, gallium and ruthenium reached clinical studies; the ruthenium compounds IT-139 (KP1339) and LX-001 (KP46) are still under development in USA and Canada. Bernhard Keppler has been awarded several prizes, among others the Heinz-Maier-Leibnitz-Prize of the German Federal Ministry of Education and Research. He is author and co-author of more than 500 publications and holds currently an H index of 65.

Walter Berger is Chair of Applied and Experimental Oncology and Deputy Head of the Institute of Cancer Research at the Medical University of Vienna, Austria. Together with Prof. Bernhard Keppler, he is founder of the interuniversity research cluster for Experimental Cancer Therapy Development. He completed his PhD in Biology at the University of Vienna in 1993. Afterwards, he worked in the industry (Hoechst Germany) for 4 years before he returned to Vienna University as assistant professor. After a postdoc period at Cambridge University, UK, he developed the research focus on translational drug development at the Institute of Cancer Research at the Medical University Vienna. In 2013 he took over the chair for Applied and Experimental Oncology at this university. In his scientific work, he focussed on the translation of molecular and cellular research approaches towards clinical oncology by developing novel therapy targets but also drug formulations. In several research networks, he is connected both with clinical oncologists and pathologists but also synthetical and analytical chemists. A major focus lies on the utilisation of metal drugs for tumor-specific drug activation and targeted therapy approaches against thoracic and paediatric as well as adult brain tumors. Walter Berger was awarded with several prizes

in the field of translational cancer research. He is author of about 200 publications and holds currently an H index of 45.

Abbreviations

3’-UTR	3’-untranslated region
AC	adenocarcinoma
ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
AGE	advanced gycation end products
AIM2	absent in melanoma 2
AIRE	autoimmune regulator
AKI	acute kidney injury
ALL	acute lymphoblastic leukaemia
ALR	absent in melanoma 2 (AIM2)-like receptor
AML	acute myelogenous leukemia
AMPK	5’-AMP-activated protein kinase
ANXA1	annexin A1
AP-1	activator protein-1
APC	antigen-presenting cell
APL	acute promyelocytic leukemia
AR	adenosine receptor
ATG7	autophagy related 7
ATO	arsenic trioxide
ATP	adenosine triphosphate
ATRA	all-trans retinoic acid
BCR	B cell receptor
BMDC	bone marrow-derived dendritic cell
BMDM	bone marrow-derived macrophage
B_{reg}	regulatory B cell
cAMP	cyclic adenosine monophosphate
CAR	chimeric antigen receptor
CCL	chemokine ligand
CCR	chemokine receptor

CD	cluster of differentiation
CDC	complement-dependent cytotoxicity
CEA	carcinoembryonic antigen
cGAS-STING	cyclic GMP-AMP synthase-stimulator of interferon genes
CIC	circulating antibody immune complex
CIC	conjugated complement C1q
CIK cell	cytokine-induced killer cell
CIN	chromosomal instability
CIPN	chemotherapy-induced peripheral neuropathy
CLR	C-type lectin receptor
CML	chronic myelogenous leukemia
CNS	central nervous system
CNT	carbon nanotube
CO	carbon monoxide
CORM	carbon monoxide (CO)-releasing molecules
COX	cyclooxygenase
CREB	cAMP responsive element-binding protein
CRT	calreticulin
CSF1	colony stimulating factor 1
CSF-1R	CSF1 receptor
CTL	cytotoxic T lymphocyte
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
Ctr1	copper transport protein 1
DAMP	damage-associated molecular pattern
DC	dendritic cell
DDR	DNA damage response
DISC	death-inducing signaling complex
DNAM-1	DNAX-accessory molecule-1
DR4/5	death receptor 4/5
DRG	dorsal root ganglia
ECM	extracellular matrix
EGCG	epigallocatechin-3-gallate
ENS	enteric nervous system
EPR	enhanced permeability and retention
ER	endoplasmic reticulum

FASL	Fas ligand
Fc	fragment crystallizable region
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GI tract	gastrointestinal tract
GM-CSF	granulocyte-macrophage colony-stimulating factor
HIF1α	hypoxia-inducible factor-1α
HLA	human leukocyte antigen
HMGB1	high-mobility group box 1
HO	heme oxygenase
HPV	human papillomavirus
HSP	heat shock protein
HSR	hypersensitivity reactions
ICAM	intercellular adhesion molecule
ICD	immunogenic cell death
IDO	indoleamine-pyrrole 2, 3-dioxygenase
IFN	interferon
Ig	immunoglobulin
IκB	Inhibitor of NFκB
IL	interleukin
iNOS	inducible nitric oxide synthase
IRF	IFN regulatory factor
ISRE	interferon-stimulated regulatory element
iT_{reg}	induced T_{reg}
Jak	Janus kinase
KIR	killer-cell immunoglobulin-like receptor
LAK cell	lymphokine-activated killer cell
LFA-1	lymphocyte function-associated antigen 1
LPS	lipopolysaccharide
MAMP	microorganism-associated molecular pattern
MAPK	mitogen-associated protein kinase
MCP-1	monocyte chemotactic protein-1
MDS	myelodysplastic syndrome
MDSC	myeloid-derived suppressor cell
MGN	multibranched gold nanoantenna
MHC	major histocompatibility complex

MICA/MICB	MHC class I-related chain A/B
Mincle	macrophage-inducible C-type lectin
MLKL	mixed lineage kinase domain-like pseudokinase
MMR	mismatch repair
moDC	monocycte-derived DC
MOSEC	murine ovarian surface epithelial cell
MPS	mononuclear phagocyte system
M_{reg}	regulatory macrophage
MSC	mesenchymal stem cell
mTEC	medullary thymic epithelial cell
NAC	N-acetyl cysteine
NADPH	nicotinamide adenine dinucleotide phosphate
NFAT	nuclear factor of activated T cells
NFκB	nuclear factor kappa B
NK cell	natural killer cell
NKG2D	natural killer group 2D
NKT cell	natural killer T cell
NLR	neutrophil-to-lymphocyte ratio
NLR	NOD-like receptor
NO	nitric oxide
NOS	nitric oxide synthase
NOX	NADPH oxidase
NP	nanoparticle
Nrf2	NF-E2-related factor 2
NSCLC	non-small cell lung cancer
Oct2	organic cation transporter 2
ODN	oligodeoxynucleotide
OHC	outer hair cell
PAMP	pathogen-associated molecular pattern
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood monocyte
PCC	pancreatic cancer cell
PD-1	programmed death-1
PDAC	pancreatic ductal adenocarcinoma
PD-L1	programmed death-ligand 1

PERK	protein kinase R-like ER kinase
PFS	progression-free survival
PG	prostaglandin
Ph chromosome	Philadelphia chromosome
PKC	protein kinase C
PMIDA	N-Phosphonomethyl iminodiacetic acid
PML	promyelocytic leukemia
PNS	peripheral nervous system
PRR	pattern recognition receptor
PSA	prostate-specific antigen
PSC	pancreatic stellate cell
RA	rheumatoid arthritis
RAGE	receptor for advanced glycation end products
RARα	retinoic acid receptor-alpha
RIG-I	retinoid acid-inducible gene I
RIPK	receptor-interacting serine/threonine kinase
RNS	reactive nitrogen species
RT	radiotherapy
SCC	squamous cell carcinoma
SCCHN	head and neck squamous cell carcinoma
SCID	severe combined immunodeficiency
SCN	sensitive cluster nanoparticle
SOD	superoxide dismutase
STAT	signal transducer and activator of transcription
TAA	tumor-associated antigen
TAM	tumor-associated macrophage
TAN	tumor-associated neutrophil
TCR	T cell receptor
T_{FH}	follicular T_H cell
TGF-β	Transforming growth factor- β
T_H	T helper lymphocyte
TIL	tumor-infiltrating lymphocyte
TLR	therapy-related leukemia
TLR	Toll-like receptor
TMB	tumor mutation burden

TME	tumor microenvironment
TNF	tumor necrosis factor
Tr1	type 1 regulatory T cell
TRAIL	TNF-related apoptosis-inducing ligand
T_{reg}	regulatory T cell
TREM2	receptor expressed on myeloid cells 2
TRL	therapy-related leukemia
TROP2/TACSTD2	tumor-associated calcium signal transducer
TRPV1	transient receptor potential vanilloid 1 channel
Trx	thioredoxin
TrxR	thioredoxin reductase
TSA	tumor-specific antigen
ULK1	unc-51 like autophagy activating kinase 1
UPR	unfolded protein response
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

References

(1) Kroemer, G.; Galluzzi, L.; Kepp, O.; Zitvogel, L. Immunogenic cell death in cancer therapy. *Annual review of immunology* **2013,** *31*, 51.

(2) Chen, D. S.; Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **2013,** *39* (1), 1.

(3) Patel, S. A.; Minn, A. J. Combination Cancer Therapy with Immune Checkpoint Blockade: Mechanisms and Strategies. *Immunity* **2018,** *48* (3), 417.

(4) Bodey, G. P.; Hersh, E. M.; Valdivieso, M.; Feid, R.; Rodriguez, V. Effects of cytotoxic and immunosuppressive agents on the immune system. *Postgraduate medicine* **1975,** *58* (7), 67.

(5) Cheson, B. D. Infectious and immunosuppressive complications of purine analog therapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1995,** *13* (9), 2431.

(6) Dale, D. C. Colony-stimulating factors for the management of neutropenia in cancer patients. *Drugs* **2002,** *62 Suppl 1*, 1.

(7) Reigle, B. S.; Dienger, M. J. Sepsis and treatment-induced immunosuppression in the patient with cancer. *Critical care nursing clinics of North America* **2003,** *15* (1), 109.

(8) van der Most, R. G.; Robinson, B. W.; Lake, R. A. Combining immunotherapy with chemotherapy to treat cancer. *Discovery medicine* **2005,** *5* (27), 265.

(9) Gandhi, L.; Rodriguez-Abreu, D.; Gadgeel, S.; Esteban, E.; Felip, E.; De Angelis, F.; Domine, M.; Clingan, P.; Hochmair, M. J.; Powell, S. F.et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* **2018**.

(10) Bracci, L.; Schiavoni, G.; Sistigu, A.; Belardelli, F. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. *Cell Death Differ* **2014,** *21* (1), 15.

(11) Brown, J. S.; Sundar, R.; Lopez, J. Combining DNA damaging therapeutics with immunotherapy: more haste, less speed. *Br J Cancer* **2018,** *118* (3), 312.

(12) Galluzzi, L.; Buque, A.; Kepp, O.; Zitvogel, L.; Kroemer, G. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. *Cancer cell* **2015,** *28* (6), 690.

(13) Gotwals, P.; Cameron, S.; Cipolletta, D.; Cremasco, V.; Crystal, A.; Hewes, B.; Mueller, B.; Quaratino, S.; Sabatos-Peyton, C.; Petruzzelli, L.et al. Prospects for combining targeted and conventional cancer therapy with immunotherapy. *Nature reviews. Cancer* **2017,** *17* (5), 286.

(14) Wu, J.; Waxman, D. J. Immunogenic chemotherapy: Dose and schedule dependence and combination with immunotherapy. *Cancer Lett* **2018,** *419*, 210.

(15) Rubner, Y.; Wunderlich, R.; Ruhle, P. F.; Kulzer, L.; Werthmoller, N.; Frey, B.; Weiss, E. M.; Keilholz, L.; Fietkau, R.; Gaipl, U. S. How does ionizing irradiation contribute to the induction of anti-tumor immunity? *Frontiers in oncology* **2012,** *2*, 75.

(16) Sharma, P.; Allison, J. P. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* **2015,** *161* (2), 205.

(17) Rivera Vargas, T.; Apetoh, L. Danger signals: Chemotherapy enhancers? *Immunological reviews* **2017,** *280* (1), 175.

(18) Langer, C. J.; Gadgeel, S. M.; Borghaei, H.; Papadimitrakopoulou, V. A.; Patnaik, A.; Powell, S. F.; Gentzler, R. D.; Martins, R. G.; Stevenson, J. P.; Jalal, S. I.et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *The Lancet. Oncology* **2016,** *17* (11), 1497.

(19) Dilruba, S.; Kalayda, G. V. Platinum-based drugs: past, present and future. *Cancer Chemother Pharmacol* **2016,** *77* (6), 1103.

(20) de Biasi, A. R.; Villena-Vargas, J.; Adusumilli, P. S. Cisplatin-induced antitumor immunomodulation: a review of preclinical and clinical evidence. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2014,** *20* (21), 5384.
(21) Hato, S. V.; Khong, A.; de Vries, I. J.; Lesterhuis, W. J. Molecular pathways: the immunogenic effects of platinum-based chemotherapeutics. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2014,** *20* (11), 2831.
(22) Terenzi, A.; Pirker, C.; Keppler, B. K.; Berger, W. Anticancer metal drugs and immunogenic cell death. *J Inorg Biochem* **2016,** *165*, 71.
(23) Bruijnincx, P. C.; Sadler, P. J. New trends for metal complexes with anticancer activity. *Curr Opin Chem Biol* **2008,** *12* (2), 197.
(24) Gasser, G.; Metzler-Nolte, N. The potential of organometallic complexes in medicinal chemistry. *Curr Opin Chem Biol* **2012,** *16* (1-2), 84.
(25) Ott, I.; Gust, R. Non platinum metal complexes as anti-cancer drugs. *Arch Pharm (Weinheim)* **2007,** *340* (3), 117.
(26) Allardyce, C. S.; Dyson, P. J. Metal-based drugs that break the rules. *Dalton Trans* **2016,** *45* (8), 3201.
(27) Johnstone, T. C.; Suntharalingam, K.; Lippard, S. J. The Next Generation of Platinum Drugs: Targeted Pt(II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs. *Chemical reviews* **2016,** *116* (5), 3436.
(28) Savage, P. A.; Leventhal, D. S.; Malchow, S. Shaping the repertoire of tumor-infiltrating effector and regulatory T cells. *Immunological reviews* **2014,** *259* (1), 245.
(29) Muenst, S.; Laubli, H.; Soysal, S. D.; Zippelius, A.; Tzankov, A.; Hoeller, S. The immune system and cancer evasion strategies: therapeutic concepts. *J Intern Med* **2016,** *279* (6), 541.
(30) Decker, W. K.; da Silva, R. F.; Sanabria, M. H.; Angelo, L. S.; Guimaraes, F.; Burt, B. M.; Kheradmand, F.; Paust, S. Cancer Immunotherapy: Historical Perspective of a Clinical Revolution and Emerging Preclinical Animal Models. *Frontiers in immunology* **2017,** *8*, 829.
(31) Burnet, F. M. The concept of immunological surveillance. *Prog Exp Tumor Res* **1970,** *13*, 1.
(32) Swann, J. B.; Smyth, M. J. Immune surveillance of tumors. *The Journal of clinical investigation* **2007,** *117* (5), 1137.
(33) Galon, J.; Angell, H. K.; Bedognetti, D.; Marincola, F. M. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. *Immunity* **2013,** *39* (1), 11.
(34) Matzinger, P. The danger model: a renewed sense of self. *Science* **2002,** *296* (5566), 301.
(35) Rizvi, N. A.; Hellmann, M. D.; Snyder, A.; Kvistborg, P.; Makarov, V.; Havel, J. J.; Lee, W.; Yuan, J.; Wong, P.; Ho, T. S.et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **2015,** *348* (6230), 124.
(36) Nebot-Bral, L.; Brandao, D.; Verlingue, L.; Rouleau, E.; Caron, O.; Despras, E.; El-Dakdouki, Y.; Champiat, S.; Aoufouchi, S.; Leary, A.et al. Hypermutated tumours in the era of immunotherapy: The paradigm of personalised medicine. *European journal of cancer* **2017,** *84*, 290.
(37) Vinay, D. S.; Ryan, E. P.; Pawelec, G.; Talib, W. H.; Stagg, J.; Elkord, E.; Lichtor, T.; Decker, W. K.; Whelan, R. L.; Kumara, H.et al. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Semin Cancer Biol* **2015,** *35 Suppl*, S185.
(38) McGranahan, N.; Furness, A. J.; Rosenthal, R.; Ramskov, S.; Lyngaa, R.; Saini, S. K.; Jamal-Hanjani, M.; Wilson, G. A.; Birkbak, N. J.; Hiley, C. T.et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* **2016,** *351* (6280), 1463.
(39) Mapara, M. Y.; Sykes, M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2004,** *22* (6), 1136.

(40) Mittal, D.; Gubin, M. M.; Schreiber, R. D.; Smyth, M. J. New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape. *Curr Opin Immunol* **2014,** *27*, 16.
(41) Vesely, M. D.; Schreiber, R. D. Cancer immunoediting: antigens, mechanisms, and implications to cancer immunotherapy. *Annals of the New York Academy of Sciences* **2013,** *1284*, 1.
(42) Dunn, G. P.; Old, L. J.; Schreiber, R. D. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* **2004,** *21* (2), 137.
(43) Galluzzi, L.; Buque, A.; Kepp, O.; Zitvogel, L.; Kroemer, G. Immunogenic cell death in cancer and infectious disease. *Nat Rev Immunol* **2017,** *17* (2), 97.
(44) Koebel, C. M.; Vermi, W.; Swann, J. B.; Zerafa, N.; Rodig, S. J.; Old, L. J.; Smyth, M. J.; Schreiber, R. D. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* **2007,** *450* (7171), 903.
(45) Fridman, W. H.; Remark, R.; Goc, J.; Giraldo, N. A.; Becht, E.; Hammond, S. A.; Damotte, D.; Dieu-Nosjean, M. C.; Sautes-Fridman, C. The immune microenvironment: a major player in human cancers. *International archives of allergy and immunology* **2014,** *164* (1), 13.
(46) Verdegaal, E. M.; de Miranda, N. F.; Visser, M.; Harryvan, T.; van Buuren, M. M.; Andersen, R. S.; Hadrup, S. R.; van der Minne, C. E.; Schotte, R.; Spits, H.et al. Neoantigen landscape dynamics during human melanoma-T cell interactions. *Nature* **2016,** *536* (7614), 91.
(47) Alberts, B. *Molecular biology of the cell*; Sixth edition. ed.; Garland Science, Taylor and Francis Group: New York, NY, 2015.
(48) Murphy, K.; Weaver, C. *Janeway's immunobiology*; 9th edition. ed.; Garland Science/Taylor & Francis Group, LLC: New York, NY, 2016.
(49) Holder, K. A.; Comeau, E. M.; Grant, M. D. Origins of natural killer cell memory: special creation or adaptive evolution. *Immunology* **2018**.
(50) Sun, J. C.; Lanier, L. L. Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination? NK Cell Memory and Immunization Strategies against Infectious Diseases and Cancer. *Cold Spring Harbor perspectives in biology* **2017**.
(51) Peng, H.; Tian, Z. Natural Killer Cell Memory: Progress and Implications. *Frontiers in immunology* **2017,** *8*, 1143.
(52) Vesely, M. D.; Kershaw, M. H.; Schreiber, R. D.; Smyth, M. J. Natural innate and adaptive immunity to cancer. *Annual review of immunology* **2011,** *29*, 235.
(53) Schrama, D.; Ritter, C.; Becker, J. C. T cell receptor repertoire usage in cancer as a surrogate marker for immune responses. *Seminars in immunopathology* **2017,** *39* (3), 255.
(54) Neefjes, J.; Jongsma, M. L.; Paul, P.; Bakke, O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol* **2011,** *11* (12), 823.
(55) Rock, K. L.; Reits, E.; Neefjes, J. Present Yourself! By MHC Class I and MHC Class II Molecules. *Trends in immunology* **2016,** *37* (11), 724.
(56) LeBien, T. W.; Tedder, T. F. B lymphocytes: how they develop and function. *Blood* **2008,** *112* (5), 1570.
(57) Gul, N.; van Egmond, M. Antibody-Dependent Phagocytosis of Tumor Cells by Macrophages: A Potent Effector Mechanism of Monoclonal Antibody Therapy of Cancer. *Cancer research* **2015,** *75* (23), 5008.
(58) Reis, E. S.; Mastellos, D. C.; Ricklin, D.; Mantovani, A.; Lambris, J. D. Complement in cancer: untangling an intricate relationship. *Nat Rev Immunol* **2017**.
(59) Galdiero, M. R.; Bonavita, E.; Barajon, I.; Garlanda, C.; Mantovani, A.; Jaillon, S. Tumor associated macrophages and neutrophils in cancer. *Immunobiology* **2013,** *218* (11), 1402.
(60) Uppendahl, L. D.; Dahl, C. M.; Miller, J. S.; Felices, M.; Geller, M. A. Natural Killer Cell-Based Immunotherapy in Gynecologic Malignancy: A Review. *Frontiers in immunology* **2017,** *8*, 1825.

(61) Malmberg, K. J.; Carlsten, M.; Bjorklund, A.; Sohlberg, E.; Bryceson, Y. T.; Ljunggren, H. G. Natural killer cell-mediated immunosurveillance of human cancer. *Seminars in immunology* **2017,** *31*, 20.
(62) Romagne, F.; Andre, P.; Spee, P.; Zahn, S.; Anfossi, N.; Gauthier, L.; Capanni, M.; Ruggeri, L.; Benson, D. M., Jr.; Blaser, B. W.et al. Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. *Blood* **2009,** *114* (13), 2667.
(63) Seliger, B.; Harders, C.; Wollscheid, U.; Staege, M. S.; Reske-Kunz, A. B.; Huber, C. Suppression of MHC class I antigens in oncogenic transformants: association with decreased recognition by cytotoxic T lymphocytes. *Experimental hematology* **1996,** *24* (11), 1275.
(64) Bradley, S. D.; Chen, Z.; Melendez, B.; Talukder, A.; Khalili, J. S.; Rodriguez-Cruz, T.; Liu, S.; Whittington, M.; Deng, W.; Li, F.et al. BRAFV600E Co-opts a Conserved MHC Class I Internalization Pathway to Diminish Antigen Presentation and CD8+ T-cell Recognition of Melanoma. *Cancer immunology research* **2015,** *3* (6), 602.
(65) Bruno, A.; Ferlazzo, G.; Albini, A.; Noonan, D. M. A think tank of TINK/TANKs: tumor-infiltrating/tumor-associated natural killer cells in tumor progression and angiogenesis. *Journal of the National Cancer Institute* **2014,** *106* (8), dju200.
(66) Moretta, L.; Montaldo, E.; Vacca, P.; Del Zotto, G.; Moretta, F.; Merli, P.; Locatelli, F.; Mingari, M. C. Human natural killer cells: origin, receptors, function, and clinical applications. *International archives of allergy and immunology* **2014,** *164* (4), 253.
(67) Raulet, D. H.; Guerra, N. Oncogenic stress sensed by the immune system: role of natural killer cell receptors. *Nat Rev Immunol* **2009,** *9* (8), 568.
(68) Vitale, M.; Cantoni, C.; Pietra, G.; Mingari, M. C.; Moretta, L. Effect of tumor cells and tumor microenvironment on NK-cell function. *European journal of immunology* **2014,** *44* (6), 1582.
(69) Sungur, C. M.; Murphy, W. J. Positive and negative regulation by NK cells in cancer. *Critical reviews in oncogenesis* **2014,** *19* (1-2), 57.
(70) Groh, V.; Wu, J.; Yee, C.; Spies, T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* **2002,** *419* (6908), 734.
(71) Garg, A. D.; Agostinis, P. Cell death and immunity in cancer: From danger signals to mimicry of pathogen defense responses. *Immunological reviews* **2017,** *280* (1), 126.
(72) da Cunha, A.; Michelin, M. A.; Murta, E. F. Pattern response of dendritic cells in the tumor microenvironment and breast cancer. *World journal of clinical oncology* **2014,** *5* (3), 495.
(73) Tang, D.; Kang, R.; Coyne, C. B.; Zeh, H. J.; Lotze, M. T. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunological reviews* **2012,** *249* (1), 158.
(74) Thaiss, C. A.; Levy, M.; Itav, S.; Elinav, E. Integration of Innate Immune Signaling. *Trends in immunology* **2016,** *37* (2), 84.
(75) Kieser, K. J.; Kagan, J. C. Multi-receptor detection of individual bacterial products by the innate immune system. *Nat Rev Immunol* **2017,** *17* (6), 376.
(76) DiLillo, D. J.; Ravetch, J. V. Fc-Receptor Interactions Regulate Both Cytotoxic and Immunomodulatory Therapeutic Antibody Effector Functions. *Cancer immunology research* **2015,** *3* (7), 704.
(77) Guilliams, M.; Bruhns, P.; Saeys, Y.; Hammad, H.; Lambrecht, B. N. The function of Fcgamma receptors in dendritic cells and macrophages. *Nat Rev Immunol* **2014,** *14* (2), 94.
(78) Lippitz, B. E. Cytokine patterns in patients with cancer: a systematic review. *The Lancet. Oncology* **2013,** *14* (6), e218.
(79) Mellman, I.; Coukos, G.; Dranoff, G. Cancer immunotherapy comes of age. *Nature* **2011,** *480* (7378), 480.
(80) Palucka, A. K.; Coussens, L. M. The Basis of Oncoimmunology. *Cell* **2016,** *164* (6), 1233.
(81) Motz, G. T.; Coukos, G. Deciphering and reversing tumor immune suppression. *Immunity* **2013,** *39* (1), 61.

(82) Kantono, M.; Guo, B. Inflammasomes and Cancer: The Dynamic Role of the Inflammasome in Tumor Development. *Frontiers in immunology* **2017,** *8*, 1132.
(83) Karki, R.; Man, S. M.; Kanneganti, T. D. Inflammasomes and Cancer. *Cancer immunology research* **2017,** *5* (2), 94.
(84) Guo, H.; Callaway, J. B.; Ting, J. P. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nature medicine* **2015,** *21* (7), 677.
(85) Westbom, C.; Thompson, J. K.; Leggett, A.; MacPherson, M.; Beuschel, S.; Pass, H.; Vacek, P.; Shukla, A. Inflammasome Modulation by Chemotherapeutics in Malignant Mesothelioma. *PloS one* **2015,** *10* (12), e0145404.
(86) Roy, S.; Trinchieri, G. Microbiota: a key orchestrator of cancer therapy. *Nature reviews. Cancer* **2017,** *17* (5), 271.
(87) Tremble, L. F.; Forde, P. F.; Soden, D. M. Clinical evaluation of macrophages in cancer: role in treatment, modulation and challenges. *Cancer Immunol Immunother* **2017,** *66* (12), 1509.
(88) Lichtnekert, J.; Kawakami, T.; Parks, W. C.; Duffield, J. S. Changes in macrophage phenotype as the immune response evolves. *Current opinion in pharmacology* **2013,** *13* (4), 555.
(89) Verstak, B.; Nagpal, K.; Bottomley, S. P.; Golenbock, D. T.; Hertzog, P. J.; Mansell, A. MyD88 adapter-like (Mal)/TIRAP interaction with TRAF6 is critical for TLR2- and TLR4-mediated NF-kappaB proinflammatory responses. *J Biol Chem* **2009,** *284* (36), 24192.
(90) Rhee, I. Diverse macrophages polarization in tumor microenvironment. *Archives of pharmacal research* **2016,** *39* (11), 1588.
(91) Galli, S. J.; Borregaard, N.; Wynn, T. A. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nature immunology* **2011,** *12* (11), 1035.
(92) van den Boorn, J. G.; Hartmann, G. Turning tumors into vaccines: co-opting the innate immune system. *Immunity* **2013,** *39* (1), 27.
(93) Gabrilovich, D. I.; Nagaraj, S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* **2009,** *9* (3), 162.
(94) Gregory, A. D.; Houghton, A. M. Tumor-associated neutrophils: new targets for cancer therapy. *Cancer research* **2011,** *71* (7), 2411.
(95) Galdiero, M. R.; Varricchi, G.; Loffredo, S.; Mantovani, A.; Marone, G. Roles of neutrophils in cancer growth and progression. *Journal of leukocyte biology* **2018,** *103* (3), 457.
(96) Kiss, M.; Van Gassen, S.; Movahedi, K.; Saeys, Y.; Laoui, D. Myeloid cell heterogeneity in cancer: not a single cell alike. *Cellular immunology* **2018**.
(97) Chao, M. P.; Jaiswal, S.; Weissman-Tsukamoto, R.; Alizadeh, A. A.; Gentles, A. J.; Volkmer, J.; Weiskopf, K.; Willingham, S. B.; Raveh, T.; Park, C. Y.et al. Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. *Sci Transl Med* **2010,** *2* (63), 63ra94.
(98) Chanmee, T.; Ontong, P.; Konno, K.; Itano, N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers* **2014,** *6* (3), 1670.
(99) Grivennikov, S. I.; Greten, F. R.; Karin, M. Immunity, inflammation, and cancer. *Cell* **2010,** *140* (6), 883.
(100) Noy, R.; Pollard, J. W. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* **2014,** *41* (1), 49.
(101) Mantovani, A.; Sica, A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol* **2010,** *22* (2), 231.
(102) Gabrilovich, D. I.; Ostrand-Rosenberg, S.; Bronte, V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* **2012,** *12* (4), 253.
(103) Rodriguez, P. C.; Hernandez, C. P.; Quiceno, D.; Dubinett, S. M.; Zabaleta, J.; Ochoa, J. B.; Gilbert, J.; Ochoa, A. C. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *The Journal of experimental medicine* **2005,** *202* (7), 931.

(104) Donkor, M. K.; Lahue, E.; Hoke, T. A.; Shafer, L. R.; Coskun, U.; Solheim, J. C.; Gulen, D.; Bishay, J.; Talmadge, J. E. Mammary tumor heterogeneity in the expansion of myeloid-derived suppressor cells. *International immunopharmacology* **2009,** *9* (7-8), 937.
(105) Mao, Y.; Poschke, I.; Wennerberg, E.; Pico de Coana, Y.; Egyhazi Brage, S.; Schultz, I.; Hansson, J.; Masucci, G.; Lundqvist, A.; Kiessling, R. Melanoma-educated CD14+ cells acquire a myeloid-derived suppressor cell phenotype through COX-2-dependent mechanisms. *Cancer research* **2013,** *73* (13), 3877.
(106) Schreiber, R. D.; Old, L. J.; Smyth, M. J. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* **2011,** *331* (6024), 1565.
(107) Kim, J. M.; Chen, D. S. Immune escape to PD-L1/PD-1 blockade: seven steps to success (or failure). *Annals of oncology : official journal of the European Society for Medical Oncology* **2016,** *27* (8), 1492.
(108) Gubin, M. M.; Zhang, X.; Schuster, H.; Caron, E.; Ward, J. P.; Noguchi, T.; Ivanova, Y.; Hundal, J.; Arthur, C. D.; Krebber, W. J.et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* **2014,** *515* (7528), 577.
(109) Dieu-Nosjean, M. C.; Giraldo, N. A.; Kaplon, H.; Germain, C.; Fridman, W. H.; Sautes-Fridman, C. Tertiary lymphoid structures, drivers of the anti-tumor responses in human cancers. *Immunological reviews* **2016,** *271* (1), 260.
(110) Pedoeem, A.; Azoulay-Alfaguter, I.; Strazza, M.; Silverman, G. J.; Mor, A. Programmed death-1 pathway in cancer and autoimmunity. *Clinical immunology* **2014,** *153* (1), 145.
(111) Chen, D. S.; Mellman, I. Elements of cancer immunity and the cancer-immune set point. *Nature* **2017,** *541* (7637), 321.
(112) Topalian, S. L.; Drake, C. G.; Pardoll, D. M. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer cell* **2015,** *27* (4), 450.
(113) Farkona, S.; Diamandis, E. P.; Blasutig, I. M. Cancer immunotherapy: the beginning of the end of cancer? *BMC Med* **2016,** *14*, 73.
(114) Boyman, O.; Sprent, J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev Immunol* **2012,** *12* (3), 180.
(115) Laidlaw, B. J.; Craft, J. E.; Kaech, S. M. The multifaceted role of CD4(+) T cells in CD8(+) T cell memory. *Nat Rev Immunol* **2016,** *16* (2), 102.
(116) Gajewski, T. F.; Woo, S. R.; Zha, Y.; Spaapen, R.; Zheng, Y.; Corrales, L.; Spranger, S. Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. *Curr Opin Immunol* **2013,** *25* (2), 268.
(117) Gajewski, T. F. The Next Hurdle in Cancer Immunotherapy: Overcoming the Non-T-Cell-Inflamed Tumor Microenvironment. *Seminars in oncology* **2015,** *42* (4), 663.
(118) Rooney, M. S.; Shukla, S. A.; Wu, C. J.; Getz, G.; Hacohen, N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* **2015,** *160* (1-2), 48.
(119) Ryan, A. E.; Shanahan, F.; O'Connell, J.; Houston, A. M. Addressing the "Fas counterattack" controversy: blocking fas ligand expression suppresses tumor immune evasion of colon cancer in vivo. *Cancer research* **2005,** *65* (21), 9817.
(120) Godfrey, D. I.; Le Nours, J.; Andrews, D. M.; Uldrich, A. P.; Rossjohn, J. Unconventional T Cell Targets for Cancer Immunotherapy. *Immunity* **2018,** *48* (3), 453.
(121) Hao, J.; Wu, X.; Xia, S.; Li, Z.; Wen, T.; Zhao, N.; Wu, Z.; Wang, P.; Zhao, L.; Yin, Z. Current progress in gammadelta T-cell biology. *Cellular & molecular immunology* **2010,** *7* (6), 409.
(122) Zou, C.; Zhao, P.; Xiao, Z.; Han, X.; Fu, F.; Fu, L. gammadelta T cells in cancer immunotherapy. *Oncotarget* **2017,** *8* (5), 8900.
(123) Nair, S.; Dhodapkar, M. V. Natural Killer T Cells in Cancer Immunotherapy. *Frontiers in immunology* **2017,** *8*, 1178.

(124) Wolf, B. J.; Choi, J. E.; Exley, M. A. Novel Approaches to Exploiting Invariant NKT Cells in Cancer Immunotherapy. *Frontiers in immunology* **2018,** *9*, 384.
(125) Schumacher, T. N.; Schreiber, R. D. Neoantigens in cancer immunotherapy. *Science* **2015,** *348* (6230), 69.
(126) Johnsen, A. K.; Templeton, D. J.; Sy, M.; Harding, C. V. Deficiency of transporter for antigen presentation (TAP) in tumor cells allows evasion of immune surveillance and increases tumorigenesis. *J Immunol* **1999,** *163* (8), 4224.
(127) Cabrera, C. M.; Jimenez, P.; Cabrera, T.; Esparza, C.; Ruiz-Cabello, F.; Garrido, F. Total loss of MHC class I in colorectal tumors can be explained by two molecular pathways: beta2-microglobulin inactivation in MSI-positive tumors and LMP7/TAP2 downregulation in MSI-negative tumors. *Tissue antigens* **2003,** *61* (3), 211.
(128) Speiser, D. E.; Ho, P. C.; Verdeil, G. Regulatory circuits of T cell function in cancer. *Nat Rev Immunol* **2016,** *16* (10), 599.
(129) Calon, A.; Espinet, E.; Palomo-Ponce, S.; Tauriello, D. V.; Iglesias, M.; Cespedes, M. V.; Sevillano, M.; Nadal, C.; Jung, P.; Zhang, X. H.et al. Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. *Cancer cell* **2012,** *22* (5), 571.
(130) Savage, P. A.; Malchow, S.; Leventhal, D. S. Basic principles of tumor-associated regulatory T cell biology. *Trends in immunology* **2013,** *34* (1), 33.
(131) Allen, M.; Louise Jones, J. Jekyll and Hyde: the role of the microenvironment on the progression of cancer. *The Journal of pathology* **2011,** *223* (2), 162.
(132) Sakaguchi, S.; Wing, K.; Onishi, Y.; Prieto-Martin, P.; Yamaguchi, T. Regulatory T cells: how do they suppress immune responses? *International immunology* **2009,** *21* (10), 1105.
(133) Nishikawa, H.; Sakaguchi, S. Regulatory T cells in cancer immunotherapy. *Curr Opin Immunol* **2014,** *27*, 1.
(134) Ward-Hartstonge, K. A.; Kemp, R. A. Regulatory T-cell heterogeneity and the cancer immune response. *Clinical & translational immunology* **2017,** *6* (9), e154.
(135) Jiang, X.; Shapiro, D. J. The immune system and inflammation in breast cancer. *Molecular and cellular endocrinology* **2014,** *382* (1), 673.
(136) Chaudhary, B.; Elkord, E. Regulatory T Cells in the Tumor Microenvironment and Cancer Progression: Role and Therapeutic Targeting. *Vaccines* **2016,** *4* (3).
(137) Zhang, S.; Wu, M.; Wang, F. Immune regulation by CD8(+) Treg cells: novel possibilities for anticancer immunotherapy. *Cellular & molecular immunology* **2018**.
(138) Zeng, H.; Zhang, R.; Jin, B.; Chen, L. Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance. *Cellular & molecular immunology* **2015,** *12* (5), 566.
(139) Kim, W. S.; Kim, H.; Kwon, K. W.; Im, S. H.; Lee, B. R.; Ha, S. J.; Shin, S. J. Cisplatin induces tolerogenic dendritic cells in response to TLR agonists via the abundant production of IL-10, thereby promoting Th2- and Tr1-biased T-cell immunity. *Oncotarget* **2016,** *7* (23), 33765.
(140) Benencia, F.; Muccioli, M.; Alnaeeli, M. Perspectives on reprograming cancer-associated dendritic cells for anti-tumor therapies. *Frontiers in oncology* **2014,** *4*, 72.
(141) Herbst, R. S.; Soria, J. C.; Kowanetz, M.; Fine, G. D.; Hamid, O.; Gordon, M. S.; Sosman, J. A.; McDermott, D. F.; Powderly, J. D.; Gettinger, S. N.et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* **2014,** *515* (7528), 563.
(142) Im, S. J.; Hashimoto, M.; Gerner, M. Y.; Lee, J.; Kissick, H. T.; Burger, M. C.; Shan, Q.; Hale, J. S.; Lee, J.; Nasti, T. H.et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* **2016,** *537* (7620), 417.
(143) Tumeh, P. C.; Harview, C. L.; Yearley, J. H.; Shintaku, I. P.; Taylor, E. J.; Robert, L.; Chmielowski, B.; Spasic, M.; Henry, G.; Ciobanu, V.et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **2014,** *515* (7528), 568.

(144) Taube, J. M.; Klein, A.; Brahmer, J. R.; Xu, H.; Pan, X.; Kim, J. H.; Chen, L.; Pardoll, D. M.; Topalian, S. L.; Anders, R. A. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2014,** *20* (19), 5064.
(145) Veglia, F.; Perego, M.; Gabrilovich, D. Myeloid-derived suppressor cells coming of age. *Nature immunology* **2018,** *19* (2), 108.
(146) Sarvaria, A.; Madrigal, J. A.; Saudemont, A. B cell regulation in cancer and anti-tumor immunity. *Cellular & molecular immunology* **2017,** *14* (8), 662.
(147) Gunderson, A. J.; Coussens, L. M. B cells and their mediators as targets for therapy in solid tumors. *Experimental cell research* **2013,** *319* (11), 1644.
(148) Harwood, N. E.; Batista, F. D. Antigen presentation to B cells. *F1000 biology reports* **2010,** *2*, 87.
(149) Moens, L.; Tangye, S. G. Cytokine-Mediated Regulation of Plasma Cell Generation: IL-21 Takes Center Stage. *Frontiers in immunology* **2014,** *5*, 65.
(150) Leonard, W. J.; Spolski, R. Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. *Nat Rev Immunol* **2005,** *5* (9), 688.
(151) Weiner, L. M.; Surana, R.; Wang, S. Monoclonal antibodies: versatile platforms for cancer immunotherapy. *Nat Rev Immunol* **2010,** *10* (5), 317.
(152) Fishelson, Z.; Donin, N.; Zell, S.; Schultz, S.; Kirschfink, M. Obstacles to cancer immunotherapy: expression of membrane complement regulatory proteins (mCRPs) in tumors. *Molecular immunology* **2003,** *40* (2-4), 109.
(153) Fremd, C.; Schuetz, F.; Sohn, C.; Beckhove, P.; Domschke, C. B cell-regulated immune responses in tumor models and cancer patients. *Oncoimmunology* **2013,** *2* (7), e25443.
(154) Linnebacher, M.; Maletzki, C. Tumor-infiltrating B cells: The ignored players in tumor immunology. *Oncoimmunology* **2012,** *1* (7), 1186.
(155) Ron, Y.; de Baetselier, P.; Gordon, J.; Feldman, M.; Segal, S. Impairment of antigen-specific T cell proliferative response in B cell suppressed mice. *Adv Exp Med Biol* **1982,** *149*, 609.
(156) Klinker, M. W.; Lundy, S. K. Multiple mechanisms of immune suppression by B lymphocytes. *Molecular medicine* **2012,** *18*, 123.
(157) Shen, M.; Sun, Q.; Wang, J.; Pan, W.; Ren, X. Positive and negative functions of B lymphocytes in tumors. *Oncotarget* **2016,** *7* (34), 55828.
(158) de Visser, K. E.; Korets, L. V.; Coussens, L. M. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer cell* **2005,** *7* (5), 411.
(159) Markiewski, M. M.; DeAngelis, R. A.; Benencia, F.; Ricklin-Lichtsteiner, S. K.; Koutoulaki, A.; Gerard, C.; Coukos, G.; Lambris, J. D. Modulation of the antitumor immune response by complement. *Nature immunology* **2008,** *9* (11), 1225.
(160) Andreu, P.; Johansson, M.; Affara, N. I.; Pucci, F.; Tan, T.; Junankar, S.; Korets, L.; Lam, J.; Tawfik, D.; DeNardo, D. G.et al. FcRgamma activation regulates inflammation-associated squamous carcinogenesis. *Cancer cell* **2010,** *17* (2), 121.
(161) Austin, C. J.; Rendina, L. M. Targeting key dioxygenases in tryptophan-kynurenine metabolism for immunomodulation and cancer chemotherapy. *Drug discovery today* **2015,** *20* (5), 609.
(162) Grohmann, U.; Bronte, V. Control of immune response by amino acid metabolism. *Immunological reviews* **2010,** *236*, 243.
(163) Prendergast, G. C.; Malachowski, W. P.; DuHadaway, J. B.; Muller, A. J. Discovery of IDO1 Inhibitors: From Bench to Bedside. *Cancer research* **2017,** *77* (24), 6795.
(164) Chabanon, R. M.; Pedrero, M.; Lefebvre, C.; Marabelle, A.; Soria, J. C.; Postel-Vinay, S. Mutational Landscape and Sensitivity to Immune Checkpoint Blockers. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2016,** *22* (17), 4309.

(165) Porta, C.; Sica, A.; Riboldi, E. Tumor-associated myeloid cells: new understandings on their metabolic regulation and their influence in cancer immunotherapy. *The FEBS journal* **2018,** *285* (4), 717.
(166) Hegde, P. S.; Karanikas, V.; Evers, S. The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2016,** *22* (8), 1865.
(167) Joyce, J. A.; Fearon, D. T. T cell exclusion, immune privilege, and the tumor microenvironment. *Science* **2015,** *348* (6230), 74.
(168) Nicolis, I.; Curis, E.; Deschamps, P.; Benazeth, S. Arsenite medicinal use, metabolism, pharmacokinetics and monitoring in human hair. *Biochimie* **2009,** *91* (10), 1260.
(169) Nobili, S.; Mini, E.; Landini, I.; Gabbiani, C.; Casini, A.; Messori, L. Gold compounds as anticancer agents: chemistry, cellular pharmacology, and preclinical studies. *Med Res Rev* **2010,** *30* (3), 550.
(170) Hoonjan, M.; Jadhav, V.; Bhatt, P. Arsenic trioxide: insights into its evolution to an anticancer agent. *J Biol Inorg Chem* **2018**.
(171) Garbutcheon-Singh, K. B.; Grant, M. P.; Harper, B. W.; Krause-Heuer, A. M.; Manohar, M.; Orkey, N.; Aldrich-Wright, J. R. Transition metal based anticancer drugs. *Curr Top Med Chem* **2011,** *11* (5), 521.
(172) Muggia, F. M.; Bonetti, A.; Hoeschele, J. D.; Rozencweig, M.; Howell, S. B. Platinum Antitumor Complexes: 50 Years Since Barnett Rosenberg's Discovery. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2015,** *33* (35), 4219.
(173) Kelland, L. The resurgence of platinum-based cancer chemotherapy. *Nature reviews. Cancer* **2007,** *7* (8), 573.
(174) Calvert, A. H.; Newell, D. R.; Gumbrell, L. A.; O'Reilly, S.; Burnell, M.; Boxall, F. E.; Siddik, Z. H.; Judson, I. R.; Gore, M. E.; Wiltshaw, E. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1989,** *7* (11), 1748.
(175) Burzynski, S. R. Treatments for astrocytic tumors in children: current and emerging strategies. *Paediatr Drugs* **2006,** *8* (3), 167.
(176) Isakoff, M. S.; Bielack, S. S.; Meltzer, P.; Gorlick, R. Osteosarcoma: Current Treatment and a Collaborative Pathway to Success. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2015,** *33* (27), 3029.
(177) Fink, D.; Nebel, S.; Aebi, S.; Zheng, H.; Cenni, B.; Nehme, A.; Christen, R. D.; Howell, S. B. The role of DNA mismatch repair in platinum drug resistance. *Cancer research* **1996,** *56* (21), 4881.
(178) Chaney, S. G.; Campbell, S. L.; Bassett, E.; Wu, Y. Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. *Crit Rev Oncol Hematol* **2005,** *53* (1), 3.
(179) Petrelli, F.; Inno, A.; Ghidini, A.; Rimassa, L.; Tomasello, G.; Labianca, R.; Barni, S.; Giscad; Cremona, H. Second line with oxaliplatin- or irinotecan-based chemotherapy for gemcitabine-pretreated pancreatic cancer: A systematic review. *European journal of cancer* **2017,** *81*, 174.
(180) McLean, J.; Rho, Y. S.; Kuruba, G.; Mamo, A.; Gilabert, M.; Kavan, T.; Panasci, L.; Melnychuk, D.; Batist, G.; Kavan, P. Clinical Practice Patterns in Chemotherapeutic Treatment Regimens for Metastatic Colorectal Cancer. *Clin Colorectal Cancer* **2016,** *15* (2), 135.
(181) Doshi, G.; Sonpavde, G.; Sternberg, C. N. Clinical and pharmacokinetic evaluation of satraplatin. *Expert opinion on drug metabolism & toxicology* **2012,** *8* (1), 103.
(182) Chen, S. J.; Zhou, G. B.; Zhang, X. W.; Mao, J. H.; de The, H.; Chen, Z. From an old remedy to a magic bullet: molecular mechanisms underlying the therapeutic effects of arsenic in fighting leukemia. *Blood* **2011,** *117* (24), 6425.
(183) de The, H.; Pandolfi, P. P.; Chen, Z. Acute Promyelocytic Leukemia: A Paradigm for Oncoprotein-Targeted Cure. *Cancer cell* **2017,** *32* (5), 552.

(184) Liang, J. X.; Zhong, H. J.; Yang, G.; Vellaisamy, K.; Ma, D. L.; Leung, C. H. Recent development of transition metal complexes with in vivo antitumor activity. *J Inorg Biochem* **2017,** *177*, 276.
(185) Lazarevic, T.; Rilak, A.; Bugarcic, Z. D. Platinum, palladium, gold and ruthenium complexes as anticancer agents: Current clinical uses, cytotoxicity studies and future perspectives. *Eur J Med Chem* **2017,** *142*, 8.
(186) Mansfield, A. S.; Fields, A. P.; Jatoi, A.; Qi, Y.; Adjei, A. A.; Erlichman, C.; Molina, J. R. Phase I dose escalation study of the PKCiota inhibitor aurothiomalate for advanced non-small-cell lung cancer, ovarian cancer, and pancreatic cancer. *Anticancer Drugs* **2013,** *24* (10), 1079.
(187) Chitambar, C. R. The therapeutic potential of iron-targeting gallium compounds in human disease: From basic research to clinical application. *Pharmacological research* **2017,** *115*, 56.
(188) Burris, H. A.; Bakewell, S.; Bendell, J. C.; Infante, J.; Jones, S. F.; Spigel, D. R.; Weiss, G. J.; Ramanathan, R. K.; Ogden, A.; Von Hoff, D. Safety and activity of IT-139, a ruthenium-based compound, in patients with advanced solid tumours: a first-in-human, open-label, dose-escalation phase I study with expansion cohort. *ESMO Open* **2016,** *1* (6), e000154.
(189) Lentz, F.; Drescher, A.; Lindauer, A.; Henke, M.; Hilger, R. A.; Hartinger, C. G.; Scheulen, M. E.; Dittrich, C.; Keppler, B. K.; Jaehde, U.et al. Pharmacokinetics of a novel anticancer ruthenium complex (KP1019, FFC14A) in a phase I dose-escalation study. *Anticancer Drugs* **2009,** *20* (2), 97.
(190) Leijen, S.; Burgers, S. A.; Baas, P.; Pluim, D.; Tibben, M.; van Werkhoven, E.; Alessio, E.; Sava, G.; Beijnen, J. H.; Schellens, J. H. Phase I/II study with ruthenium compound NAMI-A and gemcitabine in patients with non-small cell lung cancer after first line therapy. *Invest New Drugs* **2015,** *33* (1), 201.
(191) Rademaker-Lakhai, J. M.; van den Bongard, D.; Pluim, D.; Beijnen, J. H.; Schellens, J. H. A Phase I and pharmacological study with imidazolium-trans-DMSO-imidazole-tetrachlororuthenate, a novel ruthenium anticancer agent. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2004,** *10* (11), 3717.
(192) Alessio, E.; Messori, L. The Deceptively Similar Ruthenium(III) Drug Candidates KP1019 and NAMI-A Have Different Actions. What Did We Learn in the Past 30 Years? *Met Ions Life Sci* **2018,** *18*.
(193) Fuereder, T.; Berger, W. Metal drugs become targeted. *ESMO Open* **2017,** *2* (3), e000239.
(194) Jungwirth, U.; Kowol, C. R.; Keppler, B. K.; Hartinger, C. G.; Berger, W.; Heffeter, P. Anticancer activity of metal complexes: involvement of redox processes. *Antioxid Redox Signal* **2011,** *15* (4), 1085.
(195) Trachootham, D.; Alexandre, J.; Huang, P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nature reviews. Drug discovery* **2009,** *8* (7), 579.
(196) Templeton, D. M. Speciation in Metal Toxicity and Metal-Based Therapeutics. *Toxics* **2015,** *3* (2), 170.
(197) Stojanovska, V.; Sakkal, S.; Nurgali, K. Platinum-based chemotherapy: gastrointestinal immunomodulation and enteric nervous system toxicity. *Am J Physiol Gastrointest Liver Physiol* **2015,** *308* (4), G223.
(198) Soultati, A.; Mountzios, G.; Avgerinou, C.; Papaxoinis, G.; Pectasides, D.; Dimopoulos, M. A.; Papadimitriou, C. Endothelial vascular toxicity from chemotherapeutic agents: preclinical evidence and clinical implications. *Cancer Treat Rev* **2012,** *38* (5), 473.
(199) Goncalves-Ribeiro, S.; Diaz-Maroto, N. G.; Berdiel-Acer, M.; Soriano, A.; Guardiola, J.; Martinez-Villacampa, M.; Salazar, R.; Capella, G.; Villanueva, A.; Martinez-Balibrea, E.et al. Carcinoma-associated fibroblasts affect sensitivity to oxaliplatin and 5FU in colorectal cancer cells. *Oncotarget* **2016,** *7* (37), 59766.
(200) Yoshikawa, A.; Saura, R.; Matsubara, T.; Mizuno, K. A mechanism of cisplatin action: antineoplastic effect through inhibition of neovascularization. *Kobe J Med Sci* **1997,** *43* (3-4), 109.
(201) Pavic, A.; Glisic, B. D.; Vojnovic, S.; Warzajtis, B.; Savic, N. D.; Antic, M.; Radenkovic, S.; Janjic, G. V.; Nikodinovic-Runic, J.; Rychlewska, U.et al. Mononuclear gold(III) complexes with

phenanthroline ligands as efficient inhibitors of angiogenesis: A comparative study with auranofin and sunitinib. *J Inorg Biochem* **2017,** *174*, 156.
(202) Bagowski, C. P.; You, Y.; Scheffler, H.; Vlecken, D. H.; Schmitz, D. J.; Ott, I. Naphthalimide gold(I) phosphine complexes as anticancer metallodrugs. *Dalton Trans* **2009,** (48), 10799.
(203) Nazarov, A. A.; Baquie, M.; Nowak-Sliwinska, P.; Zava, O.; van Beijnum, J. R.; Groessl, M.; Chisholm, D. M.; Ahmadi, Z.; McIndoe, J. S.; Griffioen, A. W.et al. Synthesis and characterization of a new class of anti-angiogenic agents based on ruthenium clusters. *Sci Rep* **2013,** *3*, 1485.
(204) Elie, B. T.; Pechenyy, Y.; Uddin, F.; Contel, M. A heterometallic ruthenium-gold complex displays antiproliferative, antimigratory, and antiangiogenic properties and inhibits metastasis and angiogenesis-associated proteases in renal cancer. *J Biol Inorg Chem* **2018,** *23* (3), 399.
(205) Vacca, A.; Bruno, M.; Boccarelli, A.; Coluccia, M.; Ribatti, D.; Bergamo, A.; Garbisa, S.; Sartor, L.; Sava, G. Inhibition of endothelial cell functions and of angiogenesis by the metastasis inhibitor NAMI-A. *Br J Cancer* **2002,** *86* (6), 993.
(206) Alessio, E.; Mestroni, G.; Bergamo, A.; Sava, G. Ruthenium antimetastatic agents. *Curr Top Med Chem* **2004,** *4* (15), 1525.
(207) Elie, B. T.; Pechenyy, Y.; Uddin, F.; Contel, M. A heterometallic ruthenium-gold complex displays antiproliferative, antimigratory, and antiangiogenic properties and inhibits metastasis and angiogenesis-associated proteases in renal cancer. *J Biol Inorg Chem* **2018**.
(208) Rosenberg, B.; VanCamp, L.; Trosko, J. E.; Mansour, V. H. Platinum compounds: a new class of potent antitumour agents. *Nature* **1969,** *222* (5191), 385.
(209) Rosenberg, B. In *Advances in Antimicrobial and Antineoplastic Chemotherapy,*; University Park Press: Baltimore, 1972; Vol. 11.
(210) Rosenberg, B. Fundamental studies with cisplatin. *Cancer* **1985,** *55* (10), 2303.
(211) Jungwirth, U.; Xanthos, D. N.; Gojo, J.; Bytzek, A. K.; Korner, W.; Heffeter, P.; Abramkin, S. A.; Jakupec, M. A.; Hartinger, C. G.; Windberger, U.et al. Anticancer activity of methyl-substituted oxaliplatin analogs. *Mol Pharmacol* **2012,** *81* (5), 719.
(212) Shimizu, T.; Abu Lila, A. S.; Nishio, M.; Doi, Y.; Ando, H.; Ukawa, M.; Ishima, Y.; Ishida, T. Modulation of antitumor immunity contributes to the enhanced therapeutic efficacy of liposomal oxaliplatin in mouse model. *Cancer Sci* **2017,** *108* (9), 1864.
(213) Chang, C. L.; Hsu, Y. T.; Wu, C. C.; Lai, Y. Z.; Wang, C.; Yang, Y. C.; Wu, T. C.; Hung, C. F. Dose-dense chemotherapy improves mechanisms of antitumor immune response. *Cancer research* **2013,** *73* (1), 119.
(214) Michaud, M.; Martins, I.; Sukkurwala, A. Q.; Adjemian, S.; Ma, Y.; Pellegatti, P.; Shen, S.; Kepp, O.; Scoazec, M.; Mignot, G.et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science* **2011,** *334* (6062), 1573.
(215) Apetoh, L.; Ghiringhelli, F.; Tesniere, A.; Obeid, M.; Ortiz, C.; Criollo, A.; Mignot, G.; Maiuri, M. C.; Ullrich, E.; Saulnier, P.et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature medicine* **2007,** *13* (9), 1050.
(216) Riddell, I. A., Lippard, J. S. Cisplatin and Oxaliplatin: Our Current Understanding of Their Actions. *Met Ions Life Sci* **2018,** *18*.
(217) Julien, S.; Merino-Trigo, A.; Lacroix, L.; Pocard, M.; Goere, D.; Mariani, P.; Landron, S.; Bigot, L.; Nemati, F.; Dartigues, P.et al. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2012,** *18* (19), 5314.
(218) Colucci, G.; Gebbia, V.; Paoletti, G.; Giuliani, F.; Caruso, M.; Gebbia, N.; Carteni, G.; Agostara, B.; Pezzella, G.; Manzione, L.et al. Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell'Italia Meridionale. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2005,** *23* (22), 4866.

(219) Tsuchiya, S.; Yamabe, M.; Yamaguchi, Y.; Kobayashi, Y.; Konno, T.; Tada, K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer* **1980,** *26* (2), 171.
(220) Introna, M. CIK as therapeutic agents against tumors. *Journal of autoimmunity* **2017,** *85*, 32.
(221) Bellone, G.; Novarino, A.; Vizio, B.; Brondino, G.; Addeo, A.; Prati, A.; Giacobino, A.; Campra, D.; Fronda, G. R.; Ciuffreda, L. Impact of surgery and chemotherapy on cellular immunity in pancreatic carcinoma patients in view of an integration of standard cancer treatment with immunotherapy. *International journal of oncology* **2009,** *34* (6), 1701.
(222) Cassado Ados, A.; D'Imperio Lima, M. R.; Bortoluci, K. R. Revisiting mouse peritoneal macrophages: heterogeneity, development, and function. *Frontiers in immunology* **2015,** *6*, 225.
(223) Zingoni, A.; Fionda, C.; Borrelli, C.; Cippitelli, M.; Santoni, A.; Soriani, A. Natural Killer Cell Response to Chemotherapy-Stressed Cancer Cells: Role in Tumor Immunosurveillance. *Frontiers in immunology* **2017,** *8*, 1194.
(224) Kim, J. Y.; Bae, J. H.; Lee, S. H.; Lee, E. Y.; Chung, B. S.; Kim, S. H.; Kang, C. D. Induction of NKG2D ligands and subsequent enhancement of NK cell-mediated lysis of cancer cells by arsenic trioxide. *J Immunother* **2008,** *31* (5), 475.
(225) Cao, G.; Wang, J.; Zheng, X.; Wei, H.; Tian, Z.; Sun, R. Tumor Therapeutics Work as Stress Inducers to Enhance Tumor Sensitivity to Natural Killer (NK) Cell Cytolysis by Up-regulating NKp30 Ligand B7-H6. *J Biol Chem* **2015,** *290* (50), 29964.
(226) Jelencic, V.; Lenartic, M.; Wensveen, F. M.; Polic, B. NKG2D: A versatile player in the immune system. *Immunol Lett* **2017,** *189*, 48.
(227) Soriani, A.; Zingoni, A.; Cerboni, C.; Iannitto, M. L.; Ricciardi, M. R.; Di Gialleonardo, V.; Cippitelli, M.; Fionda, C.; Petrucci, M. T.; Guarini, A.et al. ATM-ATR-dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. *Blood* **2009,** *113* (15), 3503.
(228) Sangster-Guity, N.; Conrad, B. H.; Papadopoulos, N.; Bunz, F. ATR mediates cisplatin resistance in a p53 genotype-specific manner. *Oncogene* **2011,** *30* (22), 2526.
(229) Smith, J.; Tho, L. M.; Xu, N.; Gillespie, D. A. The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. *Adv Cancer Res* **2010,** *108*, 73.
(230) Lai, H.; Zhao, Z.; Li, L.; Zheng, W.; Chen, T. Antiangiogenic ruthenium(ii) benzimidazole complexes, structure-based activation of distinct signaling pathways. *Metallomics* **2015,** *7* (3), 439.
(231) Bergamo, A.; Delfino, R.; Casarsa, C.; Sava, G. CDK1 hyperphosphorylation maintenance drives the time-course of G2-M cell cycle arrest after short treatment with NAMI-A in Kb cells. *Anticancer Agents Med Chem* **2012,** *12* (8), 949.
(232) Guerriero, J. L.; Ditsworth, D.; Catanzaro, J. M.; Sabino, G.; Furie, M. B.; Kew, R. R.; Crawford, H. C.; Zong, W. X. DNA alkylating therapy induces tumor regression through an HMGB1-mediated activation of innate immunity. *J Immunol* **2011,** *186* (6), 3517.
(233) Zhang, G.; Liu, Z.; Ding, H.; Zhou, Y.; Doan, H. A.; Sin, K. W. T.; Zhu, Z. J.; Flores, R.; Wen, Y.; Gong, X.et al. Tumor induces muscle wasting in mice through releasing extracellular Hsp70 and Hsp90. *Nat Commun* **2017,** *8* (1), 589.
(234) Zhu, H.; Fang, X.; Zhang, D.; Wu, W.; Shao, M.; Wang, L.; Gu, J. Membrane-bound heat shock proteins facilitate the uptake of dying cells and cross-presentation of cellular antigen. *Apoptosis* **2016,** *21* (1), 96.
(235) Ugrinova, I.; Pasheva, E. HMGB1 Protein: A Therapeutic Target Inside and Outside the Cell. *Adv Protein Chem Struct Biol* **2017,** *107*, 37.
(236) Li, G.; Liang, X.; Lotze, M. T. HMGB1: The Central Cytokine for All Lymphoid Cells. *Frontiers in immunology* **2013,** *4*, 68.

(237) Mori, Y.; Terauchi, R.; Shirai, T.; Tsuchida, S.; Mizoshiri, N.; Arai, Y.; Kishida, T.; Fujiwara, H.; Mazda, O.; Kubo, T. Suppression of heat shock protein 70 by siRNA enhances the antitumor effects of cisplatin in cultured human osteosarcoma cells. *Cell Stress Chaperones* **2017,** *22* (5), 699.
(238) Lesterhuis, W. J.; Punt, C. J.; Hato, S. V.; Eleveld-Trancikova, D.; Jansen, B. J.; Nierkens, S.; Schreibelt, G.; de Boer, A.; Van Herpen, C. M.; Kaanders, J. H.et al. Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice. *The Journal of clinical investigation* **2011,** *121* (8), 3100.
(239) Del Zotto, G.; Marcenaro, E.; Vacca, P.; Sivori, S.; Pende, D.; Della Chiesa, M.; Moretta, F.; Ingegnere, T.; Mingari, M. C.; Moretta, A.et al. Markers and function of human NK cells in normal and pathological conditions. *Cytometry B Clin Cytom* **2017,** *92* (2), 100.
(240) Huang, X.; Venet, F.; Wang, Y. L.; Lepape, A.; Yuan, Z.; Chen, Y.; Swan, R.; Kherouf, H.; Monneret, G.; Chung, C. S.et al. PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. *Proc Natl Acad Sci U S A* **2009,** *106* (15), 6303.
(241) Vivier, E.; Tomasello, E.; Baratin, M.; Walzer, T.; Ugolini, S. Functions of natural killer cells. *Nature immunology* **2008,** *9* (5), 503.
(242) Falschlehner, C.; Schaefer, U.; Walczak, H. Following TRAIL's path in the immune system. *Immunology* **2009,** *127* (2), 145.
(243) Tauzin, S.; Debure, L.; Moreau, J. F.; Legembre, P. CD95-mediated cell signaling in cancer: mutations and post-translational modulations. *Cell Mol Life Sci* **2012,** *69* (8), 1261.
(244) Cotter, T. G. Apoptosis and cancer: the genesis of a research field. *Nature reviews. Cancer* **2009,** *9* (7), 501.
(245) Houghton, J. A. Apoptosis and drug response. *Curr Opin Oncol* **1999,** *11* (6), 475.
(246) Fong, C. W. Platinum anti-cancer drugs: Free radical mechanism of Pt-DNA adduct formation and anti-neoplastic effect. *Free Radic Biol Med* **2016,** *95*, 216.
(247) Bose, R. N.; Moghaddas, S.; Belkacemi, L.; Tripathi, S.; Adams, N. R.; Majmudar, P.; McCall, K.; Dezvareh, H.; Nislow, C. Absence of Activation of DNA Repair Genes and Excellent Efficacy of Phosphaplatins against Human Ovarian Cancers: Implications To Treat Resistant Cancers. *J Med Chem* **2015,** *58* (21), 8387.
(248) Vondalova Blanarova, O.; Jelinkova, I.; Szoor, A.; Skender, B.; Soucek, K.; Horvath, V.; Vaculova, A.; Andera, L.; Sova, P.; Szollosi, J.et al. Cisplatin and a potent platinum(IV) complex-mediated enhancement of TRAIL-induced cancer cells killing is associated with modulation of upstream events in the extrinsic apoptotic pathway. *Carcinogenesis* **2011,** *32* (1), 42.
(249) Yang, C.; Liu, H. Z.; Fu, Z. X. PEG-liposomal oxaliplatin induces apoptosis in human colorectal cancer cells via Fas/FasL and caspase-8. *Cell Biol Int* **2012,** *36* (3), 289.
(250) El-Gazzar, A.; Perco, P.; Eckelhart, E.; Anees, M.; Sexl, V.; Mayer, B.; Liu, Y.; Mikulits, W.; Horvat, R.; Pangerl, T.et al. Natural immunity enhances the activity of a DR5 agonistic antibody and carboplatin in the treatment of ovarian cancer. *Mol Cancer Ther* **2010,** *9* (4), 1007.
(251) Pasello, G.; Urso, L.; Silic-Benussi, M.; Schiavon, M.; Cavallari, I.; Marulli, G.; Nannini, N.; Rea, F.; Ciminale, V.; Favaretto, A. Synergistic antitumor activity of recombinant human Apo2L/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in combination with carboplatin and pemetrexed in malignant pleural mesothelioma. *J Thorac Oncol* **2014,** *9* (7), 1008.
(252) Frost, P. J.; Butterfield, L. H.; Dissette, V. B.; Economou, J. S.; Bonavida, B. Immunosensitization of melanoma tumor cells to non-MHC Fas-mediated killing by MART-1-specific CTL cultures. *J Immunol* **2001,** *166* (5), 3564.
(253) van der Sluis, T. C.; van Duikeren, S.; Huppelschoten, S.; Jordanova, E. S.; Beyranvand Nejad, E.; Sloots, A.; Boon, L.; Smit, V. T.; Welters, M. J.; Ossendorp, F.et al. Vaccine-induced tumor necrosis factor-producing T cells synergize with cisplatin to promote tumor cell death. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2015,** *21* (4), 781.

(254) Sin, J. I.; Kim, J. M.; Bae, S. H.; Lee, I. H.; Park, J. S.; Ryoo, H. M. Adoptive transfer of human papillomavirus E7-specific CTL enhances tumor chemoresponse through the perforin/granzyme-mediated pathway. *Molecular therapy : the journal of the American Society of Gene Therapy* **2009,** *17* (5), 906.
(255) Ramakrishnan, R.; Assudani, D.; Nagaraj, S.; Hunter, T.; Cho, H. I.; Antonia, S.; Altiok, S.; Celis, E.; Gabrilovich, D. I. Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice. *The Journal of clinical investigation* **2010,** *120* (4), 1111.
(256) Bergmann-Leitner, E. S.; Abrams, S. I. Treatment of human colon carcinoma cell lines with anti-neoplastic agents enhances their lytic sensitivity to antigen-specific CD8+ cytotoxic T lymphocytes. *Cancer Immunol Immunother* **2001,** *50* (9), 445.
(257) Gelbard, A.; Garnett, C. T.; Abrams, S. I.; Patel, V.; Gutkind, J. S.; Palena, C.; Tsang, K. Y.; Schlom, J.; Hodge, J. W. Combination chemotherapy and radiation of human squamous cell carcinoma of the head and neck augments CTL-mediated lysis. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2006,** *12* (6), 1897.
(258) Gameiro, S. R.; Caballero, J. A.; Hodge, J. W. Defining the molecular signature of chemotherapy-mediated lung tumor phenotype modulation and increased susceptibility to T-cell killing. *Cancer Biother Radiopharm* **2012,** *27* (1), 23.
(259) Medzhitov, R.; Janeway, C. A., Jr. Decoding the patterns of self and nonself by the innate immune system. *Science* **2002,** *296* (5566), 298.
(260) Abramson, J.; Husebye, E. S. Autoimmune regulator and self-tolerance - molecular and clinical aspects. *Immunological reviews* **2016,** *271* (1), 127.
(261) Perniola, R. Twenty Years of AIRE. *Frontiers in immunology* **2018,** *9*, 98.
(262) Fuller, P. J.; Verity, K. Somatostatin gene expression in the thymus gland. *J Immunol* **1989,** *143* (3), 1015.
(263) Anderton, S. M.; Wraith, D. C. Selection and fine-tuning of the autoimmune T-cell repertoire. *Nat Rev Immunol* **2002,** *2* (7), 487.
(264) Sprent, J.; Cho, J. H. Self/non-self discrimination and the problem of keeping T cells alive. *Immunology and cell biology* **2008,** *86* (1), 54.
(265) Antonia, S. J.; Vansteenkiste, J. F.; Moon, E. Immunotherapy: Beyond Anti-PD-1 and Anti-PD-L1 Therapies. *Am Soc Clin Oncol Educ Book* **2016,** *35*, e450.
(266) Rotte, A.; Jin, J. Y.; Lemaire, V. Mechanistic overview of immune checkpoints to support the rational design of their combinations in cancer immunotherapy. *Annals of oncology : official journal of the European Society for Medical Oncology* **2018,** *29* (1), 71.
(267) Timosenko, E.; Hadjinicolaou, A. V.; Cerundolo, V. Modulation of cancer-specific immune responses by amino acid degrading enzymes. *Immunotherapy* **2017,** *9* (1), 83.
(268) Braunlein, E.; Krackhardt, A. M. Identification and Characterization of Neoantigens As Well As Respective Immune Responses in Cancer Patients. *Frontiers in immunology* **2017,** *8*, 1702.
(269) Siniard, R. C.; Harada, S. Immunogenomics: using genomics to personalize cancer immunotherapy. *Virchows Archiv : an international journal of pathology* **2017,** *471* (2), 209.
(270) Wellenstein, M. D.; de Visser, K. E. Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. *Immunity* **2018,** *48* (3), 399.
(271) Davoli, T.; Uno, H.; Wooten, E. C.; Elledge, S. J. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. *Science* **2017,** *355* (6322).
(272) Senovilla, L.; Vitale, I.; Martins, I.; Tailler, M.; Pailleret, C.; Michaud, M.; Galluzzi, L.; Adjemian, S.; Kepp, O.; Niso-Santano, M.et al. An immunosurveillance mechanism controls cancer cell ploidy. *Science* **2012,** *337* (6102), 1678.
(273) Bloy, N.; Garcia, P.; Laumont, C. M.; Pitt, J. M.; Sistigu, A.; Stoll, G.; Yamazaki, T.; Bonneil, E.; Buque, A.; Humeau, J.et al. Immunogenic stress and death of cancer cells: Contribution of antigenicity vs adjuvanticity to immunosurveillance. *Immunological reviews* **2017,** *280* (1), 165.

(274) States, J. C. Disruption of Mitotic Progression by Arsenic. *Biol Trace Elem Res* **2015,** *166* (1), 34.
(275) Wasenius, V. M.; Jekunen, A.; Monni, O.; Joensuu, H.; Aebi, S.; Howell, S. B.; Knuutila, S. Comparative genomic hybridization analysis of chromosomal changes occurring during development of acquired resistance to cisplatin in human ovarian carcinoma cells. *Genes, chromosomes & cancer* **1997,** *18* (4), 286.
(276) Stordal, B.; Peters, G.; Davey, R. Similar chromosomal changes in cisplatin and oxaliplatin-resistant sublines of the H69 SCLC cell line are not associated with platinum resistance. *Genes, chromosomes & cancer* **2006,** *45* (12), 1094.
(277) Castedo, M.; Coquelle, A.; Vivet, S.; Vitale, I.; Kauffmann, A.; Dessen, P.; Pequignot, M. O.; Casares, N.; Valent, A.; Mouhamad, S.et al. Apoptosis regulation in tetraploid cancer cells. *The EMBO journal* **2006,** *25* (11), 2584.
(278) Lewis, J. D.; Reilly, B. D.; Bright, R. K. Tumor-associated antigens: from discovery to immunity. *International reviews of immunology* **2003,** *22* (2), 81.
(279) Ohtsukasa, S.; Okabe, S.; Yamashita, H.; Iwai, T.; Sugihara, K. Increased expression of CEA and MHC class I in colorectal cancer cell lines exposed to chemotherapy drugs. *J Cancer Res Clin Oncol* **2003,** *129* (12), 719.
(280) Higgins, J. P.; Bernstein, M. B.; Hodge, J. W. Enhancing immune responses to tumor-associated antigens. *Cancer biology & therapy* **2009,** *8* (15), 1440.
(281) Dempsey, J. L.; Seshadri, R. S.; Morley, A. A. Increased mutation frequency following treatment with cancer chemotherapy. *Cancer research* **1985,** *45* (6), 2873.
(282) Franzese, O.; Torino, F.; Fuggetta, M. P.; Aquino, A.; Roselli, M.; Bonmassar, E.; Giuliani, A.; D'Atri, S. Tumor immunotherapy: drug-induced neoantigens (xenogenization) and immune checkpoint inhibitors. *Oncotarget* **2017,** *8* (25), 41641.
(283) Murugaesu, N.; Wilson, G. A.; Birkbak, N. J.; Watkins, T.; McGranahan, N.; Kumar, S.; Abbassi-Ghadi, N.; Salm, M.; Mitter, R.; Horswell, S.et al. Tracking the genomic evolution of esophageal adenocarcinoma through neoadjuvant chemotherapy. *Cancer Discov* **2015,** *5* (8), 821.
(284) Huang, K. K.; Jang, K. W.; Kim, S.; Kim, H. S.; Kim, S. M.; Kwon, H. J.; Kim, H. R.; Yun, H. J.; Ahn, M. J.; Park, K. U.et al. Exome sequencing reveals recurrent REV3L mutations in cisplatin-resistant squamous cell carcinoma of head and neck. *Sci Rep* **2016,** *6*, 19552.
(285) Szikriszt, B.; Poti, A.; Pipek, O.; Krzystanek, M.; Kanu, N.; Molnar, J.; Ribli, D.; Szeltner, Z.; Tusnady, G. E.; Csabai, I.et al. A comprehensive survey of the mutagenic impact of common cancer cytotoxics. *Genome Biol* **2016,** *17*, 99.
(286) Kuczynski, E. A.; Krueger, J.; Chow, A.; Xu, P.; Man, S.; Sundaravadanam, Y.; Miller, J. K.; Krzyzanowski, P. M.; Kerbel, R. S. Impact of chemical-induced mutational load increase on immune checkpoint therapy in poorly responsive murine tumors. *Mol Cancer Ther* **2018**.
(287) Patch, A. M.; Christie, E. L.; Etemadmoghadam, D.; Garsed, D. W.; George, J.; Fereday, S.; Nones, K.; Cowin, P.; Alsop, K.; Bailey, P. J.et al. Whole-genome characterization of chemoresistant ovarian cancer. *Nature* **2015,** *521* (7553), 489.
(288) O'Donnell, T.; Christie, E. L.; Ahuja, A.; Buros, J.; Aksoy, B. A.; Bowtell, D. D. L.; Snyder, A.; Hammerbacher, J. Chemotherapy weakly contributes to predicted neoantigen expression in ovarian cancer. *BMC Cancer* **2018,** *18* (1), 87.
(289) Findlay, J. M.; Castro-Giner, F.; Makino, S.; Rayner, E.; Kartsonaki, C.; Cross, W.; Kovac, M.; Ulahannan, D.; Palles, C.; Gillies, R. S.et al. Differential clonal evolution in oesophageal cancers in response to neo-adjuvant chemotherapy. *Nat Commun* **2016,** *7*, 11111.
(290) Liu, D.; Abbosh, P.; Keliher, D.; Reardon, B.; Miao, D.; Mouw, K.; Weiner-Taylor, A.; Wankowicz, S.; Han, G.; Teo, M. Y.et al. Mutational patterns in chemotherapy resistant muscle-invasive bladder cancer. *Nat Commun* **2017,** *8* (1), 2193.
(291) Matlung, H. L.; Szilagyi, K.; Barclay, N. A.; van den Berg, T. K. The CD47-SIRPalpha signaling axis as an innate immune checkpoint in cancer. *Immunological reviews* **2017,** *276* (1), 145.

(292) Faltas, B. M.; Prandi, D.; Tagawa, S. T.; Molina, A. M.; Nanus, D. M.; Sternberg, C.; Rosenberg, J.; Mosquera, J. M.; Robinson, B.; Elemento, O.et al. Clonal evolution of chemotherapy-resistant urothelial carcinoma. *Nature genetics* **2016,** *48* (12), 1490.

(293) Wan, S.; Pestka, S.; Jubin, R. G.; Lyu, Y. L.; Tsai, Y. C.; Liu, L. F. Chemotherapeutics and radiation stimulate MHC class I expression through elevated interferon-beta signaling in breast cancer cells. *PloS one* **2012,** *7* (3), e32542.

(294) Tseng, C. W.; Hung, C. F.; Alvarez, R. D.; Trimble, C.; Huh, W. K.; Kim, D.; Chuang, C. M.; Lin, C. T.; Tsai, Y. C.; He, L.et al. Pretreatment with cisplatin enhances E7-specific CD8+ T-Cell-mediated antitumor immunity induced by DNA vaccination. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2008,** *14* (10), 3185.

(295) Nio, Y.; Hirahara, N.; Minari, Y.; Iguchi, C.; Yamasawa, K.; Toga, T.; Tamura, K. Induction of tumor-specific antitumor immunity after chemotherapy with cisplatin in mice bearing MOPC-104E plasmacytoma by modulation of MHC expression on tumor surface. *Anticancer Res* **2000,** *20* (5A), 3293.

(296) Tsai, C. C.; Qiu, J. T.; Tseng, C. W.; Hsu, Y. C. Low-dose metronomic chemotherapy with cisplatin enhanced immunity in a murine model of ectopic cervical cancer. *Clin Exp Pharmacol Physiol* **2016,** *43* (2), 251.

(297) Stanske, M.; Wienert, S.; Castillo-Tong, D. C.; Kreuzinger, C.; Vergote, I.; Lambrechts, S.; Gabra, H.; Gourley, C.; Ganapathi, R. N.; Kolaschinski, I.et al. Dynamics of the Intratumoral Immune Response during Progression of High-Grade Serous Ovarian Cancer. *Neoplasia* **2018,** *20* (3), 280.

(298) Jackaman, C.; Majewski, D.; Fox, S. A.; Nowak, A. K.; Nelson, D. J. Chemotherapy broadens the range of tumor antigens seen by cytotoxic CD8(+) T cells in vivo. *Cancer Immunol Immunother* **2012,** *61* (12), 2343.

(299) van den Elsen, P. J.; Holling, T. M.; Kuipers, H. F.; van der Stoep, N. Transcriptional regulation of antigen presentation. *Curr Opin Immunol* **2004,** *16* (1), 67.

(300) Kobayashi, K. S.; van den Elsen, P. J. NLRC5: a key regulator of MHC class I-dependent immune responses. *Nat Rev Immunol* **2012,** *12* (12), 813.

(301) Panday, A.; Inda, M. E.; Bagam, P.; Sahoo, M. K.; Osorio, D.; Batra, S. Transcription Factor NF-kappaB: An Update on Intervention Strategies. *Arch Immunol Ther Exp (Warsz)* **2016,** *64* (6), 463.

(302) Wilson, C.; Purcell, C.; Seaton, A.; Oladipo, O.; Maxwell, P. J.; O'Sullivan, J. M.; Wilson, R. H.; Johnston, P. G.; Waugh, D. J. Chemotherapy-induced CXC-chemokine/CXC-chemokine receptor signaling in metastatic prostate cancer cells confers resistance to oxaliplatin through potentiation of nuclear factor-kappaB transcription and evasion of apoptosis. *J Pharmacol Exp Ther* **2008,** *327* (3), 746.

(303) Wang, Y. T.; Liu, H. S.; Su, C. L. Curcumin-enhanced chemosensitivity of FDA-approved platinum (II)-based anti-cancer drugs involves downregulation of nuclear endonuclease G and NF-kappaB as well as induction of apoptosis and G2/M arrest. *Int J Food Sci Nutr* **2014,** *65* (3), 368.

(304) Beyranvand Nejad, E.; van der Sluis, T. C.; van Duikeren, S.; Yagita, H.; Janssen, G. M.; van Veelen, P. A.; Melief, C. J.; van der Burg, S. H.; Arens, R. Tumor Eradication by Cisplatin Is Sustained by CD80/86-Mediated Costimulation of CD8+ T Cells. *Cancer research* **2016,** *76* (20), 6017.

(305) Wu, X.; Shi, J.; Wu, Y.; Tao, Y.; Hou, J.; Meng, X.; Hu, X.; Han, Y.; Jiang, W.; Tang, S.et al. Arsenic trioxide-mediated growth inhibition of myeloma cells is associated with an extrinsic or intrinsic signaling pathway through activation of TRAIL or TRAIL receptor 2. *Cancer biology & therapy* **2010,** *10* (11), 1201.

(306) Matzinger, P. Tolerance, danger, and the extended family. *Annual review of immunology* **1994,** *12*, 991.

(307) Chen, D.; Tong, J.; Yang, L.; Wei, L.; Stolz, D. B.; Yu, J.; Zhang, J.; Zhang, L. PUMA amplifies necroptosis signaling by activating cytosolic DNA sensors. *Proc Natl Acad Sci U S A* **2018**.

(308) Erxleben, A. Mitochondria-targeting Anticancer Metal Complexes. *Curr Med Chem* **2018**.

(309) Jung, Y.; Lippard, S. J. Direct cellular responses to platinum-induced DNA damage. *Chemical reviews* **2007,** *107* (5), 1387.
(310) Brabec, V.; Novakova, O. DNA binding mode of ruthenium complexes and relationship to tumor cell toxicity. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* **2006,** *9* (3), 111.
(311) Ding, L.; Ren, J.; Zhang, D.; Li, Y.; Huang, X.; Ji, J.; Hu, Q.; Wang, H.; Ni, Y.; Hou, Y. The TLR3 Agonist Inhibit Drug Efflux and Sequentially Consolidates Low-Dose Cisplatin-Based Chemoimmunotherapy while Reducing Side Effects. *Mol Cancer Ther* **2017,** *16* (6), 1068.
(312) Sommariva, M.; De Cecco, L.; De Cesare, M.; Sfondrini, L.; Menard, S.; Melani, C.; Delia, D.; Zaffaroni, N.; Pratesi, G.; Uva, V.et al. TLR9 agonists oppositely modulate DNA repair genes in tumor versus immune cells and enhance chemotherapy effects. *Cancer research* **2011,** *71* (20), 6382.
(313) Manegold, C.; Gravenor, D.; Woytowitz, D.; Mezger, J.; Hirsh, V.; Albert, G.; Al-Adhami, M.; Readett, D.; Krieg, A. M.; Leichman, C. G. Randomized phase II trial of a toll-like receptor 9 agonist oligodeoxynucleotide, PF-3512676, in combination with first-line taxane plus platinum chemotherapy for advanced-stage non-small-cell lung cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2008,** *26* (24), 3979.
(314) Huang, B.; Zhao, J.; Unkeless, J. C.; Feng, Z. H.; Xiong, H. TLR signaling by tumor and immune cells: a double-edged sword. *Oncogene* **2008,** *27* (2), 218.
(315) Ridnour, L. A.; Cheng, R. Y.; Switzer, C. H.; Heinecke, J. L.; Ambs, S.; Glynn, S.; Young, H. A.; Trinchieri, G.; Wink, D. A. Molecular pathways: toll-like receptors in the tumor microenvironment--poor prognosis or new therapeutic opportunity. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2013,** *19* (6), 1340.
(316) Patidar, A.; Selvaraj, S.; Sarode, A.; Chauhan, P.; Chattopadhyay, D.; Saha, B. DAMP-TLR-cytokine axis dictates the fate of tumor. *Cytokine* **2018,** *104*, 114.
(317) Huang, L.; Xu, H.; Peng, G. TLR-mediated metabolic reprogramming in the tumor microenvironment: potential novel strategies for cancer immunotherapy. *Cellular & molecular immunology* **2018**.
(318) Radogna, F.; Diederich, M. Stress-induced cellular responses in immunogenic cell death: Implications for cancer immunotherapy. *Biochem Pharmacol* **2018**.
(319) Schwartzman, R. A.; Cidlowski, J. A. Apoptosis: the biochemistry and molecular biology of programmed cell death. *Endocr Rev* **1993,** *14* (2), 133.
(320) Fesik, S. W. Promoting apoptosis as a strategy for cancer drug discovery. *Nature reviews. Cancer* **2005,** *5* (11), 876.
(321) Green, D. R.; Ferguson, T.; Zitvogel, L.; Kroemer, G. Immunogenic and tolerogenic cell death. *Nat Rev Immunol* **2009,** *9* (5), 353.
(322) Lake, R. A.; Robinson, B. W. Immunotherapy and chemotherapy--a practical partnership. *Nature reviews. Cancer* **2005,** *5* (5), 397.
(323) Gebremeskel, S.; Johnston, B. Concepts and mechanisms underlying chemotherapy induced immunogenic cell death: impact on clinical studies and considerations for combined therapies. *Oncotarget* **2015,** *6* (39), 41600.
(324) Krysko, D. V.; Garg, A. D.; Kaczmarek, A.; Krysko, O.; Agostinis, P.; Vandenabeele, P. Immunogenic cell death and DAMPs in cancer therapy. *Nature reviews. Cancer* **2012,** *12* (12), 860.
(325) Galluzzi, L.; Vitale, I.; Aaronson, S. A.; Abrams, J. M.; Adam, D.; Agostinis, P.; Alnemri, E. S.; Altucci, L.; Amelio, I.; Andrews, D. W.et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ* **2018,** *25* (3), 486.
(326) Casares, N.; Pequignot, M. O.; Tesniere, A.; Ghiringhelli, F.; Roux, S.; Chaput, N.; Schmitt, E.; Hamai, A.; Hervas-Stubbs, S.; Obeid, M.et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *The Journal of experimental medicine* **2005,** *202* (12), 1691.

(327) Shan, B.; Pan, H.; Najafov, A.; Yuan, J. Necroptosis in development and diseases. *Genes Dev* **2018,** *32* (5-6), 327.
(328) Orzalli, M. H.; Kagan, J. C. Apoptosis and Necroptosis as Host Defense Strategies to Prevent Viral Infection. *Trends in cell biology* **2017,** *27* (11), 800.
(329) Zhao, C.; Chen, X.; Zang, D.; Lan, X.; Liao, S.; Yang, C.; Zhang, P.; Wu, J.; Li, X.; Liu, N.et al. A novel nickel complex works as a proteasomal deubiquitinase inhibitor for cancer therapy. *Oncogene* **2016,** *35* (45), 5916.
(330) Krumschnabel, G.; Ebner, H. L.; Hess, M. W.; Villunger, A. Apoptosis and necroptosis are induced in rainbow trout cell lines exposed to cadmium. *Aquatic toxicology* **2010,** *99* (1), 73.
(331) Wang, H.-Y.; Zhang, B. Cobalt chloride induces necroptosis in human colon cancer HT-29 cells. *Asian Pacific journal of cancer prevention : APJCP* **2015,** *16* (6), 2569.
(332) Marmol, I.; Virumbrales-Munoz, M.; Quero, J.; Sanchez-de-Diego, C.; Fernandez, L.; Ochoa, I.; Cerrada, E.; Yoldi, M. J. R. Alkynyl gold(I) complex triggers necroptosis via ROS generation in colorectal carcinoma cells. *J Inorg Biochem* **2017,** *176*, 123.
(333) Li, H.; Hu, J.; Wu, S.; Wang, L.; Cao, X.; Zhang, X.; Dai, B.; Cao, M.; Shao, R.; Zhang, R.et al. Auranofin-mediated inhibition of PI3K/AKT/mTOR axis and anticancer activity in non-small cell lung cancer cells. *Oncotarget* **2016,** *7* (3), 3548.
(334) Cabrera, A. R.; Espinosa-Bustos, C.; Faundez, M.; Melendez, J.; Jaque, P.; Daniliuc, C. G.; Aguirre, A.; Rojas, R. S.; Salas, C. O. New imidoyl-indazole platinum (II) complexes as potential anticancer agents: Synthesis, evaluation of cytotoxicity, cell death and experimental-theoretical DNA interaction studies. *J Inorg Biochem* **2017,** *174*, 90.
(335) Akhtar, M. J.; Alhadlaq, H. A.; Kumar, S.; Alrokayan, S. A.; Ahamed, M. Selective cancer-killing ability of metal-based nanoparticles: implications for cancer therapy. *Arch Toxicol* **2015,** *89* (11), 1895.
(336) Garg, A. D.; More, S.; Rufo, N.; Mece, O.; Sassano, M. L.; Agostinis, P.; Zitvogel, L.; Kroemer, G.; Galluzzi, L. Trial watch: Immunogenic cell death induction by anticancer chemotherapeutics. *Oncoimmunology* **2017,** *6* (12), e1386829.
(337) Tesniere, A.; Schlemmer, F.; Boige, V.; Kepp, O.; Martins, I.; Ghiringhelli, F.; Aymeric, L.; Michaud, M.; Apetoh, L.; Barault, L.et al. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene* **2010,** *29* (4), 482.
(338) Wong, D. Y.; Ong, W. W.; Ang, W. H. Induction of immunogenic cell death by chemotherapeutic platinum complexes. *Angewandte Chemie* **2015,** *54* (22), 6483.
(339) Zitvogel, L.; Galluzzi, L.; Smyth, M. J.; Kroemer, G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. *Immunity* **2013,** *39* (1), 74.
(340) Pitt, J. M.; Kroemer, G.; Zitvogel, L. Immunogenic and Non-immunogenic Cell Death in the Tumor Microenvironment. *Adv Exp Med Biol* **2017,** *1036*, 65.
(341) Pol, J.; Vacchelli, E.; Aranda, F.; Castoldi, F.; Eggermont, A.; Cremer, I.; Sautes-Fridman, C.; Fucikova, J.; Galon, J.; Spisek, R.et al. Trial Watch: Immunogenic cell death inducers for anticancer chemotherapy. *Oncoimmunology* **2015,** *4* (4), e1008866.
(342) Golden, E. B.; Apetoh, L. Radiotherapy and immunogenic cell death. *Semin Radiat Oncol* **2015,** *25* (1), 11.
(343) Kepp, O.; Senovilla, L.; Kroemer, G. Immunogenic cell death inducers as anticancer agents. *Oncotarget* **2014,** *5* (14), 5190.
(344) Garg, A. D.; Galluzzi, L.; Apetoh, L.; Baert, T.; Birge, R. B.; Bravo-San Pedro, J. M.; Breckpot, K.; Brough, D.; Chaurio, R.; Cirone, M.et al. Molecular and Translational Classifications of DAMPs in Immunogenic Cell Death. *Frontiers in immunology* **2015,** *6*, 588.
(345) Obeid, M.; Tesniere, A.; Ghiringhelli, F.; Fimia, G. M.; Apetoh, L.; Perfettini, J. L.; Castedo, M.; Mignot, G.; Panaretakis, T.; Casares, N.et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nature medicine* **2007,** *13* (1), 54.

(346) Obeid, M.; Tesniere, A.; Panaretakis, T.; Tufi, R.; Joza, N.; van Endert, P.; Ghiringhelli, F.; Apetoh, L.; Chaput, N.; Flament, C.et al. Ecto-calreticulin in immunogenic chemotherapy. *Immunological reviews* **2007,** *220*, 22.
(347) Fucikova, J.; Kralikova, P.; Fialova, A.; Brtnicky, T.; Rob, L.; Bartunkova, J.; Spisek, R. Human tumor cells killed by anthracyclines induce a tumor-specific immune response. *Cancer research* **2011,** *71* (14), 4821.
(348) Yamazaki, T.; Hannani, D.; Poirier-Colame, V.; Ladoire, S.; Locher, C.; Sistigu, A.; Prada, N.; Adjemian, S.; Catani, J. P.; Freudenberg, M.et al. Defective immunogenic cell death of HMGB1-deficient tumors: compensatory therapy with TLR4 agonists. *Cell Death Differ* **2014,** *21* (1), 69.
(349) Martins, I.; Wang, Y.; Michaud, M.; Ma, Y.; Sukkurwala, A. Q.; Shen, S.; Kepp, O.; Metivier, D.; Galluzzi, L.; Perfettini, J. L.et al. Molecular mechanisms of ATP secretion during immunogenic cell death. *Cell Death Differ* **2014,** *21* (1), 79.
(350) Kepp, O.; Senovilla, L.; Vitale, I.; Vacchelli, E.; Adjemian, S.; Agostinis, P.; Apetoh, L.; Aranda, F.; Barnaba, V.; Bloy, N.et al. Consensus guidelines for the detection of immunogenic cell death. *Oncoimmunology* **2014,** *3* (9), e955691.
(351) Sistigu, A.; Yamazaki, T.; Vacchelli, E.; Chaba, K.; Enot, D. P.; Adam, J.; Vitale, I.; Goubar, A.; Baracco, E. E.; Remedios, C.et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nature medicine* **2014,** *20* (11), 1301.
(352) Ghiringhelli, F.; Apetoh, L.; Tesniere, A.; Aymeric, L.; Ma, Y.; Ortiz, C.; Vermaelen, K.; Panaretakis, T.; Mignot, G.; Ullrich, E.et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. *Nature medicine* **2009,** *15* (10), 1170.
(353) Kontek, R.; Matlawska-Wasowska, K.; Kalinowska-Lis, U.; Kontek, B.; Ochocki, J. Evaluation of cytotoxicity of new trans-palladium(II) complex in human cells in vitro. *Acta poloniae pharmaceutica* **2011,** *68* (1), 127.
(354) Sukkurwala, A. Q.; Martins, I.; Wang, Y.; Schlemmer, F.; Ruckenstuhl, C.; Durchschlag, M.; Michaud, M.; Senovilla, L.; Sistigu, A.; Ma, Y.et al. Immunogenic calreticulin exposure occurs through a phylogenetically conserved stress pathway involving the chemokine CXCL8. *Cell Death Differ* **2014,** *21* (1), 59.
(355) Panaretakis, T.; Kepp, O.; Brockmeier, U.; Tesniere, A.; Bjorklund, A. C.; Chapman, D. C.; Durchschlag, M.; Joza, N.; Pierron, G.; van Endert, P.et al. Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. *The EMBO journal* **2009,** *28* (5), 578.
(356) Gardai, S. J.; McPhillips, K. A.; Frasch, S. C.; Janssen, W. J.; Starefeldt, A.; Murphy-Ullrich, J. E.; Bratton, D. L.; Oldenborg, P. A.; Michalak, M.; Henson, P. M. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell* **2005,** *123* (2), 321.
(357) Novak, I. Purinergic signalling in epithelial ion transport: regulation of secretion and absorption. *Acta Physiol (Oxf)* **2011,** *202* (3), 501.
(358) Taruno, A. ATP Release Channels. *International journal of molecular sciences* **2018,** *19* (3).
(359) Chekeni, F. B.; Elliott, M. R.; Sandilos, J. K.; Walk, S. F.; Kinchen, J. M.; Lazarowski, E. R.; Armstrong, A. J.; Penuela, S.; Laird, D. W.; Salvesen, G. S.et al. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. *Nature* **2010,** *467* (7317), 863.
(360) Jacob, F.; Perez Novo, C.; Bachert, C.; Van Crombruggen, K. Purinergic signaling in inflammatory cells: P2 receptor expression, functional effects, and modulation of inflammatory responses. *Purinergic Signal* **2013,** *9* (3), 285.
(361) Di Virgilio, F.; Dal Ben, D.; Sarti, A. C.; Giuliani, A. L.; Falzoni, S. The P2X7 Receptor in Infection and Inflammation. *Immunity* **2017,** *47* (1), 15.
(362) Chan, A. J.; Alikhan, M. A.; Odobasic, D.; Gan, P. Y.; Khouri, M. B.; Steinmetz, O. M.; Mansell, A. S.; Kitching, A. R.; Holdsworth, S. R.; Summers, S. A. Innate IL-17A-producing leukocytes promote

acute kidney injury via inflammasome and Toll-like receptor activation. *The American journal of pathology* **2014,** *184* (5), 1411.

(363) Di Blasio, S.; Wortel, I. M.; van Bladel, D. A.; de Vries, L. E.; Duiveman-de Boer, T.; Worah, K.; de Haas, N.; Buschow, S. I.; de Vries, I. J.; Figdor, C. G.et al. Human CD1c(+) DCs are critical cellular mediators of immune responses induced by immunogenic cell death. *Oncoimmunology* **2016,** *5* (8), e1192739.

(364) Martins, I.; Tesniere, A.; Kepp, O.; Michaud, M.; Schlemmer, F.; Senovilla, L.; Seror, C.; Metivier, D.; Perfettini, J. L.; Zitvogel, L.et al. Chemotherapy induces ATP release from tumor cells. *Cell Cycle* **2009,** *8* (22), 3723.

(365) Derer, A.; Spiljar, M.; Baumler, M.; Hecht, M.; Fietkau, R.; Frey, B.; Gaipl, U. S. Chemoradiation Increases PD-L1 Expression in Certain Melanoma and Glioblastoma Cells. *Frontiers in immunology* **2016,** *7*, 610.

(366) Stros, M. HMGB proteins: interactions with DNA and chromatin. *Biochim Biophys Acta* **2010,** *1799* (1-2), 101.

(367) Huang, J. C.; Zamble, D. B.; Reardon, J. T.; Lippard, S. J.; Sancar, A. HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. *Proc Natl Acad Sci U S A* **1994,** *91* (22), 10394.

(368) Lu, B.; Wang, H.; Andersson, U.; Tracey, K. J. Regulation of HMGB1 release by inflammasomes. *Protein & cell* **2013,** *4* (3), 163.

(369) Venereau, E.; Casalgrandi, M.; Schiraldi, M.; Antoine, D. J.; Cattaneo, A.; De Marchis, F.; Liu, J.; Antonelli, A.; Preti, A.; Raeli, L.et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *The Journal of experimental medicine* **2012,** *209* (9), 1519.

(370) Rojko, L.; Reiniger, L.; Teglasi, V.; Fabian, K.; Pipek, O.; Vagvolgyi, A.; Agocs, L.; Fillinger, J.; Kajdacsi, Z.; Timar, J.et al. Chemotherapy treatment is associated with altered PD-L1 expression in lung cancer patients. *J Cancer Res Clin Oncol* **2018**.

(371) Xie, J.; Mendez, J. D.; Mendez-Valenzuela, V.; Aguilar-Hernandez, M. M. Cellular signalling of the receptor for advanced glycation end products (RAGE). *Cellular signalling* **2013,** *25* (11), 2185.

(372) Schiraldi, M.; Raucci, A.; Munoz, L. M.; Livoti, E.; Celona, B.; Venereau, E.; Apuzzo, T.; De Marchis, F.; Pedotti, M.; Bachi, A.et al. HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. *The Journal of experimental medicine* **2012,** *209* (3), 551.

(373) Hernandez, C.; Huebener, P.; Pradere, J. P.; Antoine, D. J.; Friedman, R. A.; Schwabe, R. F. HMGB1 links chronic liver injury to progenitor responses and hepatocarcinogenesis. *The Journal of clinical investigation* **2018**.

(374) Taguchi, A.; Blood, D. C.; del Toro, G.; Canet, A.; Lee, D. C.; Qu, W.; Tanji, N.; Lu, Y.; Lalla, E.; Fu, C.et al. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* **2000,** *405* (6784), 354.

(375) Luo, Y.; Chihara, Y.; Fujimoto, K.; Sasahira, T.; Kuwada, M.; Fujiwara, R.; Fujii, K.; Ohmori, H.; Kuniyasu, H. High mobility group box 1 released from necrotic cells enhances regrowth and metastasis of cancer cells that have survived chemotherapy. *European journal of cancer* **2013,** *49* (3), 741.

(376) Kfoury, A.; Le Corf, K.; El Sabeh, R.; Journeaux, A.; Badran, B.; Hussein, N.; Lebecque, S.; Manie, S.; Renno, T.; Coste, I. MyD88 in DNA repair and cancer cell resistance to genotoxic drugs. *Journal of the National Cancer Institute* **2013,** *105* (13), 937.

(377) Venereau, E.; De Leo, F.; Mezzapelle, R.; Careccia, G.; Musco, G.; Bianchi, M. E. HMGB1 as biomarker and drug target. *Pharmacological research* **2016,** *111*, 534.

(378) Rufo, N.; Garg, A. D.; Agostinis, P. The Unfolded Protein Response in Immunogenic Cell Death and Cancer Immunotherapy. *Trends Cancer* **2017,** *3* (9), 643.

(379) van Vliet, A. R.; Martin, S.; Garg, A. D.; Agostinis, P. The PERKs of damage-associated molecular patterns mediating cancer immunogenicity: From sensor to the plasma membrane and beyond. *Semin Cancer Biol* **2015,** *33*, 74.
(380) Bezu, L.; Sauvat, A.; Humeau, J.; Gomes-da-Silva, L. C.; Iribarren, K.; Forveille, S.; Garcia, P.; Zhao, L.; Liu, P.; Zitvogel, L.et al. eIF2alpha phosphorylation is pathognomonic for immunogenic cell death. *Cell Death Differ* **2018**.
(381) Moserova, I.; Truxova, I.; Garg, A. D.; Tomala, J.; Agostinis, P.; Cartron, P. F.; Vosahlikova, S.; Kovar, M.; Spisek, R.; Fucikova, J. Caspase-2 and oxidative stress underlie the immunogenic potential of high hydrostatic pressure-induced cancer cell death. *Oncoimmunology* **2017,** *6* (1), e1258505.
(382) Garg, A. D.; Dudek, A. M.; Ferreira, G. B.; Verfaillie, T.; Vandenabeele, P.; Krysko, D. V.; Mathieu, C.; Agostinis, P. ROS-induced autophagy in cancer cells assists in evasion from determinants of immunogenic cell death. *Autophagy* **2013,** *9* (9), 1292.
(383) Flocke, L. S.; Trondl, R.; Jakupec, M. A.; Keppler, B. K. Molecular mode of action of NKP-1339 - a clinically investigated ruthenium-based drug - involves ER- and ROS-related effects in colon carcinoma cell lines. *Invest New Drugs* **2016,** *34* (3), 261.
(384) Schoenhacker-Alte, B.; Mohr, T.; Pirker, C.; Kryeziu, K.; Kuhn, P. S.; Buck, A.; Hofmann, T.; Gerner, C.; Hermann, G.; Koellensperger, G.et al. Sensitivity towards the GRP78 inhibitor KP1339/IT-139 is characterized by apoptosis induction via caspase 8 upon disruption of ER homeostasis. *Cancer Lett* **2017,** *404*, 79.
(385) Pfaffenbach, K. T.; Lee, A. S. The critical role of GRP78 in physiologic and pathologic stress. *Curr Opin Cell Biol* **2011,** *23* (2), 150.
(386) Gifford, J. B.; Huang, W.; Zeleniak, A. E.; Hindoyan, A.; Wu, H.; Donahue, T. R.; Hill, R. Expression of GRP78, Master Regulator of the Unfolded Protein Response, Increases Chemoresistance in Pancreatic Ductal Adenocarcinoma. *Mol Cancer Ther* **2016,** *15* (5), 1043.
(387) White, E. Deconvoluting the context-dependent role for autophagy in cancer. *Nature reviews. Cancer* **2012,** *12* (6), 401.
(388) Pietrocola, F.; Bravo-San Pedro, J. M.; Galluzzi, L.; Kroemer, G. Autophagy in natural and therapy-driven anticancer immunosurveillance. *Autophagy* **2017,** *13* (12), 2163.
(389) Dikic, I.; Elazar, Z. Mechanism and medical implications of mammalian autophagy. *Nat Rev Mol Cell Biol* **2018,** *19* (6), 349.
(390) Levy, J. M. M.; Towers, C. G.; Thorburn, A. Targeting autophagy in cancer. *Nature reviews. Cancer* **2017,** *17* (9), 528.
(391) Galluzzi, L.; Bravo-San Pedro, J. M.; Demaria, S.; Formenti, S. C.; Kroemer, G. Activating autophagy to potentiate immunogenic chemotherapy and radiation therapy. *Nat Rev Clin Oncol* **2017,** *14* (4), 247.
(392) Sahni, S.; Bae, D. H.; Jansson, P. J.; Richardson, D. R. The mechanistic role of chemically diverse metal ions in the induction of autophagy. *Pharmacological research* **2017,** *119*, 118.
(393) Zhang, Z.; Miah, M.; Culbreth, M.; Aschner, M. Autophagy in Neurodegenerative Diseases and Metal Neurotoxicity. *Neurochem Res* **2016,** *41* (1-2), 409.
(394) Tan, C. P.; Lu, Y. Y.; Ji, L. N.; Mao, Z. W. Metallomics insights into the programmed cell death induced by metal-based anticancer compounds. *Metallomics* **2014,** *6* (5), 978.
(395) Shibutani, S. T.; Saitoh, T.; Nowag, H.; Munz, C.; Yoshimori, T. Autophagy and autophagy-related proteins in the immune system. *Nature immunology* **2015,** *16* (10), 1014.
(396) Schmid, D.; Pypaert, M.; Munz, C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* **2007,** *26* (1), 79.
(397) Chiu, Y. H.; Ravichandran, K. S.; Bayliss, D. A. Intrinsic properties and regulation of Pannexin 1 channel. *Channels (Austin)* **2014,** *8* (2), 103.

(398) Tang, D.; Kang, R.; Cheh, C. W.; Livesey, K. M.; Liang, X.; Schapiro, N. E.; Benschop, R.; Sparvero, L. J.; Amoscato, A. A.; Tracey, K. J.et al. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. *Oncogene* **2010,** *29* (38), 5299.
(399) Li, D. D.; Xie, B.; Wu, X. J.; Li, J. J.; Ding, Y.; Wen, X. Z.; Zhang, X.; Zhu, S. G.; Liu, W.; Zhang, X. S.et al. Late-stage inhibition of autophagy enhances calreticulin surface exposure. *Oncotarget* **2016,** *7* (49), 80842.
(400) Dudek, A. M.; Garg, A. D.; Krysko, D. V.; De Ruysscher, D.; Agostinis, P. Inducers of immunogenic cancer cell death. *Cytokine & growth factor reviews* **2013,** *24* (4), 319.
(401) Wernitznig D, D. F. G., Kiakos K, Harrer N, Machat H, Marko D, Jakupec M, Sommergruber W, Keppler BK AACR Annual Meeting 2018, Chicago, 2018.
(402) Howells, L. M.; Mitra, A.; Manson, M. M. Comparison of oxaliplatin- and curcumin-mediated antiproliferative effects in colorectal cell lines. *Int J Cancer* **2007,** *121* (1), 175.
(403) Li, X.; Shao, C.; Shi, Y.; Han, W. Lessons learned from the blockade of immune checkpoints in cancer immunotherapy. *J Hematol Oncol* **2018,** *11* (1), 31.
(404) Pfirschke, C.; Engblom, C.; Rickelt, S.; Cortez-Retamozo, V.; Garris, C.; Pucci, F.; Yamazaki, T.; Poirier-Colame, V.; Newton, A.; Redouane, Y.et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. *Immunity* **2016,** *44* (2), 343.
(405) Kang, T. H.; Mao, C. P.; Lee, S. Y.; Chen, A.; Lee, J. H.; Kim, T. W.; Alvarez, R. D.; Roden, R. B.; Pardoll, D.; Hung, C. F.et al. Chemotherapy acts as an adjuvant to convert the tumor microenvironment into a highly permissive state for vaccination-induced antitumor immunity. *Cancer research* **2013,** *73* (8), 2493.
(406) Raghavan, B.; Martin, S. F.; Esser, P. R.; Goebeler, M.; Schmidt, M. Metal allergens nickel and cobalt facilitate TLR4 homodimerization independently of MD2. *EMBO reports* **2012,** *13* (12), 1109.
(407) Bechara, R.; Antonios, D.; Azouri, H.; Pallardy, M. Nickel Sulfate Promotes IL-17A Producing CD4+ T Cells by an IL-23-Dependent Mechanism Regulated by TLR4 and Jak-STAT Pathways. *Journal of Investigative Dermatology* **2017,** *137* (10), 2140.
(408) Kim, S. Y.; Choi, Y. J.; Joung, S. M.; Lee, B. H.; Jung, Y. S.; Lee, J. Y. Hypoxic stress up-regulates the expression of Toll-like receptor 4 in macrophages via hypoxia-inducible factor. *Immunology* **2010,** *129* (4), 516.
(409) Rachmawati, D.; Alsalem, I. W.; Bontkes, H. J.; Verstege, M. I.; Gibbs, S.; von Blomberg, B. M.; Scheper, R. J.; van Hoogstraten, I. M. Innate stimulatory capacity of high molecular weight transition metals Au (gold) and Hg (mercury). *Toxicology in vitro : an international journal published in association with BIBRA* **2015,** *29* (2), 363.
(410) Leung, C. H.; Lin, S.; Zhong, H. J.; Ma, D. L. Metal complexes as potential modulators of inflammatory and autoimmune responses. *Chem Sci* **2015,** *6* (2), 871.
(411) Miller, R. P.; Tadagavadi, R. K.; Ramesh, G.; Reeves, W. B. Mechanisms of Cisplatin nephrotoxicity. *Toxins* **2010,** *2* (11), 2490.
(412) Oun, R.; Moussa, Y. E.; Wheate, N. J. The side effects of platinum-based chemotherapy drugs: a review for chemists. *Dalton Trans* **2018**.
(413) Mayr, J.; Heffeter, P.; Groza, D.; Galvez, L.; Koellensperger, G.; Roller, A.; Alte, B.; Haider, M.; Berger, W.; Kowol, C. R.et al. An albumin-based tumor-targeted oxaliplatin prodrug with distinctly improved anticancer activity in vivo. *Chem Sci* **2017,** *8* (3), 2241.
(414) Parker, J. P.; Ude, Z.; Marmion, C. J. Exploiting developments in nanotechnology for the preferential delivery of platinum-based anti-cancer agents to tumours: targeting some of the hallmarks of cancer. *Metallomics* **2016,** *8* (1), 43.
(415) Turkson, J.; Zhang, S.; Palmer, J.; Kay, H.; Stanko, J.; Mora, L. B.; Sebti, S.; Yu, H.; Jove, R. Inhibition of constitutive signal transducer and activator of transcription 3 activation by novel platinum complexes with potent antitumor activity. *Mol Cancer Ther* **2004,** *3* (12), 1533.

(416) Hato, S. V.; Figdor, C. G.; Takahashi, S.; Pen, A. E.; Halilovic, A.; Bol, K. F.; Vasaturo, A.; Inoue, Y.; de Haas, N.; Verweij, D.et al. Direct inhibition of STAT signaling by platinum drugs contributes to their anti-cancer activity. *Oncotarget* **2017,** *8* (33), 54434.
(417) Li, H. S.; Watowich, S. S. Innate immune regulation by STAT-mediated transcriptional mechanisms. *Immunological reviews* **2014,** *261* (1), 84.
(418) Sun, C.; Mezzadra, R.; Schumacher, T. N. Regulation and Function of the PD-L1 Checkpoint. *Immunity* **2018,** *48* (3), 434.
(419) Leduc, C.; Adam, J.; Louvet, E.; Sourisseau, T.; Dorvault, N.; Bernard, M.; Maingot, E.; Faivre, L.; Cassin-Kuo, M. S.; Boissier, E.et al. TPF induction chemotherapy increases PD-L1 expression in tumour cells and immune cells in head and neck squamous cell carcinoma. *ESMO Open* **2018,** *3* (1), e000257.
(420) Qin, X.; Liu, C.; Zhou, Y.; Wang, G. Cisplatin induces programmed death-1-ligand 1(PD-L1) over-expression in hepatoma H22 cells via Erk /MAPK signaling pathway. *Cell Mol Biol (Noisy-le-grand)* **2010,** *56 Suppl*, OL1366.
(421) Yang, J. H.; Kim, H.; Roh, S. Y.; Lee, M. A.; Park, J. M.; Lee, H. H.; Park, C. H.; Lee, H. H.; Jung, E. S.; Lee, S. H.et al. Discordancy and changes in the pattern of programmed death ligand 1 expression before and after platinum-based chemotherapy in metastatic gastric cancer. *Gastric Cancer* **2018**.
(422) Tran, L.; Allen, C. T.; Xiao, R.; Moore, E.; Davis, R.; Park, S. J.; Spielbauer, K.; Van Waes, C.; Schmitt, N. C. Cisplatin Alters Antitumor Immunity and Synergizes with PD-1/PD-L1 Inhibition in Head and Neck Squamous Cell Carcinoma. *Cancer immunology research* **2017,** *5* (12), 1141.
(423) Peggs, K. S.; Quezada, S. A.; Allison, J. P. Cell intrinsic mechanisms of T-cell inhibition and application to cancer therapy. *Immunological reviews* **2008,** *224*, 141.
(424) Dyck, L.; Mills, K. H. G. Immune checkpoints and their inhibition in cancer and infectious diseases. *European journal of immunology* **2017,** *47* (5), 765.
(425) Gao, Q.; Jiang, J.; Chu, Z.; Lin, H.; Zhou, X.; Liang, X. Arsenic trioxide inhibits tumor-induced myeloid-derived suppressor cells and enhances T-cell activity. *Oncol Lett* **2017,** *13* (4), 2141.
(426) Xu, W.; Li, X.; Quan, L.; Yao, J.; Mu, G.; Guo, J.; Wang, Y. Arsenic trioxide decreases the amount and inhibits the function of regulatory T cells, which may contribute to its efficacy in the treatment of acute promyelocytic leukemia. *Leuk Lymphoma* **2018,** *59* (3), 650.
(427) Thomas-Schoemann, A.; Batteux, F.; Alexandre, J. A new strategy to target regulatory T cells in solid tumors. *Oncoimmunology* **2013,** *2* (3), e23338.
(428) Hori, S.; Nomura, T.; Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **2003,** *299* (5609), 1057.
(429) Hu, Z.; Hu, S.; Wu, Y.; Li, S.; He, C.; Xing, X.; Wang, Y.; Du, X. Accumulation and suppressive function of regulatory T cells in malignant ascites: Reducing their suppressive function using arsenic trioxide in vitro. *Oncol Lett* **2018,** *15* (4), 5384.
(430) Momcilovic, M.; Eichhorn, T.; Blazevski, J.; Schmidt, H.; Kaluderovic, G. N.; Stosic-Grujicic, S. In vitro effects of binuclear (eta (6)-p-cymene)ruthenium(II) complex containing bridging bis(nicotinate)-polyethylene glycol ester ligand on differentiation pathways of murine Th lymphocytes activated by T cell mitogen. *J Biol Inorg Chem* **2015,** *20* (3), 575.
(431) McCoy, M. J.; Lake, R. A.; van der Most, R. G.; Dick, I. M.; Nowak, A. K. Post-chemotherapy T-cell recovery is a marker of improved survival in patients with advanced thoracic malignancies. *Br J Cancer* **2012,** *107* (7), 1107.
(432) Limagne, E.; Euvrard, R.; Thibaudin, M.; Rebe, C.; Derangere, V.; Chevriaux, A.; Boidot, R.; Vegran, F.; Bonnefoy, N.; Vincent, J.et al. Accumulation of MDSC and Th17 Cells in Patients with Metastatic Colorectal Cancer Predicts the Efficacy of a FOLFOX-Bevacizumab Drug Treatment Regimen. *Cancer research* **2016,** *76* (18), 5241.
(433) Yarbro, C. H. Carboplatin: a clinical review. *Seminars in oncology nursing* **1989,** *5* (2 Suppl 1), 63.

(434) Zhu, X.; Peng, Y.; Qiu, L. Amino-functionalized nano-vesicles for enhanced anticancer efficacy and reduced myelotoxicity of carboplatin. *Colloids and surfaces. B, Biointerfaces* **2017,** *157*, 56.
(435) Moskowitz, C. H.; Bertino, J. R.; Glassman, J. R.; Hedrick, E. E.; Hunte, S.; Coady-Lyons, N.; Agus, D. B.; Goy, A.; Jurcic, J.; Noy, A.et al. Ifosfamide, carboplatin, and etoposide: a highly effective cytoreduction and peripheral-blood progenitor-cell mobilization regimen for transplant-eligible patients with non-Hodgkin's lymphoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1999,** *17* (12), 3776.
(436) Miyaji, C.; Watanabe, H.; Miyakawa, R.; Yokoyama, H.; Tsukada, C.; Ishimoto, Y.; Miyazawa, S.; Abo, T. Identification of effector cells for TNFalpha-mediated cytotoxicity against WEHI164S cells. *Cellular immunology* **2002,** *216* (1-2), 43.
(437) Kouchi, Y.; Maeda, Y.; Ohuchida, A.; Ohsawa, M. Immunotoxic effect of low dose cisplatin in mice. *J Toxicol Sci* **1996,** *21* (4), 227.
(438) Powell, C. B.; Mutch, D. G.; Kao, M. S.; Collins, J. L. Reduced natural cytotoxic cell activity in patients receiving cisplatin-based chemotherapy and in mice treated with cisplatin. *Clin Exp Immunol* **1990,** *79* (3), 424.
(439) Singh, R. A.; Sodhi, A. Expression and activation of RAS and mitogen-activated protein kinases in macrophages treated in vitro with cisplatin: regulation by kinases, phosphatases and Ca2+/calmodulin. *Immunology and cell biology* **1999,** *77* (4), 356.
(440) Shishodia, S.; Shrivastava, A.; Sodhi, A. Protein kinase C: a potential pathway of macrophage activation with cisplatin. *Immunol Lett* **1998,** *61* (2-3), 179.
(441) Kleinerman, E. S.; Zwelling, L. A.; Muchmore, A. V. Enhancement of naturally occurring human spontaneous monocyte-mediated cytotoxicity by cis-diamminedichloroplatinum(II). *Cancer research* **1980,** *40* (9), 3099.
(442) Kleinerman, E. S.; Zwelling, L. A.; Howser, D.; Barlock, A.; Young, R. C.; Decker, J. M.; Bull, J.; Muchmore, A. V. Defective monocyte killing in patients with malignancies and restoration of function during chemotherapy. *Lancet* **1980,** *2* (8204), 1102.
(443) Lichtenstein, A. K.; Pende, D. Enhancement of natural killer cytotoxicity by cis-diamminedichloroplatinum (II) in vivo and in vitro. *Cancer research* **1986,** *46* (2), 639.
(444) Collins, J. L.; Kao, M. S. The anticancer drug, cisplatin, increases the naturally occurring cell-mediated lysis of tumor cells. *Cancer Immunol Immunother* **1989,** *29* (1), 17.
(445) Judy, B. F.; Aliperti, L. A.; Predina, J. D.; Levine, D.; Kapoor, V.; Thorpe, P. E.; Albelda, S. M.; Singhal, S. Vascular endothelial-targeted therapy combined with cytotoxic chemotherapy induces inflammatory intratumoral infiltrates and inhibits tumor relapses after surgery. *Neoplasia* **2012,** *14* (4), 352.
(446) Palma, J. P.; Aggarwal, S. K. Cisplatin and carboplatin-mediated activation of murine peritoneal macrophages in vitro: production of interleukin-1 alpha and tumor necrosis factor-alpha. *Anticancer Drugs* **1995,** *6* (2), 311.
(447) Palma, J. P.; Aggarwal, S. K. Cisplatin and carboplatin mediated release of cytolytic factors in murine peritoneal macrophages in vitro. *Anticancer Drugs* **1994,** *5* (6), 615.
(448) Singh, R. A.; Sodhi, A. Expression and activation of lyn in macrophages treated in vitro with cisplatin: regulation by kinases, phosphatases and Ca2+/calmodulin. *Biochim Biophys Acta* **1998,** *1405* (1-3), 171.
(449) Tarang, S.; Sodhi, A.; Chauhan, P. Differential expression of Toll-like receptors in murine peritoneal macrophages in vitro on treatment with cisplatin. *International immunology* **2007,** *19* (5), 635.
(450) Sodhi, A.; Singh, R. A. Mechanism of NF-kappa B translocation in macrophages treated in vitro with cisplatin. *Immunol Lett* **1998,** *63* (1), 9.
(451) Stephens, J. M.; Elks, C. M. Oncostatin M: Potential Implications for Malignancy and Metabolism. *Current pharmaceutical design* **2017,** *23* (25), 3645.

(452) Singh, R. A.; Sodhi, A. Cisplatin-treated macrophages produce oncostatin M: regulation by serine/threonine and protein tyrosine kinases/phosphatases and Ca2+/calmodulin. *Immunol Lett* **1998,** *62* (3), 159.
(453) Reina, M.; Espel, E. Role of LFA-1 and ICAM-1 in Cancer. *Cancers* **2017,** *9* (11).
(454) Singh, R. A.; Sodhi, A. LFA-1-dependent tumoricidal activity of cisplatin-treated macrophages. *Immunology and cell biology* **1998,** *76* (4), 343.
(455) Shrivastava, A.; Shishodia, S.; Sodhi, A. Expression of LFA-1 adhesion molecules on cisplatin-treated macrophages. *Biochim Biophys Acta* **1998,** *1402* (3), 269.
(456) Singh, R. A.; Sodhi, A. Antigen presentation by cisplatin-activated macrophages: role of soluble factor(s) and second messengers. *Immunology and cell biology* **1998,** *76* (6), 513.
(457) Sodhi, A.; Shishodia, S.; Shrivastava, A. Cisplatin-stimulated murine bone marrow-derived macrophages secrete oncostatin M. *Immunology and cell biology* **1997,** *75* (5), 492.
(458) Hamilton, T. A.; Zhao, C.; Pavicic, P. G., Jr.; Datta, S. Myeloid colony-stimulating factors as regulators of macrophage polarization. *Frontiers in immunology* **2014,** *5*, 554.
(459) Sodhi, A.; Suresh, A. Production of reactive nitrogen intermediates by bone marrow-derived macrophages on treatment with cisplatin in vitro. *Clin Exp Immunol* **1992,** *89* (3), 502.
(460) Isidro, R. A.; Appleyard, C. B. Colonic macrophage polarization in homeostasis, inflammation, and cancer. *Am J Physiol Gastrointest Liver Physiol* **2016,** *311* (1), G59.
(461) Dijkgraaf, E. M.; Heusinkveld, M.; Tummers, B.; Vogelpoel, L. T.; Goedemans, R.; Jha, V.; Nortier, J. W.; Welters, M. J.; Kroep, J. R.; van der Burg, S. H. Chemotherapy alters monocyte differentiation to favor generation of cancer-supporting M2 macrophages in the tumor microenvironment. *Cancer research* **2013,** *73* (8), 2480.
(462) Kaminski, B. M.; Weigert, A.; Scherzberg, M. C.; Ley, S.; Gilbert, B.; Brecht, K.; Brune, B.; Steinhilber, D.; Stein, J.; Ulrich-Ruckert, S. Resveratrol-induced potentiation of the antitumor effects of oxaliplatin is accompanied by an altered cytokine profile of human monocyte-derived macrophages. *Apoptosis* **2014,** *19* (7), 1136.
(463) Guo, B.; Li, L.; Guo, J.; Liu, A.; Wu, J.; Wang, H.; Shi, J.; Pang, D.; Cao, Q. M2 tumor-associated macrophages produce interleukin-17 to suppress oxaliplatin-induced apoptosis in hepatocellular carcinoma. *Oncotarget* **2017,** *8* (27), 44465.
(464) Wu, J.; Guo, J.; Cao, Q.; Wang, Y.; Chen, J.; Wang, Z.; Yuan, Z. Autophagy impacts on oxaliplatin-induced hepatocarcinoma apoptosis via the IL-17/IL-17R-JAK2/STAT3 signaling pathway. *Oncol Lett* **2017,** *13* (2), 770.
(465) Shree, T.; Olson, O. C.; Elie, B. T.; Kester, J. C.; Garfall, A. L.; Simpson, K.; Bell-McGuinn, K. M.; Zabor, E. C.; Brogi, E.; Joyce, J. A. Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. *Genes Dev* **2011,** *25* (23), 2465.
(466) Mizutani, Y.; Bonavida, B.; Nio, Y.; Yoshida, O. Enhanced susceptibility of cis-diamminedichloroplatinum-treated K562 cells to lysis by peripheral blood lymphocytes and lymphokine activated killer cells. *Cancer* **1993,** *71* (4), 1313.
(467) Li, L.; Li, W.; Wang, C.; Yan, X.; Wang, Y.; Niu, C.; Zhang, X.; Li, M.; Tian, H.; Yao, C.et al. Adoptive transfer of natural killer cells in combination with chemotherapy improves outcomes of patients with locally advanced colon carcinoma. *Cytotherapy* **2018,** *20* (1), 134.
(468) Kiyohara, T.; Taniguchi, K.; Kubota, S.; Koga, S.; Sakuragi, T.; Saitoh, Y. Induction of lymphokine-activated killer-like cells by cancer chemotherapy. *The Journal of experimental medicine* **1988,** *168* (6), 2355.
(469) Cosiski Marana, H. R.; Santana da Silva, J.; Moreira de Andrade, J. NK cell activity in the presence of IL-12 is a prognostic assay to neoadjuvant chemotherapy in cervical cancer. *Gynecol Oncol* **2000,** *78* (3 Pt 1), 318.

(470) Markasz, L.; Stuber, G.; Vanherberghen, B.; Flaberg, E.; Olah, E.; Carbone, E.; Eksborg, S.; Klein, E.; Skribek, H.; Szekely, L. Effect of frequently used chemotherapeutic drugs on the cytotoxic activity of human natural killer cells. *Molecular Cancer Therapeutics* **2007,** *6* (2), 644.
(471) Siew, Y. Y.; Neo, S. Y.; Yew, H. C.; Lim, S. W.; Ng, Y. C.; Lew, S. M.; Seetoh, W. G.; Seow, S. V.; Koh, H. L. Oxaliplatin regulates expression of stress ligands in ovarian cancer cells and modulates their susceptibility to natural killer cell-mediated cytotoxicity. *International immunology* **2015,** *27* (12), 621.
(472) Cerboni, C.; Fionda, C.; Soriani, A.; Zingoni, A.; Doria, M.; Cippitelli, M.; Santoni, A. The DNA Damage Response: A Common Pathway in the Regulation of NKG2D and DNAM-1 Ligand Expression in Normal, Infected, and Cancer Cells. *Frontiers in immunology* **2014,** *4*, 508.
(473) Gasser, S.; Orsulic, S.; Brown, E. J.; Raulet, D. H. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature* **2005,** *436* (7054), 1186.
(474) Okita, R.; Yukawa, T.; Nojima, Y.; Maeda, A.; Saisho, S.; Shimizu, K.; Nakata, M. MHC class I chain-related molecule A and B expression is upregulated by cisplatin and associated with good prognosis in patients with non-small cell lung cancer. *Cancer Immunol Immunother* **2016,** *65* (5), 499.
(475) Fine, J. H.; Chen, P.; Mesci, A.; Allan, D. S.; Gasser, S.; Raulet, D. H.; Carlyle, J. R. Chemotherapy-induced genotoxic stress promotes sensitivity to natural killer cell cytotoxicity by enabling missing-self recognition. *Cancer research* **2010,** *70* (18), 7102.
(476) Ni, L.; Dong, C. New B7 Family Checkpoints in Human Cancers. *Mol Cancer Ther* **2017,** *16* (7), 1203.
(477) Matsuzaki, I.; Suzuki, H.; Kitamura, M.; Minamiya, Y.; Kawai, H.; Ogawa, J. Cisplatin induces fas expression in esophageal cancer cell lines and enhanced cytotoxicity in combination with LAK cells. *Oncology* **2000,** *59* (4), 336.
(478) Frost, P.; Ng, C. P.; Belldegrun, A.; Bonavida, B. Immunosensitization of prostate carcinoma cell lines for lymphocytes (CTL, TIL, LAK)-mediated apoptosis via the Fas-Fas-ligand pathway of cytotoxicity. *Cellular immunology* **1997,** *180* (1), 70.
(479) Sundelin, K.; Roberg, K.; Grenman, R.; Hakansson, L. Effects of cisplatin, alpha-interferon, and 13-cis retinoic acid on the expression of Fas (CD95), intercellular adhesion molecule-1 (ICAM-1), and epidermal growth factor receptor (EGFR) in oral cancer cell lines. *J Oral Pathol Med* **2007,** *36* (3), 177.
(480) Stewart, J. H. t.; Nguyen, D. M.; Chen, G. A.; Schrump, D. S. Induction of apoptosis in malignant pleural mesothelioma cells by activation of the Fas (Apo-1/CD95) death-signal pathway. *J Thorac Cardiovasc Surg* **2002,** *123* (2), 295.
(481) Alagkiozidis, I.; Facciabene, A.; Tsiatas, M.; Carpenito, C.; Benencia, F.; Adams, S.; Jonak, Z.; June, C. H.; Powell, D. J., Jr.; Coukos, G. Time-dependent cytotoxic drugs selectively cooperate with IL-18 for cancer chemo-immunotherapy. *J Transl Med* **2011,** *9*, 77.
(482) Song, J. H.; Song, D. K.; Pyrzynska, B.; Petruk, K. C.; Van Meir, E. G.; Hao, C. TRAIL triggers apoptosis in human malignant glioma cells through extrinsic and intrinsic pathways. *Brain Pathol* **2003,** *13* (4), 539.
(483) Yang, L.; Shen, M.; Xu, L. J.; Yang, X.; Tsai, Y.; Keng, P. C.; Chen, Y.; Lee, S. O. Enhancing NK cell-mediated cytotoxicity to cisplatin-resistant lung cancer cells via MEK/Erk signaling inhibition. *Sci Rep* **2017,** *7* (1), 7958.
(484) Yamaue, H.; Tanimura, H.; Noguchi, K.; Iwahashi, M.; Tsunoda, T.; Tani, M.; Tamai, M.; Hotta, T.; Mizobata, S.; Arii, K. Cisplatin treatment renders tumor cells more susceptible to attack by lymphokine-activated killer cells. *J Clin Lab Immunol* **1991,** *35* (4), 165.
(485) Arinaga, S.; Ueo, H.; Go, H.; Akiyoshi, T. Neoadjuvant chemoimmunotherapy with cisplatin and low-dose interleukin-2 for locally advanced esophageal carcinoma. *Cancer Biother Radiopharm* **1997,** *12* (6), 371.

(486) Meazza, C.; Cefalo, G.; Massimino, M.; Daolio, P.; Pastorino, U.; Scanagatta, P.; Morosi, C.; Podda, M.; Ferrari, A.; Terenziani, M.et al. Primary metastatic osteosarcoma: results of a prospective study in children given chemotherapy and interleukin-2. *Med Oncol* **2017,** *34* (12), 191.
(487) Basu, S.; Sodhi, A. Increased release of interleukin-1 and tumour necrosis factor by interleukin-2-induced lymphokine-activated killer cells in the presence of cisplatin and FK-565. *Immunology and cell biology* **1992,** *70 (Pt 1)*, 15.
(488) Williams, B. A.; Makrigiannis, A. P.; Blay, J.; Hoskin, D. W. Treatment of the P815 murine mastocytoma with cisplatin or etoposide up-regulates cell-surface Fas (CD95) expression and increases sensitivity to anti-Fas antibody-mediated cytotoxicity and to lysis by anti-CD3-activated killer-T cells. *Int J Cancer* **1997,** *73* (3), 416.
(489) Chen, J.; Huang, X.; Huang, G.; Chen, Y.; Chen, L.; Song, H. Preconditioning chemotherapy with cisplatin enhances the antitumor activity of cytokine-induced killer cells in a murine melanoma model. *Cancer Biother Radiopharm* **2012,** *27* (3), 210.
(490) Huang, X.; Huang, G.; Song, H.; Chen, L. Preconditioning chemotherapy with paclitaxel and cisplatin enhances the antitumor activity of cytokine induced-killer cells in a murine lung carcinoma model. *Int J Cancer* **2011,** *129* (3), 648.
(491) Huang, X.; Chen, Y. T.; Song, H. Z.; Huang, G. C.; Chen, L. B. Cisplatin pretreatment enhances anti-tumor activity of cytokine-induced killer cells. *World J Gastroenterol* **2011,** *17* (25), 3002.
(492) Jiang, J.; Xu, N.; Wu, C.; Deng, H.; Lu, M.; Li, M.; Xu, B.; Wu, J.; Wang, R.; Xu, J.et al. Treatment of advanced gastric cancer by chemotherapy combined with autologous cytokine-induced killer cells. *Anticancer Res* **2006,** *26* (3B), 2237.
(493) Merritt, R. E.; Mahtabifard, A.; Yamada, R. E.; Crystal, R. G.; Korst, R. J. Cisplatin augments cytotoxic T-lymphocyte-mediated antitumor immunity in poorly immunogenic murine lung cancer. *J Thorac Cardiovasc Surg* **2003,** *126* (5), 1609.
(494) Yoshimoto, K.; Kishida, T.; Nakano, H.; Matsui, M.; Shin-Ya, M.; Shimada, T.; Nakai, S.; Imanishi, J.; Takeuchi, M.; Hisa, Y.et al. Interleukin-28B acts synergistically with cisplatin to suppress the growth of head and neck squamous cell carcinoma. *J Immunother* **2011,** *34* (2), 139.
(495) Bernsen, M. R.; Hakansson, L.; Gustafsson, B.; Krysander, L.; Rettrup, B.; Ruiter, D.; Hakansson, A. On the biological relevance of MHC class II and B7 expression by tumour cells in melanoma metastases. *Br J Cancer* **2003,** *88* (3), 424.
(496) Ujiie, T. Increased sensitivity of tumor cells to immune defense cells following treatment with antineoplastic agents in vitro. *Jpn J Exp Med* **1989,** *59* (1), 17.
(497) Tanaka, F.; Yamaguchi, H.; Ohta, M.; Mashino, K.; Sonoda, H.; Sadanaga, N.; Inoue, H.; Mori, M. Intratumoral injection of dendritic cells after treatment of anticancer drugs induces tumor-specific antitumor effect in vivo. *Int J Cancer* **2002,** *101* (3), 265.
(498) Liu, W. M.; Fowler, D. W.; Smith, P.; Dalgleish, A. G. Pretreatment with chemotherapy can enhance the immunogenicity of tumors by promoting adaptive immune responses. *Cancer research* **2010,** *70*.
(499) Moore, D. H.; Fowler, W. C., Jr.; Olafsson, K. Class I histocompatibility antigen expression: a prognostic factor for aneuploid ovarian cancers. *Gynecol Oncol* **1990,** *38* (3), 458.
(500) Shehata, M.; Mukherjee, A.; Deen, S.; Al-Attar, A.; Durrant, L. G.; Chan, S. Human leukocyte antigen class I expression is an independent prognostic factor in advanced ovarian cancer resistant to first-line platinum chemotherapy. *Br J Cancer* **2009,** *101* (8), 1321.
(501) Rutten, M. J.; Dijk, F.; Savci-Heijink, C. D.; Buist, M. R.; Kenter, G. G.; van de Vijver, M. J.; Jordanova, E. S. HLA-G Expression Is an Independent Predictor for Improved Survival in High Grade Ovarian Carcinomas. *J Immunol Res* **2014**.
(502) Mariya, T.; Hirohashi, Y.; Torigoe, T.; Asano, T.; Kuroda, T.; Yasuda, K.; Mizuuchi, M.; Sonoda, T.; Saito, T.; Sato, N. Prognostic impact of human leukocyte antigen class I expression and association

of platinum resistance with immunologic profiles in epithelial ovarian cancer. *Cancer immunology research* **2014,** *2* (12), 1220.

(503) Shetty, V.; Nickens, Z.; Testa, J.; Hafner, J.; Sinnathamby, G.; Philip, R. Quantitative immunoproteomics analysis reveals novel MHC class I presented peptides in cisplatin-resistant ovarian cancer cells. *J Proteomics* **2012,** *75* (11), 3270.

(504) Denkert, C.; von Minckwitz, G.; Brase, J. C.; Sinn, B. V.; Gade, S.; Kronenwett, R.; Pfitzner, B. M.; Salat, C.; Loi, S.; Schmitt, W. D.et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2015,** *33* (9), 983.

(505) Coosemans, A.; Decoene, J.; Baert, T.; Laenen, A.; Kasran, A.; Verschuere, T.; Seys, S.; Vergote, I. Immunosuppressive parameters in serum of ovarian cancer patients change during the disease course. *Oncoimmunology* **2016,** *5* (4), e1111505.

(506) Vacchelli, E.; Senovilla, L.; Eggermont, A.; Fridman, W. H.; Galon, J.; Zitvogel, L.; Kroemer, G.; Galluzzi, L. Trial watch: Chemotherapy with immunogenic cell death inducers. *Oncoimmunology* **2013,** *2* (3), e23510.

(507) Kim, J. E.; Jang, M. J.; Lee, J. I.; Chung, Y. H.; Jeong, J. H.; Hung, C. F.; Kim, D. Cancer cells containing nanoscale chemotherapeutic drugs generate antiovarian cancer-specific CD4+ T cells in peritoneal space. *J Immunother* **2012,** *35* (1), 1.

(508) Apetoh, L.; Ghiringhelli, F.; Tesniere, A.; Criollo, A.; Ortiz, C.; Lidereau, R.; Mariette, C.; Chaput, N.; Mira, J. P.; Delaloge, S.et al. The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. *Immunological reviews* **2007,** *220*, 47.

(509) Martins, I.; Kepp, O.; Schlemmer, F.; Adjemian, S.; Tailler, M.; Shen, S.; Michaud, M.; Menger, L.; Gdoura, A.; Tajeddine, N.et al. Restoration of the immunogenicity of cisplatin-induced cancer cell death by endoplasmic reticulum stress. *Oncogene* **2011,** *30* (10), 1147.

(510) Michaud, M.; Sukkurwala, A. Q.; Di Sano, F.; Zitvogel, L.; Kepp, O.; Kroemer, G. Synthetic induction of immunogenic cell death by genetic stimulation of endoplasmic reticulum stress. *Oncoimmunology* **2014,** *3*, e28276.

(511) Alculumbre, S.; Raieli, S.; Hoffmann, C.; Chelbi, R.; Danlos, F. X.; Soumelis, V. Plasmacytoid pre-dendritic cells (pDC): from molecular pathways to function and disease association. *Semin Cell Dev Biol* **2018**.

(512) Kang, R.; Chen, R.; Zhang, Q.; Hou, W.; Wu, S.; Cao, L.; Huang, J.; Yu, Y.; Fan, X. G.; Yan, Z.et al. HMGB1 in health and disease. *Mol Aspects Med* **2014,** *40*, 1.

(513) Kasparkova, J.; Farrell, N.; Brabec, V. Sequence specificity, conformation, and recognition by HMG1 protein of major DNA interstrand cross-links of antitumor dinuclear platinum complexes. *J Biol Chem* **2000,** *275* (21), 15789.

(514) Dunham, S. U.; Lippard, S. J. DNA sequence context and protein composition modulate HMG-domain protein recognition of cisplatin-modified DNA. *Biochemistry* **1997,** *36* (38), 11428.

(515) Ohndorf, U. M.; Rould, M. A.; He, Q.; Pabo, C. O.; Lippard, S. J. Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins. *Nature* **1999,** *399* (6737), 708.

(516) Park, S.; Lippard, S. J. Binding interaction of HMGB4 with cisplatin-modified DNA. *Biochemistry* **2012,** *51* (34), 6728.

(517) Awuah, S. G.; Riddell, I. A.; Lippard, S. J. Repair shielding of platinum-DNA lesions in testicular germ cell tumors by high-mobility group box protein 4 imparts cisplatin hypersensitivity. *Proc Natl Acad Sci U S A* **2017,** *114* (5), 950.

(518) Winter, C.; Albers, P. Testicular germ cell tumors: pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* **2011,** *7* (1), 43.

(519) Zou, T.; Lok, C. N.; Fung, Y. M.; Che, C. M. Luminescent organoplatinum(II) complexes containing bis(N-heterocyclic carbene) ligands selectively target the endoplasmic reticulum and induce potent photo-toxicity. *Chem Commun (Camb)* **2013,** *49* (47), 5423.
(520) Arsenijevic, M.; Milovanovic, M.; Jovanovic, S.; Arsenijevic, N.; Markovic, B. S.; Gazdic, M.; Volarevic, V. In vitro and in vivo anti-tumor effects of selected platinum(IV) and dinuclear platinum(II) complexes against lung cancer cells. *J Biol Inorg Chem* **2017,** *22* (6), 807.
(521) Groza, D.; Gehrig, S.; Kudela, P.; Holcmann, M.; Pirker, C.; Dinhof, C.; Schueffl, H. H.; Sramko, M.; Hoebart, J.; Alioglu, F.et al. Bacterial ghosts as adjuvant to oxaliplatin chemotherapy in colorectal carcinomatosis. *Oncoimmunology* **2018,** *7* (5), e1424676.
(522) Chauhan, P.; Sodhi, A.; Shrivastava, A. Cisplatin primes murine peritoneal macrophages for enhanced expression of nitric oxide, proinflammatory cytokines, TLRs, transcription factors and activation of MAP kinases upon co-incubation with L929 cells. *Immunobiology* **2009,** *214* (3), 197.
(523) Van, D. N.; Roberts, C. F.; Marion, J. D.; Lepine, S.; Harikumar, K. B.; Schreiter, J.; Dumur, C. I.; Fang, X.; Spiegel, S.; Bell, J. K. Innate immune agonist, dsRNA, induces apoptosis in ovarian cancer cells and enhances the potency of cytotoxic chemotherapeutics. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2012,** *26* (8), 3188.
(524) Manegold, C.; van Zandwijk, N.; Szczesna, A.; Zatloukal, P.; Au, J. S.; Blasinska-Morawiec, M.; Serwatowski, P.; Krzakowski, M.; Jassem, J.; Tan, E. H.et al. A phase III randomized study of gemcitabine and cisplatin with or without PF-3512676 (TLR9 agonist) as first-line treatment of advanced non-small-cell lung cancer. *Annals of oncology : official journal of the European Society for Medical Oncology* **2012,** *23* (1), 72.
(525) Hu, J.; Kinn, J.; Zirakzadeh, A. A.; Sherif, A.; Norstedt, G.; Wikstrom, A. C.; Winqvist, O. The effects of chemotherapeutic drugs on human monocyte-derived dendritic cell differentiation and antigen presentation. *Clin Exp Immunol* **2013,** *172* (3), 490.
(526) Tel, J.; Hato, S. V.; Torensma, R.; Buschow, S. I.; Figdor, C. G.; Lesterhuis, W. J.; de Vries, I. J. The chemotherapeutic drug oxaliplatin differentially affects blood DC function dependent on environmental cues. *Cancer Immunol Immunother* **2012,** *61* (7), 1101.
(527) Tanaka, H.; Matsushima, H.; Mizumoto, N.; Takashima, A. Classification of Chemotherapeutic Agents Based on Their Differential In vitro Effects on Dendritic Cells. *Cancer research* **2009,** *69* (17), 6978.
(528) Gonzalez-Aparicio, M.; Alzuguren, P.; Mauleon, I.; Medina-Echeverz, J.; Hervas-Stubbs, S.; Mancheno, U.; Berraondo, P.; Crettaz, J.; Gonzalez-Aseguinolaza, G.; Prieto, J.et al. Oxaliplatin in combination with liver-specific expression of interleukin 12 reduces the immunosuppressive microenvironment of tumours and eradicates metastatic colorectal cancer in mice. *Gut* **2011,** *60* (3), 341.
(529) Lesterhuis, W. J.; de Vries, I. J.; Aarntzen, E. A.; de Boer, A.; Scharenborg, N. M.; van de Rakt, M.; van Spronsen, D. J.; Preijers, F. W.; Figdor, C. G.; Adema, G. J.et al. A pilot study on the immunogenicity of dendritic cell vaccination during adjuvant oxaliplatin/capecitabine chemotherapy in colon cancer patients. *Br J Cancer* **2010,** *103* (9), 1415.
(530) Zhao, M.; Li, H.; Li, L.; Zhang, Y. Effects of a gemcitabine plus platinum regimen combined with a dendritic cell-cytokine induced killer immunotherapy on recurrence and survival rate of non-small cell lung cancer patients. *Exp Ther Med* **2014,** *7* (5), 1403.
(531) Markasz, L.; Skribek, H.; Uhlin, M.; Otvos, R.; Flaberg, E.; Eksborg, S.; Olah, E.; Stuber, G.; Szekely, L. Effect of frequently used chemotherapeutic drugs on cytotoxic activity of human cytotoxic T-lymphocytes. *Journal of Immunotherapy* **2008,** *31* (3), 283.
(532) Correale, P.; Del Vecchio, M. T.; La Placa, M.; Montagnani, F.; Di Genova, G.; Savellini, G. G.; Terrosi, C.; Mannucci, S.; Giorgi, G.; Francini, G.et al. Chemotherapeutic drugs may be used to enhance the killing efficacy of human tumor antigen peptide-specific CTLs. *Journal of Immunotherapy* **2008,** *31* (2), 132.

(533) Tsuchikawa, T.; Md, M. M.; Yamamura, Y.; Shichinohe, T.; Hirano, S.; Kondo, S. The immunological impact of neoadjuvant chemotherapy on the tumor microenvironment of esophageal squamous cell carcinoma. *Ann Surg Oncol* **2012,** *19* (5), 1713.
(534) Hakansson, A.; Gustafsson, B.; Krysander, L.; Hjelmqvist, B.; Rettrup, B.; Hakansson, L. Biochemotherapy of metastatic malignant melanoma. Predictive value of tumour-infiltrating lymphocytes. *Br J Cancer* **2001,** *85* (12), 1871.
(535) Hakansson, A.; Hakansson, L.; Gustafsson, B.; Krysander, L.; Rettrup, B.; Ruiter, D.; Bernsen, M. R. Biochemotherapy of metastatic malignant melanoma. On down-regulation of CD28. *Cancer Immunol Immunother* **2002,** *51* (9), 499.
(536) Speiser, D. E.; Pittet, M. J.; Valmori, D.; Dunbar, R.; Rimoldi, D.; Lienard, D.; MacDonald, H. R.; Cerottini, J. C.; Cerundolo, V.; Romero, P. In vivo expression of natural killer cell inhibitory receptors by human melanoma-specific cytolytic T lymphocytes. *The Journal of experimental medicine* **1999,** *190* (6), 775.
(537) Wang, X.; Long, M.; Dong, K.; Lin, F.; Weng, Y. Y.; Ouyang, Y. R.; Liu, L.; Wei, J. X.; Chen, X.; He, T.et al. Chemotherapy agents-induced immunoresistance in lung cancer cells could be reversed by trop-2 inhibition in vitro and in vivo by interaction with MAPK signaling pathway. *Cancer biology & therapy* **2013,** *14* (12), 1123.
(538) Cook, A. M.; Lesterhuis, W. J.; Nowak, A. K.; Lake, R. A. Chemotherapy and immunotherapy: mapping the road ahead. *Curr Opin Immunol* **2016,** *39*, 23.
(539) Gameiro, S. R.; Caballero, J. A.; Higgins, J. P.; Apelian, D.; Hodge, J. W. Exploitation of differential homeostatic proliferation of T-cell subsets following chemotherapy to enhance the efficacy of vaccine-mediated antitumor responses. *Cancer Immunol Immunother* **2011,** *60* (9), 1227.
(540) Huang, X.; Cui, S.; Shu, Y. Cisplatin selectively downregulated the frequency and immunoinhibitory function of myeloid-derived suppressor cells in a murine B16 melanoma model. *Immunologic research* **2016,** *64* (1), 160.
(541) Kim, Y. H.; Choi, B. K.; Kim, K. H.; Kang, S. W.; Kwon, B. S. Combination therapy with cisplatin and anti-4-1BB: synergistic anticancer effects and amelioration of cisplatin-induced nephrotoxicity. *Cancer research* **2008,** *68* (18), 7264.
(542) Maeda, K.; Hazama, S.; Tokuno, K.; Kan, S.; Maeda, Y.; Watanabe, Y.; Kamei, R.; Shindo, Y.; Maeda, N.; Yoshimura, K.et al. Impact of chemotherapy for colorectal cancer on regulatory T-cells and tumor immunity. *Anticancer Res* **2011,** *31* (12), 4569.
(543) Gou, H. F.; Huang, J.; Shi, H. S.; Chen, X. C.; Wang, Y. S. Chemo-immunotherapy with oxaliplatin and interleukin-7 inhibits colon cancer metastasis in mice. *PloS one* **2014,** *9* (1), e85789.
(544) Wu, L.; Yun, Z.; Tagawa, T.; Rey-McIntyre, K.; Anraku, M.; de Perrot, M. Tumor cell repopulation between cycles of chemotherapy is inhibited by regulatory T-cell depletion in a murine mesothelioma model. *J Thorac Oncol* **2011,** *6* (9), 1578.
(545) Shalapour, S.; Font-Burgada, J.; Di Caro, G.; Zhong, Z.; Sanchez-Lopez, E.; Dhar, D.; Willimsky, G.; Ammirante, M.; Strasner, A.; Hansel, D. E.et al. Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature* **2015,** *521* (7550), 94.
(546) Wu, K.; Tan, M. Y.; Jiang, J. T.; Mu, X. Y.; Wang, J. R.; Zhou, W. J.; Wang, X.; Li, M. Q.; He, Y. Y.; Liu, Z. H. Cisplatin inhibits the progression of bladder cancer by selectively depleting G-MDSCs: A novel chemoimmunomodulating strategy. *Clinical immunology* **2018**.
(547) Huang, X.; Guan, D.; Shu, Y. Q.; Liu, L. K.; Ni, F. Effect of Cisplatin on the Frequency and Immuno-inhibitory Function of Myeloid-derived Suppressor Cells in A375 Melanoma Model. *Asian Pac J Cancer Prev* **2015,** *16* (10), 4329.
(548) Alexander, E. T.; Minton, A. R.; Peters, M. C.; van Ryn, J.; Gilmour, S. K. Thrombin inhibition and cisplatin block tumor progression in ovarian cancer by alleviating the immunosuppressive microenvironment. *Oncotarget* **2016,** *7* (51), 85291.

(549) Kanterman, J.; Sade-Feldman, M.; Biton, M.; Ish-Shalom, E.; Lasry, A.; Goldshtein, A.; Hubert, A.; Baniyash, M. Adverse immunoregulatory effects of 5FU and CPT11 chemotherapy on myeloid-derived suppressor cells and colorectal cancer outcomes. *Cancer research* **2014,** *74* (21), 6022.
(550) Maleki Vareki, S.; Rytelewski, M.; Figueredo, R.; Chen, D.; Ferguson, P. J.; Vincent, M.; Min, W.; Zheng, X.; Koropatnick, J. Indoleamine 2,3-dioxygenase mediates immune-independent human tumor cell resistance to olaparib, gamma radiation, and cisplatin. *Oncotarget* **2014,** *5* (9), 2778.
(551) Miyazaki, T.; Moritake, K.; Yamada, K.; Hara, N.; Osago, H.; Shibata, T.; Akiyama, Y.; Tsuchiya, M. Indoleamine 2,3-dioxygenase as a new target for malignant glioma therapy Laboratory investigation. *J Neurosurg* **2009,** *111* (2), 230.
(552) Muller, A. J.; DuHadaway, J. B.; Donover, P. S.; Sutanto-Ward, E.; Prendergast, G. C. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nature medicine* **2005,** *11* (3), 312.
(553) Lu, J.; Liu, X.; Liao, Y. P.; Salazar, F.; Sun, B.; Jiang, W.; Chang, C. H.; Jiang, J.; Wang, X.; Wu, A. M.et al. Nano-enabled pancreas cancer immunotherapy using immunogenic cell death and reversing immunosuppression. *Nat Commun* **2017,** *8* (1), 1811.
(554) Wang, N.; Wang, Z.; Xu, Z.; Chen, X.; Zhu, G. A cisplatin-loaded immuno-chemotherapeutic nanohybrid bearing immune checkpoint inhibitors for enhanced cervical cancer therapy. *Angewandte Chemie* **2018**.
(555) Awuah, S. G.; Zheng, Y. R.; Bruno, P. M.; Hemann, M. T.; Lippard, S. J. A Pt(IV) Pro-drug Preferentially Targets Indoleamine-2,3-dioxygenase, Providing Enhanced Ovarian Cancer Immuno-Chemotherapy. *J Am Chem Soc* **2015,** *137* (47), 14854.
(556) Stewart, R.; Morrow, M.; Hammond, S. A.; Mulgrew, K.; Marcus, D.; Poon, E.; Watkins, A.; Mullins, S.; Chodorge, M.; Andrews, J.et al. Identification and Characterization of MEDI4736, an Antagonistic Anti-PD-L1 Monoclonal Antibody. *Cancer immunology research* **2015,** *3* (9), 1052.
(557) Wu, L. C.; Yun, Z. H.; Tagawa, T.; Rey-McIntyre, K.; de Perrot, M. CTLA-4 Blockade Expands Infiltrating T Cells and Inhibits Cancer Cell Repopulation during the Intervals of Chemotherapy in Murine Mesothelioma. *Molecular Cancer Therapeutics* **2012,** *11* (8), 1809.
(558) Lesterhuis, W. J.; Salmons, J.; Nowak, A. K.; Rozali, E. N.; Khong, A.; Dick, I. M.; Harken, J. A.; Robinson, B. W.; Lake, R. A. Synergistic effect of CTLA-4 blockade and cancer chemotherapy in the induction of anti-tumor immunity. *PloS one* **2013,** *8* (4), e61895.
(559) Song, W.; Shen, L.; Wang, Y.; Liu, Q.; Goodwin, T. J.; Li, J.; Dorosheva, O.; Liu, T.; Liu, R.; Huang, L. Synergistic and low adverse effect cancer immunotherapy by immunogenic chemotherapy and locally expressed PD-L1 trap. *Nat Commun* **2018,** *9* (1), 2237.
(560) Vonderheide, R. H.; Burg, J. M.; Mick, R.; Trosko, J. A.; Li, D.; Shaik, M. N.; Tolcher, A. W.; Hamid, O. Phase I study of the CD40 agonist antibody CP-870,893 combined with carboplatin and paclitaxel in patients with advanced solid tumors. *Oncoimmunology* **2013,** *2* (1), e23033.
(561) Nowak, A. K.; Cook, A. M.; McDonnell, A. M.; Millward, M. J.; Creaney, J.; Francis, R. J.; Hasani, A.; Segal, A.; Musk, A. W.; Turlach, B. A.et al. A phase 1b clinical trial of the CD40-activating antibody CP-870,893 in combination with cisplatin and pemetrexed in malignant pleural mesothelioma. *Annals of oncology : official journal of the European Society for Medical Oncology* **2015,** *26* (12), 2483.
(562) Chester, C.; Sanmamed, M. F.; Wang, J.; Melero, I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. *Blood* **2018,** *131* (1), 49.
(563) Wei, H.; Zhao, L.; Li, W.; Fan, K.; Qian, W.; Hou, S.; Wang, H.; Dai, M.; Hellstrom, I.; Hellstrom, K. E.et al. Combinatorial PD-1 blockade and CD137 activation has therapeutic efficacy in murine cancer models and synergizes with cisplatin. *PloS one* **2013,** *8* (12), e84927.
(564) Wong, D. Y. Q.; Yeo, C. H. F.; Ang, W. H. Immuno-Chemotherapeutic Platinum(IV) Prodrugs of Cisplatin as Multimodal Anticancer Agents. *Angew Chem Int Edit* **2014,** *53* (26), 6752.

(565) Prevete, N.; Liotti, F.; Marone, G.; Melillo, R. M.; de Paulis, A. Formyl peptide receptors at the interface of inflammation, angiogenesis and tumor growth. *Pharmacological research* **2015,** *102*, 184.
(566) Carter, D. E.; Aposhian, H. V.; Gandolfi, A. J. The metabolism of inorganic arsenic oxides, gallium arsenide, and arsine: a toxicochemical review. *Toxicology and applied pharmacology* **2003,** *193* (3), 309.
(567) Abdul, K. S.; Jayasinghe, S. S.; Chandana, E. P.; Jayasumana, C.; De Silva, P. M. Arsenic and human health effects: A review. *Environmental toxicology and pharmacology* **2015,** *40* (3), 828.
(568) Dangleben, N. L.; Skibola, C. F.; Smith, M. T. Arsenic immunotoxicity: a review. *Environmental health : a global access science source* **2013,** *12* (1), 73.
(569) Zhang, J.; Zhang, Y.; Wang, W.; Li, C.; Zhang, Z. Double-Sided Personality: Effects of Arsenic Trioxide on Inflammation. *Inflammation* **2018**.
(570) Arslan, B.; Djamgoz, M. B. A.; Akun, E. ARSENIC: A Review on Exposure Pathways, Accumulation, Mobility and Transmission into the Human Food Chain. *Reviews of environmental contamination and toxicology* **2017,** *243*, 27.
(571) Lee, C.-H.; Liao, W.-T.; Yu, H.-S. Aberrant immune responses in arsenical skin cancers. *The Kaohsiung journal of medical sciences* **2011,** *27* (9), 396.
(572) Miller, W. H.; Schipper, H. M.; Lee, J. S.; Singer, J.; Waxman, S. Mechanisms of action of arsenic trioxide. *Cancer research* **2002,** *62* (14), 3893.
(573) Shackelford, D.; Kenific, C.; Blusztajn, A.; Waxman, S.; Ren, R. Targeted degradation of the AML1/MDS1/EVI1 oncoprotein by arsenic trioxide. *Cancer research* **2006,** *66* (23), 11360.
(574) Jing, Y.; Dai, J.; Chalmers-Redman, R. M.; Tatton, W. G.; Waxman, S. Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. *Blood* **1999,** *94* (6), 2102.
(575) Kumagai, Y.; Sumi, D. Arsenic: signal transduction, transcription factor, and biotransformation involved in cellular response and toxicity. *Annu. Rev. Pharmacol. Toxicol.* **2007,** *47*, 243.
(576) Zheng, Y.; Yamaguchi, H.; Tian, C.; Lee, M. W.; Tang, H.; Wang, H.-G.; Chen, Q. Arsenic trioxide (As 2 O 3) induces apoptosis through activation of Bax in hematopoietic cells. *Oncogene* **2005,** *24* (20), 3339.
(577) Chen, G.-Q.; Zhu, J.; Shi, X.-G.; Ni, J.; Zhong, H.; Si, G.; Jin, X.; Tang, W.; Li, X.; Xong, S. In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia: As2O3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. *Blood* **1996,** *88* (3), 1052.
(578) Wang, J.; Li, L.; Cang, H.; Shi, G.; Yi, J. NADPH oxidase-derived reactive oxygen species are responsible for the high susceptibility to arsenic cytotoxicity in acute promyelocytic leukemia cells. *Leuk Res* **2008,** *32* (3), 429.
(579) Torka, P.; Al Ustwani, O.; Wetzler, M.; Wang, E. S.; Griffiths, E. A. Swallowing a bitter pill-oral arsenic trioxide for acute promyelocytic leukemia. *Blood reviews* **2016,** *30* (3), 201.
(580) Ferrario, D.; Gribaldo, L.; Hartung, T. Arsenic exposure and immunotoxicity: a review including the possible influence of age and sex. *Current environmental health reports* **2016,** *3* (1), 1.
(581) Lage, C. R.; Nayak, A.; Kim, C. H. Arsenic ecotoxicology and innate immunity. *Integrative and Comparative Biology* **2006,** *46* (6), 1040.
(582) Haque, R.; Chaudhary, A.; Sadaf, N. Immunomodulatory Role of Arsenic in Regulatory T Cells. *Endocrine, metabolic & immune disorders drug targets* **2017,** *17* (3), 176.
(583) Morzadec, C.; Macoch, M.; Robineau, M.; Sparfel, L.; Fardel, O.; Vernhet, L. Inorganic arsenic represses interleukin-17A expression in human activated Th17 lymphocytes. *Toxicol Appl Pharmacol* **2012,** *262* (3), 217.

(584) Biswas, R.; Ghosh, P.; Banerjee, N.; Das, J.; Sau, T.; Banerjee, A.; Roy, S.; Ganguly, S.; Chatterjee, M.; Mukherjee, A. Analysis of T-cell proliferation and cytokine secretion in the individuals exposed to arsenic. *Human & experimental toxicology* **2008,** *27* (5), 381.
(585) Lemarie, A.; Morzadec, C.; Bourdonnay, E.; Fardel, O.; Vernhet, L. Human macrophages constitute targets for immunotoxic inorganic arsenic. *J Immunol* **2006,** *177* (5), 3019.
(586) VanDenBerg, K. R.; Freeborn, R. A.; Liu, S.; Kennedy, R. C.; Zagorski, J. W.; Rockwell, C. E. Inhibition of early T cell cytokine production by arsenic trioxide occurs independently of Nrf2. *PloS one* **2017,** *12* (10), e0185579.
(587) Burchiel, S. W.; Mitchell, L. A.; Lauer, F. T.; Sun, X.; McDonald, J. D.; Hudson, L. G.; Liu, K. J. Immunotoxicity and biodistribution analysis of arsenic trioxide in C57Bl/6 mice following a 2-week inhalation exposure. *Toxicol Appl Pharmacol* **2009,** *241* (3), 253.
(588) Mehrzad, J.; Mahmudy Gharaie, M. H.; Taheri, M. Effects of arsenic on porcine dendritic cells in vitro. *Journal of immunotoxicology* **2017,** *14* (1), 1.
(589) Yan, G.; Xi, Y.; Xu, S.; Lin, Y.; Chen, J.; Dai, H.; Xia, J.; Li, C.; Li, Q.; Li, Z. Inhibition of accelerated rejection mediated by alloreactive CD4+ memory T cells and prolonged allograft survival by arsenic trioxide. *Immunological investigations* **2013,** *42* (5), 438.
(590) Li, C.; Guan, T.; Gao, C.; Lin, Y.; Yan, G.; Zhu, M.; Lv, C.; Xia, J.; Qi, Z. Arsenic trioxide inhibits accelerated allograft rejection mediated by alloreactive CD8+ memory T cells and prolongs allograft survival time. *Transplant immunology* **2015,** *33* (1), 30.
(591) Li, K.; Zhang, L.; Xiang, X.; Gong, S.; Ma, L.; Xu, L.; Wang, G.; Liu, Y.; Ji, X.; Liu, S. Arsenic trioxide alleviates airway hyperresponsiveness and promotes apoptosis of CD4+ T lymphocytes: evidence for involvement of the ER stress–CHOP pathway. *Irish journal of medical science* **2013,** *182* (4), 573.
(592) Kerbauy, D. M.; Lesnikov, V.; Abbasi, N.; Seal, S.; Scott, B.; Deeg, H. J. NF-kappaB and FLIP in arsenic trioxide (ATO)-induced apoptosis in myelodysplastic syndromes (MDSs). *Blood* **2005,** *106* (12), 3917.
(593) Estrov, Z.; Manna, S. K.; Harris, D.; Van, Q.; Estey, E. H.; Kantarjian, H. M.; Talpaz, M.; Aggarwal, B. B. Phenylarsine oxide blocks interleukin-1beta-induced activation of the nuclear transcription factor NF-kappaB, inhibits proliferation, and induces apoptosis of acute myelogenous leukemia cells. *Blood* **1999,** *94* (8), 2844.
(594) Deaglio, S.; Canella, D.; Baj, G.; Arnulfo, A.; Waxman, S.; Malavasi, F. Evidence of an immunologic mechanism behind the therapeutical effects of arsenic trioxide (As2O3) on myeloma cells. *Leukemia research* **2001,** *25* (3), 227.
(595) Baj, G.; Arnulfo, A.; Deaglio, S.; Mallone, R.; Vigone, A.; De Cesaris, M. G.; Surico, N.; Malavasi, F.; Ferrero, E. Arsenic trioxide and breast cancer: analysis of the apoptotic, differentiative and immunomodulatory effects. *Breast cancer research and treatment* **2002,** *73* (1), 61.
(596) Thomas-Schoemann, A.; Batteux, F.; Mongaret, C.; Nicco, C.; Chereau, C.; Annereau, M.; Dauphin, A.; Goldwasser, F.; Weill, B.; Lemare, F.et al. Arsenic trioxide exerts antitumor activity through regulatory T cell depletion mediated by oxidative stress in a murine model of colon cancer. *J Immunol* **2012,** *189* (11), 5171.
(597) Wang, L.; Hu, X.; Xu, Y.; Liu, Z. Arsenic trioxide inhibits lung metastasis of mouse colon cancer via reducing the infiltration of regulatory T cells. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* **2016,** *37* (11), 15165.
(598) Wang, L.; Liang, W.; Peng, N.; Hu, X.; Xu, Y.; Liu, Z. The synergistic antitumor effect of arsenic trioxide combined with cytotoxic T cells in pulmonary metastasis model of colon cancer. *Oncotarget* **2017,** *8* (65), 109609.
(599) Wang, L.; Wang, R.; Fan, L.; Liang, W.; Liang, K.; Xu, Y.; Peng, G.; Ye, Q. Arsenic trioxide is an immune adjuvant in liver cancer treatment. *Molecular immunology* **2017,** *81*, 118.

(600) Kim, J.-Y.; Bae, J.-H.; Lee, S.-H.; Lee, E.-Y.; Chung, B.-S.; Kim, S.-H.; Kang, C.-D. Induction of NKG2D ligands and subsequent enhancement of NK cell-mediated lysis of cancer cells by arsenic trioxide. *Journal of Immunotherapy* **2008,** *31* (5), 475.
(601) Luo, L.; Qiao, H.; Meng, F.; Dong, X.; Zhou, B.; Jiang, H.; Kanwar, J. R.; Krissansen, G. W.; Sun, X. Arsenic trioxide synergizes with B7H3-mediated immunotherapy to eradicate hepatocellular carcinomas. *Int J Cancer* **2006,** *118* (7), 1823.
(602) Sun, X.; Vale, M.; Leung, E.; Kanwar, J. R.; Gupta, R.; Krissansen, G. W. Mouse B7-H3 induces antitumor immunity. *Gene therapy* **2003,** *10* (20), 1728.
(603) Leitner, J.; Klauser, C.; Pickl, W. F.; Stockl, J.; Majdic, O.; Bardet, A. F.; Kreil, D. P.; Dong, C.; Yamazaki, T.; Zlabinger, G.et al. B7-H3 is a potent inhibitor of human T-cell activation: No evidence for B7-H3 and TREML2 interaction. *European journal of immunology* **2009,** *39* (7), 1754.
(604) Collier, W. A., Krauss, F. . Zur experimentellen Therapie von Tumoren. *Zeitschrift für Krebsforschung* **1931,** *34* (1), 526.
(605) Jakupec, M. A.; Galanski, M.; Arion, V. B.; Hartinger, C. G.; Keppler, B. K. Antitumour metal compounds: more than theme and variations. *Dalton Trans* **2008,** (2), 183.
(606) Bergamo, A.; Gaiddon, C.; Schellens, J. H.; Beijnen, J. H.; Sava, G. Approaching tumour therapy beyond platinum drugs: status of the art and perspectives of ruthenium drug candidates. *J Inorg Biochem* **2012,** *106* (1), 90.
(607) Bergamo, A.; Sava, G. Ruthenium complexes can target determinants of tumour malignancy. *Dalton Trans* **2007,** (13), 1267.
(608) Zheng, K.; Wu, Q.; Wang, C.; Tan, W.; Mei, W. Ruthenium(II) Complexes as Potential Apoptosis Inducers in Chemotherapy. *Anticancer Agents Med Chem* **2017,** *17* (1), 29.
(609) Babak, M. V.; Meier, S. M.; Huber, K. V. M.; Reynisson, J.; Legin, A. A.; Jakupec, M. A.; Roller, A.; Stukalov, A.; Gridling, M.; Bennett, K. L.et al. Target profiling of an antimetastatic RAPTA agent by chemical proteomics: relevance to the mode of action. *Chem Sci* **2015,** *6* (4), 2449.
(610) Zeng, L.; Gupta, P.; Chen, Y.; Wang, E.; Ji, L.; Chao, H.; Chen, Z. S. The development of anticancer ruthenium(ii) complexes: from single molecule compounds to nanomaterials. *Chem Soc Rev* **2017,** *46* (19), 5771.
(611) Frik, M.; Martinez, A.; Elie, B. T.; Gonzalo, O.; Ramirez de Mingo, D.; Sanau, M.; Sanchez-Delgado, R.; Sadhukha, T.; Prabha, S.; Ramos, J. W.et al. In vitro and in vivo evaluation of water-soluble iminophosphorane ruthenium(II) compounds. A potential chemotherapeutic agent for triple negative breast cancer. *J Med Chem* **2014,** *57* (23), 9995.
(612) Mari, C.; Gasser, G. Lightening up Ruthenium Complexes to Fight Cancer? *Chimia (Aarau)* **2015,** *69* (4), 176.
(613) Guo, W.; Zheng, W.; Luo, Q.; Li, X.; Zhao, Y.; Xiong, S.; Wang, F. Transferrin serves as a mediator to deliver organometallic ruthenium(II) anticancer complexes into cells. *Inorg Chem* **2013,** *52* (9), 5328.
(614) Bergamo, A.; Sava, G. Ruthenium anticancer compounds: myths and realities of the emerging metal-based drugs. *Dalton Trans* **2011,** *40* (31), 7817.
(615) Hartinger, C. G.; Jakupec, M. A.; Zorbas-Seifried, S.; Groessl, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P. J.; Keppler, B. K. KP1019, a new redox-active anticancer agent--preclinical development and results of a clinical phase I study in tumor patients. *Chemistry & biodiversity* **2008,** *5* (10), 2140.
(616) Pal, M.; Nandi, U.; Mukherjee, D. Detailed account on activation mechanisms of ruthenium coordination complexes and their role as antineoplastic agents. *Eur J Med Chem* **2018,** *150*, 419.
(617) Heffeter, P.; Jungwirth, U.; Jakupec, M.; Hartinger, C.; Galanski, M.; Elbling, L.; Micksche, M.; Keppler, B.; Berger, W. Resistance against novel anticancer metal compounds: differences and similarities. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* **2008,** *11* (1-2), 1.
(618) Kostova, I. Ruthenium complexes as anticancer agents. *Curr Med Chem* **2006,** *13* (9), 1085.

(619) Matczuk, M.; Kupiec, M.; Legat, J.; Pawlak, K.; Timerbaev, A. R.; Jarosz, M. A shotgun metalloproteomic approach enables identification of proteins involved in the speciation of a ruthenium anticancer drug in the cytosol of cancer cells. *The Analyst* **2015,** *140* (10), 3492.
(620) Meier, S. M.; Kreutz, D.; Winter, L.; Klose, M. H. M.; Cseh, K.; Weiss, T.; Bileck, A.; Alte, B.; Mader, J. C.; Jana, S.et al. An Organoruthenium Anticancer Agent Shows Unexpected Target Selectivity For Plectin. *Angewandte Chemie* **2017,** *56* (28), 8267.
(621) Su, W.; Tang, Z.; Li, P. Development of Arene Ruthenium Antitumor Complexes. *Mini reviews in medicinal chemistry* **2016,** *16* (10), 787.
(622) Thota, S.; Rodrigues, D. A.; Crans, D. C.; Barreiro, E. J. Ru(II) Compounds: Next-Generation Anticancer Metallotherapeutics? *J Med Chem* **2018**.
(623) Alessio, E. Thirty years of the drug candidate NAMI-A and the myths in the field of ruthenium anticancer compounds: a personal perspective. *Eur J Inorg Chem* **2017**, 1549.
(624) Licona, C.; Spaety, M. E.; Capuozzo, A.; Ali, M.; Santamaria, R.; Armant, O.; Delalande, F.; Van Dorsselaer, A.; Cianferani, S.; Spencer, J.et al. A ruthenium anticancer compound interacts with histones and impacts differently on epigenetic and death pathways compared to cisplatin. *Oncotarget* **2017,** *8* (2), 2568.
(625) Adhireksan, Z.; Davey, G. E.; Campomanes, P.; Groessl, M.; Clavel, C. M.; Yu, H.; Nazarov, A. A.; Yeo, C. H.; Ang, W. H.; Droge, P.et al. Ligand substitutions between ruthenium-cymene compounds can control protein versus DNA targeting and anticancer activity. *Nat Commun* **2014,** *5*, 3462.
(626) Malecot, C. O.; Bito, V.; Argibay, J. A. Ruthenium red as an effective blocker of calcium and sodium currents in guinea-pig isolated ventricular heart cells. *Br J Pharmacol* **1998,** *124* (3), 465.
(627) Dwyer, D. S.; Gordon, K.; Jones, B. Ruthenium Red potently inhibits immune responses both in vitro and in vivo. *Int J Immunopharmacol* **1995,** *17* (11), 931.
(628) Newcomb, J. R.; Rivnay, B.; Bastos, C. M.; Ocain, T. D.; Gordon, K.; Gregory, P.; Turci, S. M.; Sterne, K. A.; Jesson, M.; Krieger, J.et al. In vitro immunomodulatory activity of ruthenium complexes. *Inflamm Res* **2003,** *52* (6), 263.
(629) Costa, M. S.; Goncalves, Y. G.; Nunes, D. C. O.; Napolitano, D. R.; Maia, P. I. S.; Rodrigues, R. S.; Rodrigues, V. M.; Von Poelhsitz, G.; Yoneyama, K. A. G. Anti-Leishmania activity of new ruthenium(II) complexes: Effect on parasite-host interaction. *J Inorg Biochem* **2017,** *175*, 225.
(630) Santos-Silva, T.; Mukhopadhyay, A.; Seixas, J. D.; Bernardes, G. J.; Romao, C. C.; Romao, M. J. Towards improved therapeutic CORMs: understanding the reactivity of CORM-3 with proteins. *Curr Med Chem* **2011,** *18* (22), 3361.
(631) Abraham, N. G.; Kappas, A. Pharmacological and clinical aspects of heme oxygenase. *Pharmacological reviews* **2008,** *60* (1), 79.
(632) Motterlini, R.; Otterbein, L. E. The therapeutic potential of carbon monoxide. *Nature reviews. Drug discovery* **2010,** *9* (9), 728.
(633) Otterbein, L. E.; Bach, F. H.; Alam, J.; Soares, M.; Tao Lu, H.; Wysk, M.; Davis, R. J.; Flavell, R. A.; Choi, A. M. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nature medicine* **2000,** *6* (4), 422.
(634) Sawle, P.; Foresti, R.; Mann, B. E.; Johnson, T. R.; Green, C. J.; Motterlini, R. Carbon monoxide-releasing molecules (CO-RMs) attenuate the inflammatory response elicited by lipopolysaccharide in RAW264.7 murine macrophages. *Br J Pharmacol* **2005,** *145* (6), 800.
(635) Romao, C. C.; Blattler, W. A.; Seixas, J. D.; Bernardes, G. J. Developing drug molecules for therapy with carbon monoxide. *Chem Soc Rev* **2012,** *41* (9), 3571.
(636) Guo, Y.; Stein, A. B.; Wu, W. J.; Tan, W.; Zhu, X.; Li, Q. H.; Dawn, B.; Motterlini, R.; Bolli, R. Administration of a CO-releasing molecule at the time of reperfusion reduces infarct size in vivo. *American journal of physiology. Heart and circulatory physiology* **2004,** *286* (5), H1649.

(637) Di Pascoli, M.; Rodella, L.; Sacerdoti, D.; Bolognesi, M.; Turkseven, S.; Abraham, N. G. Chronic CO levels have [corrected] a beneficial effect on vascular relaxation in diabetes. *Biochem Biophys Res Commun* **2006,** *340* (3), 935.
(638) Vera, T.; Henegar, J. R.; Drummond, H. A.; Rimoldi, J. M.; Stec, D. E. Protective effect of carbon monoxide-releasing compounds in ischemia-induced acute renal failure. *Journal of the American Society of Nephrology : JASN* **2005,** *16* (4), 950.
(639) Tamasi, G.; Merlino, A.; Scaletti, F.; Heffeter, P.; Legin, A. A.; Jakupec, M. A.; Berger, W.; Messori, L.; Keppler, B. K.; Cini, R. {Ru(CO)x}-Core complexes with benzimidazole ligands: synthesis, X-ray structure and evaluation of anticancer activity in vivo. *Dalton Trans* **2017,** *46* (9), 3025.
(640) Lee, D. W.; Shin, H. Y.; Jeong, J. H.; Han, J.; Ryu, S.; Nakahira, K.; Moon, J. S. Carbon monoxide regulates glycolysis-dependent NLRP3 inflammasome activation in macrophages. *Biochem Biophys Res Commun* **2017,** *493* (2), 957.
(641) Choi, E. Y.; Choe, S. H.; Hyeon, J. Y.; Choi, J. I.; Choi, I. S.; Kim, S. J. Carbon monoxide-releasing molecule-3 suppresses Prevotella intermedia lipopolysaccharide-induced production of nitric oxide and interleukin-1beta in murine macrophages. *Eur J Pharmacol* **2015,** *764*, 22.
(642) Yamamoto-Oka, H.; Mizuguchi, S.; Toda, M.; Minamiyama, Y.; Takemura, S.; Shibata, T.; Cepinskas, G.; Nishiyama, N. Carbon monoxide-releasing molecule, CORM-3, modulates alveolar macrophage M1/M2 phenotype in vitro. *Inflammopharmacology* **2018,** *26* (2), 435.
(643) Yoon, Y. E.; Lee, K. S.; Lee, Y. J.; Lee, H. H.; Han, W. K. Renoprotective Effects of Carbon Monoxide-Releasing Molecule 3 in Ischemia-Reperfusion Injury and Cisplatin-Induced Toxicity. *Transplantation proceedings* **2017,** *49* (5), 1175.
(644) Silveira-Lacerda Ede, P.; Vilanova-Costa, C. A.; Pereira Fde, C.; Hamaguchi, A.; Pavanin, L. A.; Goulart, L. R.; Homsi-Brandenburgo, M. I.; Soares, A. M.; dos Santos, W. B.; Nomizo, A. The ruthenium complex cis-(Dichloro)Tetraammineruthenium(III) chloride presents immune stimulatory activity on human peripheral blood mononuclear cells. *Biol Trace Elem Res* **2010,** *133* (3), 270.
(645) Sava, G.; Bergamo, A. Ruthenium-based compounds and tumour growth control (review). *International journal of oncology* **2000,** *17* (2), 353.
(646) Kreuser, E. D.; Keppler, B. K.; Berdel, W. E.; Piest, A.; Thiel, E. Synergistic antitumor interactions between newly synthesized ruthenium complexes and cytokines in human colon carcinoma cell lines. *Seminars in oncology* **1992,** *19* (2 Suppl 3), 73.
(647) Sava, G.; Salerno, G.; Bergamo, A.; Cocchietto, M.; Gagliardi, R.; Alessio, E.; Mestroni, G. Reduction of Lung Metastases by Na[trans-RuCl(4)(DMSO)Im] is not Coupled With the Induction of Chemical Xenogenization. *Met Based Drugs* **1996,** *3* (2), 67.
(648) Brescacin, L.; Masi, A.; Sava, G.; Bergamo, A. Effects of the ruthenium-based drug NAMI-A on the roles played by TGF-beta1 in the metastatic process. *J Biol Inorg Chem* **2015,** *20* (7), 1163.
(649) Bergamo, A.; Pelillo, C.; Chambery, A.; Sava, G. Influence of components of tumour microenvironment on the response of HCT-116 colorectal cancer to the ruthenium-based drug NAMI-A. *J Inorg Biochem* **2017,** *168*, 90.
(650) Bacac, M.; Vadori, M.; Sava, G.; Pacor, S. Cocultures of metastatic and host immune cells: selective effects of NAMI-A for tumor cells. *Cancer Immunol Immunother* **2004,** *53* (12), 1101.
(651) Magnarin, M.; Bergamo, A.; Carotenuto, M. E.; Zorzet, S.; Sava, G. Increase of tumour infiltrating lymphocytes in mice treated with antimetastatic doses of NAMI-A. *Anticancer Res* **2000,** *20* (5A), 2939.
(652) Sava, G.; Capozzi, I.; Clerici, K.; Gagliardi, G.; Alessio, E.; Mestroni, G. Pharmacological control of lung metastases of solid tumours by a novel ruthenium complex. *Clinical & experimental metastasis* **1998,** *16* (4), 371.

(653) Gagliardi, R.; Sava, G.; Pacor, S.; Mestroni, G.; Alessio, E. Antimetastatic action and toxicity on healthy tissues of Na[trans-RuCl4(DMSO)Im] in the mouse. *Clinical & experimental metastasis* **1994,** *12* (2), 93.
(654) Meng, X.; Leyva, M. L.; Jenny, M.; Gross, I.; Benosman, S.; Fricker, B.; Harlepp, S.; Hebraud, P.; Boos, A.; Wlosik, P.et al. A ruthenium-containing organometallic compound reduces tumor growth through induction of the endoplasmic reticulum stress gene CHOP. *Cancer research* **2009,** *69* (13), 5458.
(655) Montani, M.; Pazmay, G. V. B.; Hysi, A.; Lupidi, G.; Pettinari, R.; Gambini, V.; Tilio, M.; Marchetti, F.; Pettinari, C.; Ferraro, S.et al. The water soluble ruthenium(II) organometallic compound [Ru(p-cymene)(bis(3,5 dimethylpyrazol-1-yl)methane)Cl]Cl suppresses triple negative breast cancer growth by inhibiting tumor infiltration of regulatory T cells. *Pharmacological research* **2016,** *107*, 282.
(656) Kaluderovic, G. N.; Krajnovic, T.; Momcilovic, M.; Stosic-Grujicic, S.; Mijatovic, S.; Maksimovic-Ivanic, D.; Hey-Hawkins, E. Ruthenium(II) p-cymene complex bearing 2,2'-dipyridylamine targets caspase 3 deficient MCF-7 breast cancer cells without disruption of antitumor immune response. *J Inorg Biochem* **2015,** *153*, 315.
(657) Hu, D. E.; Dyke, S. O.; Moore, A. M.; Thomsen, L. L.; Brindle, K. M. Tumor cell-derived nitric oxide is involved in the immune-rejection of an immunogenic murine lymphoma. *Cancer research* **2004,** *64* (1), 152.
(658) Castellarin, A.; Zorzet, S.; Bergamo, A.; Sava, G. Pharmacological Activities of Ruthenium Complexes Related to Their NO Scavenging Properties. *International journal of molecular sciences* **2016,** *17* (8).
(659) Hegde, P. S.; Wallin, J. J.; Mancao, C. Predictive markers of anti-VEGF and emerging role of angiogenesis inhibitors as immunotherapeutics. *Semin Cancer Biol* **2017**.
(660) Eisler, R. Chrysotherapy: a synoptic review. *Inflamm Res* **2003,** *52* (12), 487.
(661) Faa, G.; Gerosa, C.; Fanni, D.; Lachowicz, J. I.; Nurchi, V. M. Gold - Old Drug with New Potentials. *Curr Med Chem* **2018,** *25* (1), 75.
(662) Merchant, B. Gold, the noble metal and the paradoxes of its toxicology. *Biologicals* **1998,** *26* (1), 49.
(663) Casini, A.; Messori, L. Molecular mechanisms and proposed targets for selected anticancer gold compounds. *Curr Top Med Chem* **2011,** *11* (21), 2647.
(664) Hamilton, J.; McInnes, I. B.; Thomson, E. A.; Porter, D.; Hunter, J. A.; Madhok, R.; Capell, H. A. Comparative study of intramuscular gold and methotrexate in a rheumatoid arthritis population from a socially deprived area. *Ann Rheum Dis* **2001,** *60* (6), 566.
(665) Jurgens, S.; Casini, A. Mechanistic Insights into Gold Organometallic Compounds and their Biomedical Applications. *Chimia (Aarau)* **2017,** *71* (3), 92.
(666) Graham, G. G.; Kettle, A. J. The activation of gold complexes by cyanide produced by polymorphonuclear leukocytes. III. The formation of aurocyanide by myeloperoxidase. *Biochem Pharmacol* **1998,** *56* (3), 307.
(667) Shaw, I. C. Gold-based therapeutic agents. *Chemical reviews* **1999,** *99* (9), 2589.
(668) Mirabelli, C. K.; Hill, D. T.; Faucette, L. F.; McCabe, F. L.; Girard, G. R.; Bryan, D. B.; Sutton, B. M.; Bartus, J. O.; Crooke, S. T.; Johnson, R. K. Antitumor activity of bis(diphenylphosphino)alkanes, their gold(I) coordination complexes, and related compounds. *J Med Chem* **1987,** *30* (12), 2181.
(669) Mirabelli, C. K.; Sung, C. M.; Zimmerman, J. P.; Hill, D. T.; Mong, S.; Crooke, S. T. Interactions of gold coordination complexes with DNA. *Biochem Pharmacol* **1986,** *35* (9), 1427.
(670) Porchia, M.; Pellei, M.; Marinelli, M.; Tisato, F.; Del Bello, F.; Santini, C. New insights in Au-NHCs complexes as anticancer agents. *Eur J Med Chem* **2018,** *146*, 709.
(671) Nardon, C.; Boscutti, G.; Fregona, D. Beyond platinums: gold complexes as anticancer agents. *Anticancer Res* **2014,** *34* (1), 487.

(672) Tiekink, E. R. Anti-cancer potential of gold complexes. *Inflammopharmacology* **2008,** *16* (3), 138.
(673) Madeira, J. M.; Gibson, D. L.; Kean, W. F.; Klegeris, A. The biological activity of auranofin: implications for novel treatment of diseases. *Inflammopharmacology* **2012,** *20* (6), 297.
(674) Han, S.; Kim, K.; Kim, H.; Kwon, J.; Lee, Y. H.; Lee, C. K.; Song, Y.; Lee, S. J.; Ha, N.; Kim, K. Auranofin inhibits overproduction of pro-inflammatory cytokines, cyclooxygenase expression and PGE2 production in macrophages. *Archives of pharmacal research* **2008,** *31* (1), 67.
(675) Sato, H.; Yamaguchi, M.; Shibasaki, T.; Ishii, T.; Bannai, S. Induction of stress proteins in mouse peritoneal macrophages by the anti-rheumatic agents gold sodium thiomalate and auranofin. *Biochem Pharmacol* **1995,** *49* (10), 1453.
(676) Kim, N. H.; Oh, M. K.; Park, H. J.; Kim, I. S. Auranofin, a gold(I)-containing antirheumatic compound, activates Keap1/Nrf2 signaling via Rac1/iNOS signal and mitogen-activated protein kinase activation. *J Pharmacol Sci* **2010,** *113* (3), 246.
(677) Youn, H. S.; Lee, J. Y.; Saitoh, S. I.; Miyake, K.; Hwang, D. H. Auranofin, as an anti-rheumatic gold compound, suppresses LPS-induced homodimerization of TLR4. *Biochem Biophys Res Commun* **2006,** *350* (4), 866.
(678) Griem, P.; Panthel, K.; Kalbacher, H.; Gleichmann, E. Alteration of a model antigen by Au(III) leads to T cell sensitization to cryptic peptides. *European journal of immunology* **1996,** *26* (2), 279.
(679) Han, S.; Kim, K.; Song, Y.; Kim, H.; Kwon, J.; Lee, Y. H.; Lee, C. K.; Lee, S. J.; Ha, N.; Kim, K. Auranofin, an immunosuppressive drug, inhibits MHC class I and MHC class II pathways of antigen presentation in dendritic cells. *Archives of pharmacal research* **2008,** *31* (3), 370.
(680) Sanders, K. M.; Carlson, P. L.; Littman, B. H. Effects of gold sodium thiomalate on interferon stimulation of C2 synthesis and HLA-DR expression by human monocytes. *Arthritis Rheum* **1987,** *30* (9), 1032.
(681) Yang, J. P.; Merin, J. P.; Nakano, T.; Kato, T.; Kitade, Y.; Okamoto, T. Inhibition of the DNA-binding activity of NF-kappa B by gold compounds in vitro. *FEBS Lett* **1995,** *361* (1), 89.
(682) Jeon, K. I.; Byun, M. S.; Jue, D. M. Gold compound auranofin inhibits IkappaB kinase (IKK) by modifying Cys-179 of IKKbeta subunit. *Exp Mol Med* **2003,** *35* (2), 61.
(683) Zeligs, K. P.; Neuman, M. K.; Annunziata, C. M. Molecular Pathways: The Balance between Cancer and the Immune System Challenges the Therapeutic Specificity of Targeting Nuclear Factor-kappaB Signaling for Cancer Treatment. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2016,** *22* (17), 4302.
(684) Gerondakis, S.; Siebenlist, U. Roles of the NF-kappaB pathway in lymphocyte development and function. *Cold Spring Harbor perspectives in biology* **2010,** *2* (5), a000182.
(685) Lim, P. S.; Sutton, C. R.; Rao, S. Protein kinase C in the immune system: from signalling to chromatin regulation. *Immunology* **2015,** *146* (4), 508.
(686) Zetterstrom, C. K.; Jiang, W.; Wahamaa, H.; Ostberg, T.; Aveberger, A. C.; Schierbeck, H.; Lotze, M. T.; Andersson, U.; Pisetsky, D. S.; Erlandsson Harris, H. Pivotal advance: inhibition of HMGB1 nuclear translocation as a mechanism for the anti-rheumatic effects of gold sodium thiomalate. *Journal of leukocyte biology* **2008,** *83* (1), 31.
(687) Madeira, J. M.; Bajwa, E.; Stuart, M. J.; Hashioka, S.; Klegeris, A. Gold drug auranofin could reduce neuroinflammation by inhibiting microglia cytotoxic secretions and primed respiratory burst. *J Neuroimmunol* **2014,** *276* (1-2), 71.
(688) Handel, M. L.; Watts, C. K.; deFazio, A.; Day, R. O.; Sutherland, R. L. Inhibition of AP-1 binding and transcription by gold and selenium involving conserved cysteine residues in Jun and Fos. *Proc Natl Acad Sci U S A* **1995,** *92* (10), 4497.
(689) Kim, N. H.; Lee, M. Y.; Park, S. J.; Choi, J. S.; Oh, M. K.; Kim, I. S. Auranofin blocks interleukin-6 signalling by inhibiting phosphorylation of JAK1 and STAT3. *Immunology* **2007,** *122* (4), 607.
(690) Jeon, K. I.; Jeong, J. Y.; Jue, D. M. Thiol-reactive metal compounds inhibit NF-kappa B activation by blocking I kappa B kinase. *J Immunol* **2000,** *164* (11), 5981.

(691) Yamada, R.; Sano, H.; Hla, T.; Hashiramoto, A.; Fukui, W.; Miyazaki, S.; Kohno, M.; Tsubouchi, Y.; Kusaka, Y.; Kondo, M. Auranofin inhibits interleukin-1beta-induced transcript of cyclooxygenase-2 on cultured human synoviocytes. *Eur J Pharmacol* **1999,** *385* (1), 71.
(692) Nakaya, A.; Sagawa, M.; Muto, A.; Uchida, H.; Ikeda, Y.; Kizaki, M. The gold compound auranofin induces apoptosis of human multiple myeloma cells through both down-regulation of STAT3 and inhibition of NF-kappaB activity. *Leuk Res* **2011,** *35* (2), 243.
(693) Parente, J. E.; Walsh, M. P.; Girard, P. R.; Kuo, J. F.; Ng, D. S.; Wong, K. Effects of gold coordination complexes on neutrophil function are mediated via inhibition of protein kinase C. *Mol Pharmacol* **1989,** *35* (1), 26.
(694) Hashimoto, K.; Whitehurst, C. E.; Lipsky, P. E. Synergistic inhibition of T cell proliferation by gold sodium thiomalate and auranofin. *J Rheumatol* **1994,** *21* (6), 1020.
(695) Lazarevic, M. B.; Yan, K.; Swedler, W. I.; Rasenick, M. M.; Skosey, J. L. Effect of gold compounds on the activity of adenylyl cyclase in human lymphocyte membranes. *Arthritis Rheum* **1992,** *35* (8), 857.
(696) Griem, P.; Takahashi, K.; Kalbacher, H.; Gleichmann, E. The antirheumatic drug disodium aurothiomalate inhibits CD4+ T cell recognition of peptides containing two or more cysteine residues. *J Immunol* **1995,** *155* (3), 1575.
(697) Wang, Q.; Janzen, N.; Ramachandran, C.; Jirik, F. Mechanism of inhibition of protein-tyrosine phosphatases by disodium aurothiomalate. *Biochem Pharmacol* **1997,** *54* (6), 703.
(698) Hirohata, S. Inhibition of human B cell activation by gold compounds. *Clin Immunol Immunopathol* **1996,** *81* (2), 175.
(699) Kiely, P. D.; Helbert, M. R.; Miles, J.; Oliveira, D. B. Immunosuppressant effect of gold on IgG subclasses and IgE; evidence for sparing of Th2 responses. *Clin Exp Immunol* **2000,** *120* (2), 369.
(700) Vanco, J.; Galikova, J.; Hosek, J.; Dvorak, Z.; Parakova, L.; Travnicek, Z. Gold(I) complexes of 9-deazahypoxanthine as selective antitumor and anti-inflammatory agents. *PloS one* **2014,** *9* (10), e109901.
(701) Kim, N. H.; Park, H. J.; Oh, M. K.; Kim, I. S. Antiproliferative effect of gold(I) compound auranofin through inhibition of STAT3 and telomerase activity in MDA-MB 231 human breast cancer cells. *BMB Rep* **2013,** *46* (1), 59.
(702) Markowska, A.; Kasprzak, B.; Jaszczynska-Nowinka, K.; Lubin, J.; Markowska, J. Noble metals in oncology. *Contemp Oncol (Pozn)* **2015,** *19* (4), 271.
(703) Stafford, W. C.; Peng, X.; Olofsson, M. H.; Zhang, X.; Luci, D. K.; Lu, L.; Cheng, Q.; Tresaugues, L.; Dexheimer, T. S.; Coussens, N. P.et al. Irreversible inhibition of cytosolic thioredoxin reductase 1 as a mechanistic basis for anticancer therapy. *Sci Transl Med* **2018,** *10* (428).
(704) Arner, E. S.; Holmgren, A. The thioredoxin system in cancer. *Semin Cancer Biol* **2006,** *16* (6), 420.
(705) Hou, G. X.; Liu, P. P.; Zhang, S.; Yang, M.; Liao, J.; Yang, J.; Hu, Y.; Jiang, W. Q.; Wen, S.; Huang, P. Elimination of stem-like cancer cell side-population by auranofin through modulation of ROS and glycolysis. *Cell Death Dis* **2018,** *9* (2), 89.
(706) Talbot, S.; Nelson, R.; Self, W. T. Arsenic trioxide and auranofin inhibit selenoprotein synthesis: implications for chemotherapy for acute promyelocytic leukaemia. *Br J Pharmacol* **2008,** *154* (5), 940.
(707) Oommen, D.; Yiannakis, D.; Jha, A. N. BRCA1 deficiency increases the sensitivity of ovarian cancer cells to auranofin. *Mutat Res* **2016,** *784-785*, 8.
(708) Papaioannou, M.; Mylonas, I.; Kast, R. E.; Bruning, A. Disulfiram/copper causes redox-related proteotoxicity and concomitant heat shock response in ovarian cancer cells that is augmented by auranofin-mediated thioredoxin inhibition. *Oncoscience* **2014,** *1* (1), 21.
(709) Jatoi, A.; Radecki Breitkopf, C.; Foster, N. R.; Block, M. S.; Grudem, M.; Wahner Hendrickson, A.; Carlson, R. E.; Barrette, B.; Karlin, N.; Fields, A. P. A mixed-methods feasibility trial of protein kinase

C iota inhibition with auranofin in asymptomatic ovarian cancer patients. *Oncology* **2015,** *88* (4), 208.
(710) Kast, R. E. Glioblastoma invasion, cathepsin B, and the potential for both to be inhibited by auranofin, an old anti-rheumatoid arthritis drug. *Cent Eur Neurosurg* **2010,** *71* (3), 139.
(711) Park, S. H.; Lee, J. H.; Berek, J. S.; Hu, M. C. Auranofin displays anticancer activity against ovarian cancer cells through FOXO3 activation independent of p53. *International journal of oncology* **2014,** *45* (4), 1691.
(712) Heimburger, M.; Lerner, R.; Palmblad, J. Effects of antirheumatic drugs on adhesiveness of endothelial cells and neutrophils. *Biochem Pharmacol* **1998,** *56* (12), 1661.
(713) Newman, P. M.; To, S. S.; Robinson, B. G.; Hyland, V. J.; Schrieber, L. Effect of gold sodium thiomalate and its thiomalate component on the in vitro expression of endothelial cell adhesion molecules. *The Journal of clinical investigation* **1994,** *94* (5), 1864.
(714) He, M. F.; Gao, X. P.; Li, S. C.; He, Z. H.; Chen, N.; Wang, Y. B.; She, J. X. Anti-angiogenic effect of auranofin on HUVECs in vitro and zebrafish in vivo. *Eur J Pharmacol* **2014,** *740*, 240.
(715) Chen, X.; Zhou, H. J.; Huang, Q.; Lu, L.; Min, W. Novel action and mechanism of auranofin in inhibition of vascular endothelial growth factor receptor-3-dependent lymphangiogenesis. *Anticancer Agents Med Chem* **2014,** *14* (7), 946.
(716) Park, S. J.; Lee, A. N.; Youn, H. S. TBK1-targeted suppression of TRIF-dependent signaling pathway of toll-like receptor 3 by auranofin. *Archives of pharmacal research* **2010,** *33* (6), 939.
(717) Williame, L. M.; Joos, R.; Proot, F.; Immesoete, C. Gold-induced aplastic anemia. *Clin Rheumatol* **1987,** *6* (4), 600.
(718) Yan, A.; Davis, P. Gold induced marrow suppression: a review of 10 cases. *J Rheumatol* **1990,** *17* (1), 47.
(719) Stillman, A. E.; Dubey, D. P. Immune functions and gold-induced enterocolitis. *Dig Dis Sci* **1988,** *33* (8), 1046.
(720) Uhm, W. S.; Yoo, D. H.; Lee, J. H.; Kim, T. H.; Jun, J. B.; Lee, I. H.; Bae, S. C.; Kim, S. Y. Injectable gold-induced hepatitis and neutropenia in rheumatoid arthritis. *Korean J Intern Med* **2000,** *15* (2), 156.
(721) Schuhmann, D.; Kubicka-Muranyi, M.; Mirtschewa, J.; Gunther, J.; Kind, P.; Gleichmann, E. Adverse immune reactions to gold. I. Chronic treatment with an Au(I) drug sensitizes mouse T cells not to Au(I), but to Au(III) and induces autoantibody formation. *J Immunol* **1990,** *145* (7), 2132.
(722) Goebel, C.; Kubicka-Muranyi, M.; Tonn, T.; Gonzalez, J.; Gleichmann, E. Phagocytes render chemicals immunogenic: oxidation of gold(I) to the T cell-sensitizing gold(III) metabolite generated by mononuclear phagocytes. *Arch Toxicol* **1995,** *69* (7), 450.
(723) Qureshi, A. A.; Rosenblatt, D. S.; Cooper, B. A. Inherited disorders of cobalamin metabolism. *Crit Rev Oncol Hematol* **1994,** *17* (2), 133.
(724) Paustenbach, D. J.; Tvermoes, B. E.; Unice, K. M.; Finley, B. L.; Kerger, B. D. A review of the health hazards posed by cobalt. *Critical reviews in toxicology* **2013,** *43* (4), 316.
(725) Zywiel, M. G.; Cherian, J. J.; Banerjee, S.; Cheung, A. C.; Wong, F.; Butany, J.; Gilbert, C.; Overgaard, C.; Syed, K.; Jacobs, J. J.et al. Systemic cobalt toxicity from total hip arthroplasties: review of a rare condition Part 2. measurement, risk factors, and step-wise approach to treatment. *The bone & joint journal* **2016,** *98-B* (1), 14.
(726) Beyersmann, D.; Hartwig, A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Arch Toxicol* **2008,** *82* (8), 493.
(727) Kasprzak, K. S.; Zastawny, T. H.; North, S. L.; Riggs, C. W.; Diwan, B. A.; Rice, J. M.; Dizdaroglu, M. Oxidative DNA base damage in renal, hepatic, and pulmonary chromatin of rats after intraperitoneal injection of cobalt(II) acetate. *Chemical research in toxicology* **1994,** *7* (3), 329.
(728) Kopera, E.; Schwerdtle, T.; Hartwig, A.; Bal, W. Co(II) and Cd(II) substitute for Zn(II) in the zinc finger derived from the DNA repair protein XPA, demonstrating a variety of potential mechanisms of toxicity. *Chemical research in toxicology* **2004,** *17* (11), 1452.

(729) Leyssens, L.; Vinck, B.; Van Der Straeten, C.; Wuyts, F.; Maes, L. Cobalt toxicity in humans-A review of the potential sources and systemic health effects. *Toxicology* **2017,** *387*, 43.
(730) Van Der Straeten, C.; Van Quickenborne, D.; De Roest, B.; Calistri, A.; Victor, J.; De Smet, K. Metal ion levels from well-functioning Birmingham Hip Resurfacings decline significantly at ten years. *The bone & joint journal* **2013,** *95-B* (10), 1332.
(731) Lison, D.; Lauwerys, R. Cobalt bioavailability from hard metal particles. Further evidence that cobalt alone is not responsible for the toxicity of hard metal particles. *Archives of toxicology* **1994,** *68* (8), 528.
(732) Baskey, S. J.; Lehoux, E. A.; Catelas, I. Effects of cobalt and chromium ions on lymphocyte migration. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **2017,** *35* (4), 916.
(733) Munteanu, C. R.; Suntharalingam, K. Advances in cobalt complexes as anticancer agents. *Dalton transactions (Cambridge, England : 2003)* **2015,** *44* (31), 13796.
(734) Ruiz-Sanchez, P.; Konig, C.; Ferrari, S.; Alberto, R. Vitamin B(1)(2) as a carrier for targeted platinum delivery: in vitro cytotoxicity and mechanistic studies. *J Biol Inorg Chem* **2011,** *16* (1), 33.
(735) Karnthaler-Benbakka, C.; Groza, D.; Kryeziu, K.; Pichler, V.; Roller, A.; Berger, W.; Heffeter, P.; Kowol, C. R. Tumor-targeting of EGFR inhibitors by hypoxia-mediated activation. *Angewandte Chemie* **2014,** *53* (47), 12930.
(736) Sun, Z.; Mohamed, M. A. A.; Park, S. Y.; Yi, T. H. Fucosterol protects cobalt chloride induced inflammation by the inhibition of hypoxia-inducible factor through PI3K/Akt pathway. *International immunopharmacology* **2015,** *29* (2), 642.
(737) Rath, S.; Anand, A.; Ghosh, N.; Das, L.; Kokate, S. B.; Dixit, P.; Majhi, S.; Rout, N.; Singh, S. P.; Bhattacharyya, A. Cobalt chloride-mediated protein kinase Calpha (PKCalpha) phosphorylation induces hypoxia-inducible factor 1alpha (HIF1alpha) in the nucleus of gastric cancer cell. *Biochemical and biophysical research communications* **2016,** *471* (1), 205.
(738) Davis, J. E.; Fields, J. P. Experimental production of polycythemia in humans by administration of cobalt chloride. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)* **1958,** *99* (2), 493.
(739) Ebert, B.; Jelkmann, W. Intolerability of cobalt salt as erythropoietic agent. *Drug testing and analysis* **2014,** *6* (3), 185.
(740) Yuan, Y.; Hilliard, G.; Ferguson, T.; Millhorn, D. E. Cobalt inhibits the interaction between hypoxia-inducible factor-alpha and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor-alpha. *J Biol Chem* **2003,** *278* (18), 15911.
(741) Wilson, H. M.; Welikson, R. E.; Luo, J.; Kean, T. J.; Cao, B.; Dennis, J. E.; Allen, M. D. Can Cytoprotective Cobalt Protoporphyrin Protect Skeletal Muscle and Muscle-derived Stem Cells From Ischemic Injury? *Clinical orthopaedics and related research* **2015,** *473* (9), 2908.
(742) Luo, J.; Weaver, M. S.; Cao, B.; Dennis, J. E.; Van Biber, B.; Laflamme, M. A.; Allen, M. D. Cobalt protoporphyrin pretreatment protects human embryonic stem cell-derived cardiomyocytes from hypoxia/reoxygenation injury in vitro and increases graft size and vascularization in vivo. *Stem cells translational medicine* **2014,** *3* (6), 734.
(743) Issan, Y.; Kornowski, R.; Aravot, D.; Shainberg, A.; Laniado-Schwartzman, M.; Sodhi, K.; Abraham, N. G.; Hochhauser, E. Heme oxygenase-1 induction improves cardiac function following myocardial ischemia by reducing oxidative stress. *PloS one* **2014,** *9* (3), e92246.
(744) Marelli, G.; Erreni, M.; Anselmo, A.; Taverniti, V.; Guglielmetti, S.; Mantovani, A.; Allavena, P. Heme-oxygenase-1 Production by Intestinal CX3CR1(+) Macrophages Helps to Resolve Inflammation and Prevents Carcinogenesis. *Cancer research* **2017,** *77* (16), 4472.
(745) Sierra-Filardi, E.; Vega, M. A.; Sanchez-Mateos, P.; Corbi, A. L.; Puig-Kroger, A. Heme Oxygenase-1 expression in M-CSF-polarized M2 macrophages contributes to LPS-induced IL-10 release. *Immunobiology* **2010,** *215* (9-10), 788.

(746) Hu, A. P.; Du, J. M.; Li, J. Y.; Liu, J. W. Oridonin promotes CD4+/CD25+ Treg differentiation, modulates Th1/Th2 balance and induces HO-1 in rat splenic lymphocytes. *Inflamm Res* **2008,** *57* (4), 163.
(747) Webb, T. J.; Carey, G. B.; East, J. E.; Sun, W.; Bollino, D. R.; Kimball, A. S.; Brutkiewicz, R. R. Alterations in cellular metabolism modulate CD1d-mediated NKT-cell responses. *Pathogens and disease* **2016,** *74* (6).
(748) Lawrence, H.; Mawdesley, A. E.; Holland, J. P.; Kirby, J. A.; Deehan, D. J.; Tyson-Capper, A. J. Targeting Toll-like receptor 4 prevents cobalt-mediated inflammation. *Oncotarget* **2016,** *7* (7), 7578.
(749) Pettersson, M.; Pettersson, J.; Thoren, M. M.; Johansson, A. Effect of cobalt ions on the interaction between macrophages and titanium. *Journal of biomedical materials research. Part A* **2018**.
(750) Wang, X. D.; Liu, C. X.; Wu, Y. L.; Chen, J. [Hypoxia and its simulant CoCl(2) down-regulates Foxp3 expression independent from HIF-1alpha]. *Zhongguo shi yan xue ye xue za zhi* **2009,** *17* (3), 533.
(751) Paladini, F.; Cocco, E.; Potolicchio, I.; Fazekasova, H.; Lombardi, G.; Fiorillo, M. T.; Sorrentino, R. Divergent effect of cobalt and beryllium salts on the fate of peripheral blood monocytes and T lymphocytes. *Toxicological sciences : an official journal of the Society of Toxicology* **2011,** *119* (2), 257.
(752) Zhu, H.; Liu, Y.; Hong, H.; Wang, W.; Liu, F. Protective effects of Zn(2+) against cobalt nanoparticles and cobalt chloride-induced cytotoxicity of RAW 264.7cells via ROS pathway. *Biochem Biophys Res Commun* **2017,** *486* (2), 357.
(753) Ott, I.; Schmidt, K.; Kircher, B.; Schumacher, P.; Wiglenda, T.; Gust, R. Antitumor-active cobalt-alkyne complexes derived from acetylsalicylic acid: studies on the mode of drug action. *J Med Chem* **2005,** *48* (2), 622.
(754) Obermoser, V.; Baecker, D.; Schuster, C.; Braun, V.; Kircher, B.; Gust, R. Chlorinated cobalt alkyne complexes derived from acetylsalicylic acid as new specific antitumor agents. *Dalton Trans* **2018,** *47* (12), 4341.
(755) Lin, C. Y.; Power, P. P. Complexes of Ni(i): a "rare" oxidation state of growing importance. *Chem Soc Rev* **2017,** *46* (17), 5347.
(756) Patil, S. A.; Patil, S. A.; Patil, R.; Keri, R. S.; Budagumpi, S.; Balakrishna, G. R.; Tacke, M. N-heterocyclic carbene metal complexes as bio-organometallic antimicrobial and anticancer drugs. *Future Med Chem* **2015,** *7* (10), 1305.
(757) Saad, E. A.; Hassanien, M. M.; El-Lban, F. W. Nickel(II) diacetyl monoxime-2-pyridyl hydrazone complex can inhibit Ehrlich solid tumor growth in mice: A potential new antitumor drug. *Biochemical and biophysical research communications* **2017,** *484* (3), 579.
(758) Pahontu, E.; Julea, F.; Rosu, T.; Purcarea, V.; Chumakov, Y.; Petrenco, P.; Gulea, A. Antibacterial, antifungal and in vitro antileukaemia activity of metal complexes with thiosemicarbazones. *Journal of cellular and molecular medicine* **2015,** *19* (4), 865.
(759) Shawish, H. B.; Wong, W. Y.; Wong, Y. L.; Loh, S. W.; Looi, C. Y.; Hassandarvish, P.; Phan, A. Y.; Wong, W. F.; Wang, H.; Paterson, I. C.et al. Nickel(II) complex of polyhydroxybenzaldehyde N4-thiosemicarbazone exhibits anti-inflammatory activity by inhibiting NF-kappaB transactivation. *PloS one* **2014,** *9* (6), e100933.
(760) Banerjee, K.; Biswas, M. K.; Choudhuri, S. K. A newly synthesized nickel chelate can selectively target and overcome multidrug resistance in cancer through redox imbalance both in vivo and in vitro. *J Biol Inorg Chem* **2017,** *22* (8), 1223.
(761) Li, Z.; Yan, H.; Chang, G.; Hong, M.; Dou, J.; Niu, M. Cu(II), Ni(II) complexes derived from chiral Schiff-base ligands: Synthesis, characterization, cytotoxicity, protein and DNA-binding properties. *Journal of photochemistry and photobiology. B, Biology* **2016,** *163*, 403.
(762) Flamme, M.; Cressey, P. B.; Lu, C.; Bruno, P. M.; Eskandari, A.; Hemann, M. T.; Hogarth, G.; Suntharalingam, K. Induction of Necroptosis in Cancer Stem Cells using a Nickel(II)-

Dithiocarbamate Phenanthroline Complex. *Chemistry (Weinheim an der Bergstrasse, Germany)* **2017,** *23* (40), 9674.

(763) Saito, M.; Arakaki, R.; Yamada, A.; Tsunematsu, T.; Kudo, Y.; Ishimaru, N. Molecular Mechanisms of Nickel Allergy. *International journal of molecular sciences* **2016,** *17* (2).

(764) Schmidt, M.; Goebeler, M. Immunology of metal allergies. *Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG* **2015,** *13* (7), 653.

(765) Tilakaratne, D.; Sidhu, S. Heavy metal (monoclonal) bands: a link between cutaneous T-cell lymphoma and contact allergy to potassium dichromate, nickel and cobalt? *The Australasian journal of dermatology* **2015,** *56* (1), 59.

(766) Ali, A.; Suhail, M.; Mathew, S.; Shah, M. A.; Harakeh, S. M.; Ahmad, S.; Kazmi, Z.; Alhamdan, M. A. R.; Chaudhary, A.; Damanhouri, G. A.et al. Nanomaterial Induced Immune Responses and Cytotoxicity. *Journal of nanoscience and nanotechnology* **2016,** *16* (1), 40.

(767) Ding, J.; Huang, Y.; Ning, B.; Gong, W.; Li, J.; Wang, H.; Chen, C.-Y.; Huang, C. TNF-alpha induction by nickel compounds is specific through ERKs/AP-1-dependent pathway in human bronchial epithelial cells. *Current cancer drug targets* **2009,** *9* (1), 81.

(768) McFadden, J. P.; Puangpet, P.; Basketter, D. A.; Dearman, R. J.; Kimber, I. Why does allergic contact dermatitis exist? *British Journal of Dermatology* **2013,** *168* (4), 692.

(769) Kaplan, D. H.; Igyártó, B. Z.; Gaspari, A. A. Early immune events in the induction of allergic contact dermatitis. *Nature Reviews Immunology* **2012,** *12* (2), 114.

(770) Honda, T.; Egawa, G.; Grabbe, S.; Kabashima, K. Update of Immune Events in the Murine Contact Hypersensitivity Model: Toward the Understanding of Allergic Contact Dermatitis. *Journal of Investigative Dermatology* **2013,** *133* (2), 303.

(771) Dhingra, N.; Shemer, A.; Correa da Rosa, J.; Rozenblit, M.; Fuentes-Duculan, J.; Gittler, J. K.; Finney, R.; Czarnowicki, T.; Zheng, X.; Xu, H.et al. Molecular profiling of contact dermatitis skin identifies allergen-dependent differences in immune response. *The Journal of allergy and clinical immunology* **2014,** *134* (2), 362.

(772) Freitas, M.; Fernandes, E. Zinc, cadmium and nickel increase the activation of NF-κB and the release of cytokines from THP-1 monocytic cells. *Metallomics : integrated biometal science* **2011,** *3* (11), 1238.

(773) Gölz, L.; Buerfent, B. C.; Hofmann, A.; Rühl, H.; Fricker, N.; Stamminger, W.; Oldenburg, J.; Deschner, J.; Hoerauf, A.; Nöthen, M. M.et al. Genome-wide transcriptome induced by nickel in human monocytes. *Acta Biomaterialia* **2016,** *43*, 369.

(774) Xu, H.; DiIulio, N. A.; Fairchild, R. L. T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon gamma-producing (Tc1) effector CD8+ T cells and interleukin (Il) 4/Il-10-producing (Th2) negative regulatory CD4+ T cells. *The Journal of experimental medicine* **1996,** *183* (3), 1001.

(775) Turbica, I.; Gallais, Y.; Gueguen, C.; Tharinger, H.; Al Sabbagh, C.; Gorges, R.; Gary-Gouy, H.; Kerdine-Römer, S.; Pallardy, M.; Mascarell, L.et al. Ectosomes from neutrophil-like cells down-regulate nickel-induced dendritic cell maturation and promote Th2 polarization. *Journal of leukocyte biology* **2015,** *97* (4), 737.

(776) Balmert, S. C.; Donahue, C.; Vu, J. R.; Erdos, G.; Falo, L. D., Jr.; Little, S. R. In vivo induction of regulatory T cells promotes allergen tolerance and suppresses allergic contact dermatitis. *Journal of controlled release : official journal of the Controlled Release Society* **2017,** *261*, 223.

(777) Jakob, A.; Mussotter, F.; Ohnesorge, S.; Dietz, L.; Pardo, J.; Haidl, I. D.; Thierse, H.-J. Immunoproteomic identification and characterization of Ni2+-regulated proteins implicates Ni2+in the induction of monocyte cell death. *Cell death & disease* **2017,** *8* (3), e2684.

(778) Zarei, M. H.; Hosseini Shirazi, S. F.; Aghvami, M.; Salimi, A.; Pourahmad, J. Analysis of cytotoxic effects of nickel on human blood lymphocytes. *Toxicology mechanisms and methods* **2018,** *28* (2), 79.
(779) Cameron, K. S.; Buchner, V.; Tchounwou, P. B. Exploring the molecular mechanisms of nickel-induced genotoxicity and carcinogenicity: a literature review. *Reviews on environmental health* **2011,** *26* (2), 81.
(780) Li, M. X.; Zhang, L. Z.; Zhang, D.; Ji, B. S.; Zhao, J. W. Synthesis, crystal structures, and biological evaluation of manganese(II) and nickel(II) complexes of 4-cyclohexyl-1-(1-(pyrazin-2-yl)ethylidene)thiosemicarbazide. *European journal of medicinal chemistry* **2011,** *46* (9), 4383.
(781) Babahan, I.; Eyduran, F.; Coban, E. P.; Orhan, N.; Kazar, D.; Biyik, H. Spectroscopic and biological approach of Ni(II), Cu(II) and Co(II) complexes of 4-methoxy/ethoxybenzaldehyde thiosemicarbazone glyoxime. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2014,** *121*, 205.
(782) Li, J.; Davidson, G.; Huang, Y.; Jiang, B.-H.; Shi, X.; Costa, M.; Huang, C. Nickel compounds act through phosphatidylinositol-3-kinase/Akt-dependent, p70(S6k)-independent pathway to induce hypoxia inducible factor transactivation and Cap43 expression in mouse epidermal Cl41 cells. *Cancer research* **2004,** *64* (1), 94.
(783) Shawish, H. B.; Wong, W. Y.; Wong, Y. L.; Loh, S. W.; Looi, C. Y.; Hassandarvish, P.; Phan, A. Y. L.; Wong, W. F.; Wang, H.; Paterson, I. C.et al. Nickel(II) complex of polyhydroxybenzaldehyde N4-thiosemicarbazone exhibits anti-inflammatory activity by inhibiting NF-κB transactivation. *PloS one* **2014,** *9* (6), e100933.
(784) Chen, Z.; Wang, X.; Zhu, Y.; Li, Y.; Guo, Z. Selective guanosine binding and cytotoxicity of a benzimidazole derived dinickel complex. *J Inorg Biochem* **2007,** *101* (11-12), 1894.
(785) Evangelou, A. M. Vanadium in cancer treatment. *Crit Rev Oncol Hematol* **2002,** *42* (3), 249.
(786) Crans, D. C.; Yang, L.; Haase, A.; Yang, X. Health Benefits of Vanadium and Its Potential as an Anticancer Agent. *Metal ions in life sciences* **2018,** *18*.
(787) Tsave, O.; Petanidis, S.; Kioseoglou, E.; Yavropoulou, M. P.; Yovos, J. G.; Anestakis, D.; Tsepa, A.; Salifoglou, A. Role of Vanadium in Cellular and Molecular Immunology: Association with Immune-Related Inflammation and Pharmacotoxicology Mechanisms. *Oxidative medicine and cellular longevity* **2016,** *2016*, 4013639.
(788) Tang, H.; Sun, Y.; Xiu, Q.; Lu, H.; Han, H. Cyclooxygenase-2 induction requires activation of nuclear factor of activated T-cells in Beas-2B cells after vanadium exposure and plays an anti-apoptotic role. *Archives of biochemistry and biophysics* **2007,** *468* (1), 92.
(789) Rehder, D. Perspectives for vanadium in health issues. *Future medicinal chemistry* **2016,** *8* (3), 325.
(790) Taroni, A. V for vanadium. *Nature chemistry* **2017,** *9* (6), 602.
(791) Selman, M.; Rousso, C.; Bergeron, A.; Son, H. H.; Krishnan, R.; El-Sayes, N. A.; Varette, O.; Chen, A.; Le Boeuf, F.; Tzelepis, F.et al. Multi-modal Potentiation of Oncolytic Virotherapy by Vanadium Compounds. *Molecular therapy : the journal of the American Society of Gene Therapy* **2018,** *26* (1), 56.
(792) Levina, A.; Lay, P. A. Stabilities and Biological Activities of Vanadium Drugs: What is the Nature of the Active Species? *Chemistry - An Asian Journal* **2017,** *12* (14), 1692.
(793) Korbecki, J.; Baranowska-Bosiacka, I.; Gutowska, I.; Chlubek, D. Biochemical and medical importance of vanadium compounds. *Acta biochimica Polonica* **2012,** *59* (2), 195.
(794) Mukherjee, B.; Patra, B.; Mahapatra, S.; Banerjee, P.; Tiwari, A.; Chatterjee, M. Vanadium--an element of atypical biological significance. *Toxicology letters* **2004,** *150* (2), 135.
(795) Jaspers, I.; Samet, J. M.; Erzurum, S.; Reed, W. Vanadium-induced kappaB-dependent transcription depends upon peroxide-induced activation of the p38 mitogen-activated protein kinase. *Am J Respir Cell Mol Biol* **2000,** *23* (1), 95.

(796) Bishayee, A.; Waghray, A.; Patel, M. A.; Chatterjee, M. Vanadium in the detection, prevention and treatment of cancer: the in vivo evidence. *Cancer Lett* **2010,** *294* (1), 1.
(797) Morinville, A.; Maysinger, D.; Shaver, A. From Vanadis to Atropos: vanadium compounds as pharmacological tools in cell death signalling. *Trends in pharmacological sciences* **1998,** *19* (11), 452.
(798) Crans, D. C.; Smee, J. J.; Gaidamauskas, E.; Yang, L. The chemistry and biochemistry of vanadium and the biological activities exerted by vanadium compounds. *Chemical reviews* **2004,** *104* (2), 849.
(799) Zwolak, I. Vanadium carcinogenic, immunotoxic and neurotoxic effects: a review of in vitro studies. *Toxicology mechanisms and methods* **2014,** *24* (1), 1.
(800) Gallardo-Vera, F.; Diaz, D.; Tapia-Rodriguez, M.; Fortoul van der Goes, T.; Masso, F.; Rendon-Huerta, E.; Montaño, L. F. Vanadium pentoxide prevents NK-92MI cell proliferation and IFNγ secretion through sustained JAK3 phosphorylation. *Journal of immunotoxicology* **2016,** *13* (1), 27.
(801) Cui, W.; Cui, H.; Peng, X.; Fang, J.; Zuo, Z.; Liu, X.; Wu, B. Excess dietary vanadium induces the changes of subsets and proliferation of splenic T cells in broilers. *Biol Trace Elem Res* **2011,** *143* (2), 932.
(802) Cui, W.; Cui, H.-M.; Peng, X.; Zuo, Z.; Liu, X.; Wu, B. Effect of vanadium on the subset and proliferation of peripheral blood T cells, and serum interleukin-2 content in broilers. *Biological trace element research* **2011,** *141* (1-3), 192.
(803) Ustarroz-Cano, M.; Garcia-Pelaez, I.; Cervantes-Yepez, S.; Lopez-Valdez, N.; Fortoul, T. I. Thymic cytoarchitecture changes in mice exposed to vanadium. *Journal of immunotoxicology* **2017,** *14* (1), 9.
(804) Ustarroz-Cano, M.; Garcia-Pelaez, I.; Pinon-Zarate, G.; Herrera-Enriquez, M.; Soldevila, G.; Fortoul, T. I. CD11c decrease in mouse thymic dendritic cells after vanadium inhalation. *J Immunotoxicol* **2012,** *9* (4), 374.
(805) Krueger, A. A missing link in thymic dendritic cell development. *European journal of immunology* **2011,** *41* (8), 2145.
(806) Lee, K.; Shen, X.; König, R. Effects of cadmium and vanadium ions on antigen-induced signaling in CD4(+) T cells. *Toxicology* **2001,** *169* (1), 53.
(807) Cohen, M. D.; Parsons, E.; Schlesinger, R. B.; Zelikoff, J. T. Immunotoxicity of in vitro vanadium exposures: effects on interleukin-1, tumor necrosis factor-alpha, and prostaglandin E2 production by WEHI-3 macrophages. *International journal of immunopharmacology* **1993,** *15* (3), 437.
(808) Gallardo-Vera, F.; Diaz, D.; Tapia-Rodriguez, M.; Fortoul van der Goes, T.; Masso, F.; Rendon-Huerta, E.; Montano, L. F. Vanadium pentoxide prevents NK-92MI cell proliferation and IFNgamma secretion through sustained JAK3 phosphorylation. *J Immunotoxicol* **2016,** *13* (1), 27.
(809) Olivares-Navarrete, R.; Hyzy, S. L.; Slosar, P. J.; Schneider, J. M.; Schwartz, Z.; Boyan, B. D. Implant materials generate different peri-implant inflammatory factors: poly-ether-ether-ketone promotes fibrosis and microtextured titanium promotes osteogenic factors. *Spine (Phila Pa 1976)* **2015,** *40* (6), 399.
(810) Basu, A.; Bhattacharjee, A.; Hajra, S.; Samanta, A.; Bhattacharya, S. Ameliorative effect of an oxovanadium (IV) complex against oxidative stress and nephrotoxicity induced by cisplatin. *Redox report : communications in free radical research* **2017,** *22* (6), 377.
(811) Basu, A.; Singha Roy, S.; Bhattacharjee, A.; Bhuniya, A.; Baral, R.; Biswas, J.; Bhattacharya, S. Vanadium(III)-L-cysteine protects cisplatin-induced nephropathy through activation of Nrf2/HO-1 pathway. *Free radical research* **2016,** *50* (1), 39.
(812) Srivastava, S.; Kumar, N.; Roy, P. Role of ERK/NFkappaB in vanadium (IV) oxide mediated osteoblast differentiation in C3H10t1/2 cells. *Biochimie* **2014,** *101*, 132.
(813) Castaldi, L.; Serra, C.; Moretti, F.; Messina, G.; Paoletti, R.; Sampaolesi, M.; Torgovnick, A.; Baiocchi, M.; Padula, F.; Pisaniello, A.et al. Bisperoxovanadium, a phospho-tyrosine phosphatase inhibitor,

reprograms myogenic cells to acquire a pluripotent, circulating phenotype. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2007,** *21* (13), 3573.
(814) Imbert, V.; Rupec, R. A.; Livolsi, A.; Pahl, H. L.; Traenckner, E. B.; Mueller-Dieckmann, C.; Farahifar, D.; Rossi, B.; Auberger, P.; Baeuerle, P. A.et al. Tyrosine phosphorylation of I kappa B-alpha activates NF-kappa B without proteolytic degradation of I kappa B-alpha. *Cell* **1996,** *86* (5), 787.
(815) Ha, D.; Joo, H.; Ahn, G.; Kim, M. J.; Bing, S. J.; An, S.; Kim, H.; Kang, K.-g.; Lim, Y.-K.; Jee, Y. Jeju ground water containing vanadium induced immune activation on splenocytes of low dose γ-rays-irradiated mice. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* **2012,** *50* (6), 2097.
(816) Chen, F.; Demers, L. M.; Vallyathan, V.; Ding, M.; Lu, Y.; Castranova, V.; Shi, X. Vanadate induction of NF-kappaB involves IkappaB kinase beta and SAPK/ERK kinase 1 in macrophages. *The Journal of biological chemistry* **1999,** *274* (29), 20307.
(817) Ashiq, U.; Jamal, R. A.; Mesaik, M. A.; Mahroof-Tahir, M.; Shahid, S.; Khan, K. M. Synthesis, immunomodulation and cytotoxic effects of vanadium (IV) complexes. *Medicinal chemistry* **2014,** *10* (3), 287.
(818) Öhrström, L. Rhodium roles. *Nature chemistry* **2016,** *8* (1), 90.
(819) Katsaros, N.; Anagnostopoulou, A. Rhodium and its compounds as potential agents in cancer treatment. *Critical reviews in oncology/hematology* **2002,** *42* (3), 297.
(820) Leung, C.-H.; Yang, H.; Ma, V. P.-Y.; Chan, D. S.-H.; Zhong, H.-J.; Li, Y.-W.; Fong, W.-F.; Ma, D.-L. Inhibition of Janus kinase 2 by cyclometalated rhodium complexes. *MedChemComm* **2012,** *3* (6), 696.
(821) Kang, T. S.; Wang, W.; Zhong, H. J.; Liang, J. X.; Ko, C. N.; Lu, J. J.; Chen, X. P.; Ma, D. L.; Leung, C. H. A rhodium(III)-based inhibitor of autotaxin with antiproliferative activity. *Biochim Biophys Acta* **2017,** *1861* (2), 256.
(822) Yang, C.; Wang, W.; Liang, J. X.; Li, G.; Vellaisamy, K.; Wong, C. Y.; Ma, D. L.; Leung, C. H. A Rhodium(III)-Based Inhibitor of Lysine-Specific Histone Demethylase 1 as an Epigenetic Modulator in Prostate Cancer Cells. *J Med Chem* **2017,** *60* (6), 2597.
(823) Valdes-Rives, S. A.; Gonzalez-Arenas, A. Autotaxin-Lysophosphatidic Acid: From Inflammation to Cancer Development. *Mediators of inflammation* **2017,** *2017*, 9173090.
(824) Desoize, B. Metals and metal compounds in cancer treatment. *Anticancer Res* **2004,** *24* (3a), 1529.
(825) Cascales, M.; Martin-Sanz, P.; Craciunescu, D. G.; Mayo, I.; Aguilar, A.; Robles-Chillida, E. M.; Cascales, C. Alterations in hepatic peroxidation mechanisms in thioacetamide-induced tumors in rats. Effect of a rhodium(III) complex. *Carcinogenesis* **1991,** *12* (2), 233.
(826) Geldmacher, Y.; Oleszak, M.; Sheldrick, W. S. Rhodium(III) and iridium(III) complexes as anticancer agents. *Inorganica Chimica Acta* **2012,** *393*, 84.
(827) Leung, C.-H.; Zhong, H.-J.; Chan, D. S.-H.; Ma, D.-L. Bioactive iridium and rhodium complexes as therapeutic agents. *Coordination Chemistry Reviews* **2013,** *257* (11-12), 1764.
(828) Zhang, P.; Sadler, P. J. Advances in the design of organometallic anticancer complexes. *Journal of Organometallic Chemistry* **2017,** *839*, 5.
(829) Sava, G.; Zorzet, S.; Mestroni, G.; Zassinovich, G. Antineoplastic activity of planar rhodium(I) complexes in mice bearing Lewis lung carcinoma and P388 leukemia. *Anticancer Res* **1985,** *5* (3), 249.
(830) Wang, X.; Li, Y.; Lin, M.; Jin, J.; Huang, Z. Rhodium (II) complex with 2-benzoylpyridine, a novel potential chemotherapeutic drug, induces cell cycle arrest and apoptosis in HepG2 cells. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine* **2017,** *30* (6), 903.
(831) Iavicoli, I.; Sgambato, A.; Fontana, L.; Marinaccio, A.; Leso, V.; Corbi, M.; Bergamaschi, A.; Calabrese, E. J. Effects of sub-acute exposure to rhodium (as Rh (III) chloride hydrate) on cytokines in female Wistar rats. *Bulletin of environmental contamination and toxicology* **2012,** *89* (3), 686.

(832) Boscolo, P.; Di Giampaolo, L.; Reale, M.; Castellani, M. L.; Ritavolpe, A.; Carmignani, M.; Ponti, J.; Paganelli, R.; Sabbioni, E.; Conti, P.et al. Different effects of platinum, palladium, and rhodium salts on lymphocyte proliferation and cytokine release. *Annals of clinical and laboratory science* **2004,** *34* (3), 299.

(833) Aresta, M.; De Fazio, M.; Fumarulo, R.; Giordano, D.; Pantaleo, R.; Riccardi, S. Biological activity of metal complexes. V: Influence of Pd(II), Pt(II), and Rh(I) on the macrophages chemotaxis. *Biochem Biophys Res Commun* **1982,** *104* (1), 121.

(834) Liu, L.-J.; Lin, S.; Chan, D. S.-H.; Vong, C. T.; Hoi, P. M.; Wong, C.-Y.; Ma, D.-L.; Leung, C.-H. A rhodium(III) complex inhibits LPS-induced nitric oxide production and angiogenic activity in cellulo. *Journal of Inorganic Biochemistry* **2014,** *140*, 23.

(835) Moragues, M. E.; Brines, R.; Terencio, M.; Sancenon, F.; Martinez-Manez, R.; Alcaraz, M. CO-releasing binuclear rhodium complexes as inhibitors of nitric oxide generation in stimulated macrophages. *Inorg Chem* **2013,** *52* (24), 13806.

(836) Rodriguez-Cabezas, M. N.; Mesa-Valle, C. M.; Azzouz, S.; Moraleda-Lindez, V.; Craciunescu, D.; Gutierrez-Rios, M. T.; De Frutos, M. I.; Osuna, A. In vitro and in vivo activity of new rhodium (III) complexes against Leishmania donovani. *Pharmacology* **2001,** *63* (2), 112.

(837) Merget, R.; Sander, I.; van Kampen, V.; Raulf-Heimsoth, M.; Ulmer, H. M.; Kulzer, R.; Bruening, T. Occupational immediate-type asthma and rhinitis due to rhodium salts. *American journal of industrial medicine* **2010,** *53* (1), 42.

(838) Goossens, A.; Cattaert, N.; Nemery, B.; Boey, L.; De Graef, E. Occupational allergic contact dermatitis caused by rhodium solutions. *Contact dermatitis* **2011,** *64* (3), 158.

(839) Paolucci, C.; Ponti, J.; Fabbri, M. V.; Breda, D.; Sabbioni, E.; Burastero, S. E. Platinum group elements enhance the allergic immune response by acting on dendritic cells. *International archives of allergy and immunology* **2007,** *143* (3), 225.

(840) Bordignon, V.; Palamara, F.; Cordiali-Fei, P.; Vento, A.; Aiello, A.; Picardo, M.; Ensoli, F.; Cristaudo, A. Nickel, palladium and rhodium induced IFN-gamma and IL-10 production as assessed by in vitro ELISpot-analysis in contact dermatitis patients. *BMC immunology* **2008,** *9*, 19.

(841) McCall, K. A.; Huang, C.; Fierke, C. A. Function and mechanism of zinc metalloenzymes. *The Journal of nutrition* **2000,** *130* (5S Suppl), 1437S.

(842) Prasad, A. S. Zinc: an antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS)* **2014,** *28* (4), 364.

(843) Prasad, A. S. Impact of the discovery of human zinc deficiency on health. *Journal of the American College of Nutrition* **2009,** *28* (3), 257.

(844) Prasad, A. S.; Beck, F. W. J.; Snell, D. C.; Kucuk, O. Zinc in cancer prevention. *Nutrition and cancer* **2009,** *61* (6), 879.

(845) Brieger, A.; Rink, L.; Haase, H. Differential regulation of TLR-dependent MyD88 and TRIF signaling pathways by free zinc ions. *J Immunol* **2013,** *191* (4), 1808.

(846) Dierichs, L.; Kloubert, V.; Rink, L. Cellular zinc homeostasis modulates polarization of THP-1-derived macrophages. *European journal of nutrition* **2017**.

(847) George, M. M.; Subramanian Vignesh, K.; Landero Figueroa, J. A.; Caruso, J. A.; Deepe, G. S., Jr. Zinc Induces Dendritic Cell Tolerogenic Phenotype and Skews Regulatory T Cell-Th17 Balance. *J Immunol* **2016,** *197* (5), 1864.

(848) Yamasaki, S.; Hasegawa, A.; Hojyo, S.; Ohashi, W.; Fukada, T.; Nishida, K.; Hirano, T. A novel role of the L-type calcium channel alpha1D subunit as a gatekeeper for intracellular zinc signaling: zinc wave. *PloS one* **2012,** *7* (6), e39654.

(849) Ugajin, T.; Nishida, K.; Yamasaki, S.; Suzuki, J.; Mita, M.; Kubo, M.; Yokozeki, H.; Hirano, T. Zinc-binding metallothioneins are key modulators of IL-4 production by basophils. *Molecular immunology* **2015,** *66* (2), 180.

(850) Ooi, T. C.; Chan, K. M.; Sharif, R. Zinc L-carnosine suppresses inflammatory responses in lipopolysaccharide-induced RAW 264.7 murine macrophages cell line via activation of Nrf2/HO-1 signaling pathway. *Immunopharmacology and immunotoxicology* **2017,** *39* (5), 259.
(851) Toren, A.; Pismenyuk, T.; Yalon, M.; Freedman, S.; Simon, A. J.; Fisher, T.; Moshe, I.; Reichardt, J. K. V.; Constantini, S.; Mardor, Y.et al. Zinc enhances temozolomide cytotoxicity in glioblastoma multiforme model systems. *Oncotarget* **2016,** *7* (46), 74860.
(852) Aude-Garcia, C.; Dalzon, B.; Ravanat, J.-L.; Collin-Faure, V.; Diemer, H.; Strub, J. M.; Cianferani, S.; Van Dorsselaer, A.; Carrière, M.; Rabilloud, T. A combined proteomic and targeted analysis unravels new toxic mechanisms for zinc oxide nanoparticles in macrophages. *Journal of proteomics* **2016,** *134*, 174.
(853) Li, X.-S.; Ke, M.-R.; Zhang, M.-F.; Tang, Q.-Q.; Zheng, B.-Y.; Huang, J.-D. A non-aggregated and tumour-associated macrophage-targeted photosensitiser for photodynamic therapy: a novel zinc(ii) phthalocyanine containing octa-sulphonates. *Chemical Communications* **2015,** *51* (22), 4704.
(854) Li, X.; Shenashen, M. A.; Wang, X.; Ito, A.; Taniguchi, A.; Ei-Safty, S. A. Hierarchically porous, and Cu- and Zn-containing γ-AlOOH mesostrands as adjuvants for cancer immunotherapy. *Scientific reports* **2017,** *7* (1), 16749.
(855) Yun, J.-W.; Yoon, J.-H.; Kang, B.-C.; Cho, N.-H.; Seok, S. H.; Min, S.-K.; Min, J. H.; Che, J.-H.; Kim, Y. K. The toxicity and distribution of iron oxide-zinc oxide core-shell nanoparticles in C57BL/6 mice after repeated subcutaneous administration. *Journal of applied toxicology : JAT* **2015,** *35* (6), 593.
(856) Martinez, V. R.; Aguirre, M. V.; Todaro, J. S.; Piro, O. E.; Echeverria, G. A.; Ferrer, E. G.; Williams, P. A. M. Azilsartan and its Zn(II) complex. Synthesis, anticancer mechanisms of action and binding to bovine serum albumin. *Toxicology in vitro : an international journal published in association with BIBRA* **2018,** *48*, 205.
(857) Banerjee, A.; Banerjee, K.; Sinha, A.; Das, S.; Majumder, S.; Majumdar, S.; Choudhuri, S. K. A zinc Schiff base complex inhibits cancer progression both in vivo and in vitro by inducing apoptosis. *Environmental toxicology and pharmacology* **2017,** *56*, 383.
(858) Chellan, P.; Sadler, P. J. The elements of life and medicines. *Philosophical transactions. Series A, Mathematical, physical, and engineering sciences* **2015,** *373* (2037).
(859) Stiban, J.; So, M.; Kaguni, L. S. Iron-Sulfur Clusters in Mitochondrial Metabolism: Multifaceted Roles of a Simple Cofactor. *Biochemistry. Biokhimiia* **2016,** *81* (10), 1066.
(860) Danielson, P. B. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. *Current drug metabolism* **2002,** *3* (6), 561.
(861) Chelikani, P.; Fita, I.; Loewen, P. C. Diversity of structures and properties among catalases. *Cell Mol Life Sci* **2004,** *61* (2), 192.
(862) Elledge, S. J.; Zhou, Z.; Allen, J. B. Ribonucleotide reductase: regulation, regulation, regulation. *Trends in biochemical sciences* **1992,** *17* (3), 119.
(863) Wang, L.; Cherayil, B. J. Ironing out the wrinkles in host defense: interactions between iron homeostasis and innate immunity. *Journal of innate immunity* **2009,** *1* (5), 455.
(864) Inoue, H.; Kobayashi, K.; Ndong, M.; Yamamoto, Y.; Katsumata, S.; Suzuki, K.; Uehara, M. Activation of Nrf2/Keap1 signaling and autophagy induction against oxidative stress in heart in iron deficiency. *Bioscience, biotechnology, and biochemistry* **2015,** *79* (8), 1366.
(865) Zohora, F.; Bidad, K.; Pourpak, Z.; Moin, M. Biological and Immunological Aspects of Iron Deficiency Anemia in Cancer Development: A Narrative Review. *Nutr Cancer* **2018,** *70* (4), 546.
(866) Kopf-Maier, P.; Kopf, H.; Neuse, E. W. Ferricenium complexes: a new type of water-soluble antitumor agent. *J Cancer Res Clin Oncol* **1984,** *108* (3), 336.
(867) Gasser, G.; Ott, I.; Metzler-Nolte, N. Organometallic anticancer compounds. *J Med Chem* **2011,** *54* (1), 3.
(868) Jaouen, G.; Vessieres, A.; Top, S. Ferrocifen type anti cancer drugs. *Chem Soc Rev* **2015,** *44* (24), 8802.

(869) Wang, Y.; Pigeon, P.; Top, S.; McGlinchey, M. J.; Jaouen, G. Organometallic Antitumor Compounds: Ferrocifens as Precursors to Quinone Methides. *Angewandte Chemie* **2015,** *54* (35), 10230.
(870) Kovjazin, R.; Eldar, T.; Patya, M.; Vanichkin, A.; Lander, H. M.; Novogrodsky, A. Ferrocene-induced lymphocyte activation and anti-tumor activity is mediated by redox-sensitive signaling. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2003,** *17* (3), 467.
(871) Gasiorowski, K.; Brokos, J. B.; Szyba, K.; Wozniak, D.; Fraser, D. M.; Zakeeruddin, S. M.; Graetzel, M. Evaluation of genotoxic and immunotoxic activities of potential glucose biosensor components: ferrocenes. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine* **1999,** *12* (1), 19.
(872) Zhang, B.; Qiu, J.; Wu, C.; Li, Y.; Liu, Z. Anti-tumor and immunomodulatory activity of iron hepta-tungsten phosphate oxygen clusters complex. *International immunopharmacology* **2015,** *29* (2), 293.
(873) Bruyere, C.; Mathieu, V.; Vessieres, A.; Pigeon, P.; Top, S.; Jaouen, G.; Kiss, R. Ferrocifen derivatives that induce senescence in cancer cells: selected examples. *J Inorg Biochem* **2014,** *141*, 144.
(874) Costa da Silva, M.; Breckwoldt, M. O.; Vinchi, F.; Correia, M. P.; Stojanovic, A.; Thielmann, C. M.; Meister, M.; Muley, T.; Warth, A.; Platten, M.et al. Iron Induces Anti-tumor Activity in Tumor-Associated Macrophages. *Frontiers in immunology* **2017,** *8*, 1479.
(875) Chitambar, C. R. Gallium and its competing roles with iron in biological systems. *Biochim Biophys Acta* **2016,** *1863* (8), 2044.
(876) Chitambar, C. R. 10 Gallium Complexes as Anticancer Drugs. *Met Ions Life Sci* **2018,** *18*.
(877) Hofheinz, R. D.; Dittrich, C.; Jakupec, M. A.; Drescher, A.; Jaehde, U.; Gneist, M.; Graf von Keyserlingk, N.; Keppler, B. K.; Hochhaus, A. Early results from a phase I study on orally administered tris(8-quinolinolato)gallium(III) (FFC11, KP46) in patients with solid tumors--a CESAR study (Central European Society for Anticancer Drug Research--EWIV). *International journal of clinical pharmacology and therapeutics* **2005,** *43* (12), 590.
(878) Webster, L. K.; Olver, I. N.; Stokes, K. H.; Sephton, R. G.; Hillcoat, B. L.; Bishop, J. F. A pharmacokinetic and phase II study of gallium nitrate in patients with non-small cell lung cancer. *Cancer Chemother Pharmacol* **2000,** *45* (1), 55.
(879) Qi, J.; Zheng, Y.; Qian, K.; Tian, L.; Zhang, G. X.; Cheng, Z.; Wang, Y. Synthesis, crystal structure and antiproliferative mechanisms of 2-acetylpyridine-thiosemicarbazones Ga(III) with a greater selectivity against tumor cells. *J Inorg Biochem* **2017,** *177*, 110.
(880) Heffeter, P.; Pirker, C.; Kowol, C. R.; Herrman, G.; Dornetshuber, R.; Miklos, W.; Jungwirth, U.; Koellensperger, G.; Keppler, B. K.; Berger, W. Impact of terminal dimethylation on the resistance profile of alpha-N-heterocyclic thiosemicarbazones. *Biochem Pharmacol* **2012,** *83* (12), 1623.
(881) Silva, F.; Marques, F.; Santos, I. C.; Paulo, A.; Rodrigues, A. S.; Rueff, J.; Santos, I. Synthesis, characterization and cytotoxic activity of gallium(III) complexes anchored by tridentate pyrazole-based ligands. *J Inorg Biochem* **2010,** *104* (5), 523.
(882) Betoulle, S.; Etienne, J. C.; Vernet, G. Acute immunotoxicity of gallium to carp (Cyprinus carpio L.). *Bulletin of environmental contamination and toxicology* **2002,** *68* (6), 817.
(883) Apseloff, G.; Hackshaw, K. V.; Whitacre, C.; Weisbrode, S. E.; Gerber, N. Gallium nitrate suppresses lupus in MRL/lpr mice. *Naunyn-Schmiedeberg's archives of pharmacology* **1997,** *356* (4), 517.
(884) Choi, J. H.; Lee, J. H.; Roh, K. H.; Seo, S. K.; Choi, I. W.; Park, S. G.; Lim, J. G.; Lee, W. J.; Kim, M. H.; Cho, K. R.et al. Gallium nitrate ameliorates type II collagen-induced arthritis in mice. *International immunopharmacology* **2014,** *20* (1), 269.
(885) Wu, X.; Wang, T. W.; Lessmann, G. M.; Saleh, J.; Liu, X.; Chitambar, C. R.; Hwang, S. T. Gallium maltolate inhibits human cutaneous T-cell lymphoma tumor development in mice. *The Journal of investigative dermatology* **2015,** *135* (3), 877.

(886) Jurowska, A.; Jurowski, K.; Szklarzewicz, J.; Buszewski, B.; Kalenik, T.; Piekoszewski, W. Molybdenum Metallopharmaceuticals Candidate Compounds - The "Renaissance" of Molybdenum Metallodrugs? *Current medicinal chemistry* **2016,** *23* (29), 3322.
(887) Pearson, M. J.; Williams, R. L.; Floyd, H.; Bodansky, D.; Grover, L. M.; Davis, E. T.; Lord, J. M. The effects of cobalt-chromium-molybdenum wear debris in vitro on serum cytokine profiles and T cell repertoire. *Biomaterials* **2015,** *67*, 232.
(888) Denoyer, D.; Clatworthy, S. A. S.; Cater, M. A. Copper Complexes in Cancer Therapy. *Metal ions in life sciences* **2018,** *18*.
(889) Jain, S.; Cohen, J.; Ward, M. M.; Kornhauser, N.; Chuang, E.; Cigler, T.; Moore, A.; Donovan, D.; Lam, C.; Cobham, M. V.et al. Tetrathiomolybdate-associated copper depletion decreases circulating endothelial progenitor cells in women with breast cancer at high risk of relapse. *Annals of oncology : official journal of the European Society for Medical Oncology* **2013,** *24* (6), 1491.
(890) Pass, H. I.; Brewer, G. J.; Dick, R.; Carbone, M.; Merajver, S. A phase II trial of tetrathiomolybdate after surgery for malignant mesothelioma: final results. *The Annals of thoracic surgery* **2008,** *86* (2), 383.
(891) Wei, H.; Frei, B.; Beckman, J. S.; Zhang, W. J. Copper chelation by tetrathiomolybdate inhibits lipopolysaccharide-induced inflammatory responses in vivo. *American journal of physiology. Heart and circulatory physiology* **2011,** *301* (3), H712.
(892) Deigendesch, N.; Zychlinsky, A.; Meissner, F. Copper Regulates the Canonical NLRP3 Inflammasome. *J Immunol* **2018,** *200* (5), 1607.
(893) Lee, K.; Briehl, M. M.; Mazar, A. P.; Batinic-Haberle, I.; Reboucas, J. S.; Glinsmann-Gibson, B.; Rimsza, L. M.; Tome, M. E. The copper chelator ATN-224 induces peroxynitrite-dependent cell death in hematological malignancies. *Free Radic Biol Med* **2013,** *60*, 157.
(894) Wei, H.; Zhang, W. J.; McMillen, T. S.; Leboeuf, R. C.; Frei, B. Copper chelation by tetrathiomolybdate inhibits vascular inflammation and atherosclerotic lesion development in apolipoprotein E-deficient mice. *Atherosclerosis* **2012,** *223* (2), 306.
(895) Joimel, U.; Gest, C.; Soria, J.; Pritchard, L. L.; Alexandre, J.; Laurent, M.; Blot, E.; Cazin, L.; Vannier, J. P.; Varin, R.et al. Stimulation of angiogenesis resulting from cooperation between macrophages and MDA-MB-231 breast cancer cells: proposed molecular mechanism and effect of tetrathiomolybdate. *BMC Cancer* **2010,** *10*, 375.
(896) Crowe, A.; Jackaman, C.; Beddoes, K. M.; Ricciardo, B.; Nelson, D. J. Rapid copper acquisition by developing murine mesothelioma: decreasing bioavailable copper slows tumor growth, normalizes vessels and promotes T cell infiltration. *PloS one* **2013,** *8* (8), e73684.
(897) Hanif, M.; Babak, M. V.; Hartinger, C. G. Development of anticancer agents: wizardry with osmium. *Drug discovery today* **2014,** *19* (10), 1640.
(898) Zhang, J.; Pitto-Barry, A.; Shang, L.; Barry, N. P. E. Anti-inflammatory activity of electron-deficient organometallics. *Royal Society open science* **2017,** *4* (11), 170786.
(899) Kostova, I.; Kiefer, W.; Momekov, G. Cytotoxic activity of Gd(III)- and Dy(III)-complexes. *Archiv der Pharmazie* **2007,** *340* (12), 642.
(900) Kostova, I.; Momekov, G.; Stancheva, P. New Samarium(III), Gadolinium(III), and Dysprosium(III) Complexes of Coumarin-3-Carboxylic Acid as Antiproliferative Agents. *Metal-based drugs* **2007,** *2007*, 15925.
(901) Suominen, M. I.; Rissanen, J. P.; Kakonen, R.; Fagerlund, K. M.; Alhoniemi, E.; Mumberg, D.; Ziegelbauer, K.; Halleen, J. M.; Kakonen, S. M.; Scholz, A. Survival benefit with radium-223 dichloride in a mouse model of breast cancer bone metastasis. *Journal of the National Cancer Institute* **2013,** *105* (12), 908.
(902) Chakraborty, M.; Wansley, E. K.; Carrasquillo, J. A.; Yu, S.; Paik, C. H.; Camphausen, K.; Becker, M. D.; Goeckeler, W. F.; Schlom, J.; Hodge, J. W. The use of chelated radionuclide (samarium-153-ethylenediaminetetramethylenephosphonate) to modulate phenotype of tumor cells and

enhance T cell-mediated killing. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2008,** *14* (13), 4241.

(903) Picciotto, M.; Franchina, T.; Russo, A.; Ricciardi, G. R. R.; Provazza, G.; Sava, S.; Baldari, S.; Caffo, O.; Adamo, V. Emerging role of Radium-223 in the growing therapeutic armamentarium of metastatic castration-resistant prostate cancer. *Expert opinion on pharmacotherapy* **2017,** *18* (9), 899.

(904) Parker, C. C.; Coleman, R. E.; Sartor, O.; Vogelzang, N. J.; Bottomley, D.; Heinrich, D.; Helle, S. I.; O'Sullivan, J. M.; Fosså, S. D.; Chodacki, A.et al. Three-year Safety of Radium-223 Dichloride in Patients with Castration-resistant Prostate Cancer and Symptomatic Bone Metastases from Phase 3 Randomized Alpharadin in Symptomatic Prostate Cancer Trial. *European urology* **2017,** *73* (3), 427.

(905) Vogelzang, N. J.; Coleman, R. E.; Michalski, J. M.; Nilsson, S.; O'Sullivan, J. M.; Parker, C.; Widmark, A.; Thuresson, M.; Xu, L.; Germino, J.et al. Hematologic Safety of Radium-223 Dichloride: Baseline Prognostic Factors Associated With Myelosuppression in the ALSYMPCA Trial. *Clinical genitourinary cancer* **2017,** *15* (1), 42.

(906) Sartor, O. Potential synergistic implications for stromal-targeted radiopharmaceuticals in bone-metastatic prostate cancer. *Asian journal of andrology* **2011,** *13* (3), 366.

(907) Heery, C. R.; Madan, R. A.; Stein, M. N.; Stadler, W. M.; Di Paola, R. S.; Rauckhorst, M.; Steinberg, S. M.; Marté, J. L.; Chen, C. C.; Grenga, I.et al. Samarium-153-EDTMP (Quadramet®) with or without vaccine in metastatic castration-resistant prostate cancer: A randomized Phase 2 trial. *Oncotarget* **2016,** *7* (42), 69014.

(908) Isaacsson Velho, P.; Antonarakis, E. S. PD-1/PD-L1 pathway inhibitors in advanced prostate cancer. *Expert Rev Clin Pharmacol* **2018,** *11* (5), 475.

(909) Tse, B. W.; Jovanovic, L.; Nelson, C. C.; de Souza, P.; Power, C. A.; Russell, P. J. From bench to bedside: immunotherapy for prostate cancer. *BioMed research international* **2014,** *2014*, 981434.

(910) Wani, W. A.; Prashar, S.; Shreaz, S.; Gómez-Ruiz, S. Nanostructured materials functionalized with metal complexes: In search of alternatives for administering anticancer metallodrugs. *Coordination Chemistry Reviews* **2016,** *312*, 67.

(911) Chen, H.; Zhang, W.; Zhu, G.; Xie, J.; Chen, X. Rethinking cancer nanotheranostics. *Nat Rev Mater* **2017,** *2*.

(912) Dobrovolskaia, M. A. Pre-clinical immunotoxicity studies of nanotechnology-formulated drugs: Challenges, considerations and strategy. *Journal of controlled release : official journal of the Controlled Release Society* **2015,** *220* (Pt B), 571.

(913) Hussain, S.; Vanoirbeek, J. A. J.; Hoet, P. H. M. Interactions of nanomaterials with the immune system. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* **2012,** *4* (2), 169.

(914) Dobrovolskaia, M. A.; Shurin, M.; Shvedova, A. A. Current understanding of interactions between nanoparticles and the immune system. *Toxicology and Applied Pharmacology* **2016,** *299*, 78.

(915) Shi, J.; Kantoff, P. W.; Wooster, R.; Farokhzad, O. C. Cancer nanomedicine: progress, challenges and opportunities. *Nature reviews. Cancer* **2017,** *17* (1), 20.

(916) Immordino, M. L.; Dosio, F.; Cattel, L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. *Int J Nanomedicine* **2006,** *1* (3), 297.

(917) Jiang, W.; von Roemeling, C. A.; Chen, Y.; Qie, Y.; Liu, X.; Chen, J.; Kim, B. Y. S. Designing nanomedicine for immuno-oncology. *Nature Biomedical Engineering* **2017,** *1*, 0029.

(918) Serda, R. E. Particle platforms for cancer immunotherapy. *Int J Nanomedicine* **2013,** *8*, 1683.

(919) Pallardy, M. J.; Turbica, I.; Biola-Vidamment, A. Why the Immune System Should Be Concerned by Nanomaterials? *Frontiers in immunology* **2017,** *8* (544).

(920) Petrarca, C.; Clemente, E.; Amato, V.; Pedata, P.; Sabbioni, E.; Bernardini, G.; Iavicoli, I.; Cortese, S.; Niu, Q.; Otsuki, T.et al. Engineered metal based nanoparticles and innate immunity. *Clinical and Molecular Allergy : CMA* **2015,** *13* (1), 13.

(921) Eriksson, M.; Hassan, S.; Larsson, R.; Linder, S.; Ramqvist, T.; Lovborg, H.; Vikinge, T.; Figgemeier, E.; Muller, J.; Stetefeld, J.et al. Utilization of a right-handed coiled-coil protein from archaebacterium Staphylothermus marinus as a carrier for cisplatin. *Anticancer Res* **2009,** *29* (1), 11.

(922) Li, J.; Pant, A.; Chin, C. F.; Ang, W. H.; Ménard-Moyon, C.; Nayak, T. R.; Gibson, D.; Ramaprabhu, S.; Panczyk, T.; Bianco, A.et al. In vivo biodistribution of platinum-based drugs encapsulated into multi-walled carbon nanotubes. *Nanomedicine: Nanotechnology, Biology and Medicine* **2014,** *10* (7), 1465.

(923) Zhao, X.; Yang, K.; Zhao, R.; Ji, T.; Wang, X.; Yang, X.; Zhang, Y.; Cheng, K.; Liu, S.; Hao, J.et al. Inducing enhanced immunogenic cell death with nanocarrier-based drug delivery systems for pancreatic cancer therapy. *Biomaterials* **2016,** *102*, 187.

(924) Fan, W.; Yung, B.; Huang, P.; Chen, X. Nanotechnology for Multimodal Synergistic Cancer Therapy. *Chemical reviews* **2017,** *117* (22), 13566.

(925) Abu Lila, A. S.; Kizuki, S.; Doi, Y.; Suzuki, T.; Ishida, T.; Kiwada, H. Oxaliplatin encapsulated in PEG-coated cationic liposomes induces significant tumor growth suppression via a dual-targeting approach in a murine solid tumor model. *Journal of Controlled Release* **2009,** *137* (1), 8.

(926) Doi, Y.; Okada, T.; Matsumoto, H.; Ichihara, M.; Ishida, T.; Kiwada, H. Combination therapy of metronomic S-1 dosing with oxaliplatin-containing polyethylene glycol-coated liposome improves antitumor activity in a murine colorectal tumor model. *Cancer Science* **2010,** *101* (11), 2470.

(927) Shen, S.; Li, H.-J.; Chen, K.-G.; Wang, Y.-C.; Yang, X.-Z.; Lian, Z.-X.; Du, J.-Z.; Wang, J. Spatial Targeting of Tumor-Associated Macrophages and Tumor Cells with a pH-Sensitive Cluster Nanocarrier for Cancer Chemoimmunotherapy. *Nano Letters* **2017,** *17* (6), 3822.

(928) Nakasone, Elizabeth S.; Askautrud, Hanne A.; Kees, T.; Park, J.-H.; Plaks, V.; Ewald, Andrew J.; Fein, M.; Rasch, Morten G.; Tan, Y.-X.; Qiu, J.et al. Imaging Tumor-Stroma Interactions during Chemotherapy Reveals Contributions of the Microenvironment to Resistance. *Cancer cell 21* (4), 488.

(929) Hume, D. A.; MacDonald, K. P. A. Therapeutic applications of macrophage colony-stimulating factor-1 (CSF-1) and antagonists of CSF-1 receptor (CSF-1R) signaling. *Blood* **2012,** *119* (8), 1810.

(930) Li, H.-J.; Du, J.-Z.; Liu, J.; Du, X.-J.; Shen, S.; Zhu, Y.-H.; Wang, X.; Ye, X.; Nie, S.; Wang, J. Smart Superstructures with Ultrahigh pH-Sensitivity for Targeting Acidic Tumor Microenvironment: Instantaneous Size Switching and Improved Tumor Penetration. *ACS Nano* **2016,** *10* (7), 6753.

(931) Shao, K.; Singha, S.; Clemente-Casares, X.; Tsai, S.; Yang, Y.; Santamaria, P. Nanoparticle-Based Immunotherapy for Cancer. *ACS Nano* **2015,** *9* (1), 16.

(932) Jain, S.; Hirst, D. G.; O'Sullivan, J. M. Gold nanoparticles as novel agents for cancer therapy. *The British Journal of Radiology* **2012,** *85* (1010), 101.

(933) Lim, Z.-Z. J.; Li, J.-E. J.; Ng, C.-T.; Yung, L.-Y. L.; Bay, B.-H. Gold nanoparticles in cancer therapy. *Acta Pharmacologica Sinica* **2011,** *32*, 983.

(934) Dykman, L. A.; Khlebtsov, N. G. Immunological properties of gold nanoparticles. *Chemical Science* **2017,** *8* (3), 1719.

(935) Cruz, L. J.; Rueda, F.; Cordobilla, B.; Simón, L.; Hosta, L.; Albericio, F.; Domingo, J. C. Targeting Nanosystems to Human DCs via Fc Receptor as an Effective Strategy to Deliver Antigen for Immunotherapy. *Molecular Pharmaceutics* **2011,** *8* (1), 104.

(936) Saha, S.; Xiong, X.; Chakraborty, P. K.; Shameer, K.; Arvizo, R. R.; Kudgus, R. A.; Dwivedi, S. K. D.; Hossen, M. N.; Gillies, E. M.; Robertson, J. D.et al. Gold Nanoparticle Reprograms Pancreatic Tumor Microenvironment and Inhibits Tumor Growth. *ACS Nano* **2016,** *10* (12), 10636.

(937) Webb, J. A.; Ou, Y.-C.; Faley, S.; Paul, E. P.; Hittinger, J. P.; Cutright, C. C.; Lin, E. C.; Bellan, L. M.; Bardhan, R. Theranostic Gold Nanoantennas for Simultaneous Multiplexed Raman Imaging of Immunomarkers and Photothermal Therapy. *ACS Omega* **2017,** *2* (7), 3583.

(938) Chen, N.; Wei, M.; Sun, Y.; Li, F.; Pei, H.; Li, X.; Su, S.; He, Y.; Wang, L.; Shi, J.et al. Self-Assembly of Poly-Adenine-Tailed CpG Oligonucleotide-Gold Nanoparticle Nanoconjugates with Immunostimulatory Activity. *Small* **2014,** *10* (2), 368.
(939) Lee, S. B.; Lee, Y. J.; Cho, S. J.; Kim, S. K.; Lee, S. W.; Lee, J.; Lim, D. K.; Jeon, Y. H. Antigen-Free Radionuclide-Embedded Gold Nanoparticles for Dendritic Cell Maturation, Tracking, and Strong Antitumor Immunity. *Adv Healthc Mater* **2018,** *7* (9), e1701369.
(940) Chattopadhyay, S.; Dash, S. K.; Ghosh, T.; Das, D.; Pramanik, P.; Roy, S. Surface modification of cobalt oxide nanoparticles using phosphonomethyl iminodiacetic acid followed by folic acid: a biocompatible vehicle for targeted anticancer drug delivery. *Cancer nanotechnology* **2013,** *4* (4-5), 103.
(941) Chattopadhyay, S.; Dash, S. K.; Ghosh, T.; Das, S.; Tripathy, S.; Mandal, D.; Das, D.; Pramanik, P.; Roy, S. Anticancer and immunostimulatory role of encapsulated tumor antigen containing cobalt oxide nanoparticles. *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry* **2013,** *18* (8), 957.
(942) Chattopadhyay, S.; Dash, S. K.; Kar Mahapatra, S.; Tripathy, S.; Ghosh, T.; Das, B.; Das, D.; Pramanik, P.; Roy, S. Chitosan-modified cobalt oxide nanoparticles stimulate TNF-alpha-mediated apoptosis in human leukemic cells. *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry* **2014,** *19* (3), 399.
(943) Chattopadhyay, S.; Dash, S. K.; Mandal, D.; Das, B.; Tripathy, S.; Dey, A.; Pramanik, P.; Roy, S. Metal based nanoparticles as cancer antigen delivery vehicles for macrophage based antitumor vaccine. *Vaccine* **2016,** *34* (7), 957.
(944) Laurent, S.; Saei, A. A.; Behzadi, S.; Panahifar, A.; Mahmoudi, M. Superparamagnetic iron oxide nanoparticles for delivery of therapeutic agents: opportunities and challenges. *Expert Opin Drug Deliv* **2014,** *11* (9), 1449.
(945) Arami, H.; Khandhar, A.; Liggitt, D.; Krishnan, K. M. In vivo delivery, pharmacokinetics, biodistribution and toxicity of iron oxide nanoparticles. *Chem Soc Rev* **2015,** *44* (23), 8576.
(946) Revia, R. A.; Zhang, M. Magnetite nanoparticles for cancer diagnosis, treatment, and treatment monitoring: recent advances. *Materials Today* **2016,** *19* (3), 157.
(947) Zanganeh, S.; Hutter, G.; Spitler, R.; Lenkov, O.; Mahmoudi, M.; Shaw, A.; Pajarinen, J. S.; Nejadnik, H.; Goodman, S.; Moseley, M.et al. Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. *Nature Nanotechnology* **2016,** *11*, 986.
(948) Perica, K.; De León Medero, A.; Durai, M.; Chiu, Y. L.; Bieler, J. G.; Sibener, L.; Niemöller, M.; Assenmacher, M.; Richter, A.; Edidin, M.et al. Nanoscale artificial antigen presenting cells for T cell immunotherapy. *Nanomedicine: Nanotechnology, Biology and Medicine* **2014,** *10* (1), 119.
(949) Zhu, M.; Tian, X.; Song, X.; Li, Y.; Tian, Y.; Zhao, Y.; Nie, G. Nanoparticle-Induced Exosomes Target Antigen-Presenting Cells to Initiate Th1-Type Immune Activation. *Small* **2012,** *8* (18), 2841.
(950) Balogh, L. P. Caging cancer. *Nanomedicine : nanotechnology, biology, and medicine* **2015,** *11* (4), 867.
(951) Liu, Y.; Chen, C.; Qian, P.; Lu, X.; Sun, B.; Zhang, X.; Wang, L.; Gao, X.; Li, H.; Chen, Z.et al. Gd-metallofullerenol nanomaterial as non-toxic breast cancer stem cell-specific inhibitor. *Nature communications* **2015,** *6* (1), 5988.
(952) Li, Y.; Tian, Y.; Nie, G. Antineoplastic activities of $Gd@C_{82}(OH)_{22}$ nanoparticles: tumor microenvironment regulation. *Science China. Life sciences* **2012,** *55* (10), 884.
(953) Song, Y.; Jin, J.; Li, J.; He, R.; Zhang, M.; Chang, Y.; Chen, K.; Wang, Y.; Sun, B.; Xing, G. Gd@C82(OH)22 nanoparticles constrain macrophages migration into tumor tissue to prevent metastasis. *Journal of nanoscience and nanotechnology* **2014,** *14* (6), 4022.
(954) Tang, J.; Chen, Z.; Sun, B.; Dong, J.; Liu, J.; Zhou, H.; Wang, L.; Bai, R.; Miao, Q.; Zhao, Y.et al. Polyhydroxylated fullerenols regulate macrophage for cancer adoptive immunotherapy and

greatly inhibit the tumor metastasis. *Nanomedicine: Nanotechnology, Biology and Medicine* **2016,** *12* (4), 945.

(955) Liu, Y.; Jiao, F.; Qiu, Y.; Li, W.; Lao, F.; Zhou, G.; Sun, B.; Xing, G.; Dong, J.; Zhao, Y.et al. The effect of Gd@C82(OH)22 nanoparticles on the release of Th1/Th2 cytokines and induction of TNF-alpha mediated cellular immunity. *Biomaterials* **2009,** *30* (23-24), 3934.

(956) Yang, D.; Zhao, Y.; Guo, H.; Li, Y.; Tewary, P.; Xing, G.; Hou, W.; Oppenheim, J. J.; Zhang, N. [Gd@C(82)(OH)(22)](n) nanoparticles induce dendritic cell maturation and activate Th1 immune responses. *ACS nano* **2010,** *4* (2), 1178.

(957) Allaire, J. M.; Crowley, S. M.; Law, H. T.; Chang, S. Y.; Ko, H. J.; Vallance, B. A. The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. *Trends in immunology* **2018**.

(958) Gopalakrishnan, V.; Helmink, B. A.; Spencer, C. N.; Reuben, A.; Wargo, J. A. The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. *Cancer cell* **2018,** *33* (4), 570.

(959) Haase, S.; Haghikia, A.; Wilck, N.; Muller, D. N.; Linker, R. A. Impacts of microbiome metabolites on immune regulation and autoimmunity. *Immunology* **2018**.

(960) Viaud, S.; Daillere, R.; Boneca, I. G.; Lepage, P.; Langella, P.; Chamaillard, M.; Pittet, M. J.; Ghiringhelli, F.; Trinchieri, G.; Goldszmid, R.et al. Gut microbiome and anticancer immune response: really hot Sh*t! *Cell Death Differ* **2015,** *22* (2), 199.

(961) Pellegrini, C.; Antonioli, L.; Colucci, R.; Blandizzi, C.; Fornai, M. Interplay among gut microbiota, intestinal mucosal barrier and enteric neuro-immune system: a common path to neurodegenerative diseases? *Acta Neuropathol* **2018**.

(962) Walsh, J.; Griffin, B. T.; Clarke, G.; Hyland, N. P. Drug-Gut Microbiota Interactions: Implications for Neuropharmacology. *Br J Pharmacol* **2018**.

(963) Bosch, T. C.; McFall-Ngai, M. J. Metaorganisms as the new frontier. *Zoology* **2011,** *114* (4), 185.

(964) Dzutsev, A.; Goldszmid, R. S.; Viaud, S.; Zitvogel, L.; Trinchieri, G. The role of the microbiota in inflammation, carcinogenesis, and cancer therapy. *European journal of immunology* **2015,** *45* (1), 17.

(965) Chalazonitis, A.; Rao, M. Enteric nervous system manifestations of neurodegenerative disease. *Brain research* **2018**.

(966) Holmes, E.; Li, J. V.; Marchesi, J. R.; Nicholson, J. K. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell metabolism* **2012,** *16* (5), 559.

(967) Nicholson, J. K.; Holmes, E.; Kinross, J.; Burcelin, R.; Gibson, G.; Jia, W.; Pettersson, S. Host-gut microbiota metabolic interactions. *Science* **2012,** *336* (6086), 1262.

(968) Nicholson, J. K.; Holmes, E.; Wilson, I. D. Gut microorganisms, mammalian metabolism and personalized health care. *Nature reviews. Microbiology* **2005,** *3* (5), 431.

(969) Li, H.; He, J.; Jia, W. The influence of gut microbiota on drug metabolism and toxicity. *Expert opinion on drug metabolism & toxicology* **2016,** *12* (1), 31.

(970) Gopalakrishnan, V.; Spencer, C. N.; Nezi, L.; Reuben, A.; Andrews, M. C.; Karpinets, T. V.; Prieto, P. A.; Vicente, D.; Hoffman, K.; Wei, S. C.et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **2018,** *359* (6371), 97.

(971) Bashiardes, S.; Tuganbaev, T.; Federici, S.; Elinav, E. The microbiome in anti-cancer therapy. *Seminars in immunology* **2017,** *32*, 74.

(972) Wallington, M.; Saxon, E. B.; Bomb, M.; Smittenaar, R.; Wickenden, M.; McPhail, S.; Rashbass, J.; Chao, D.; Dewar, J.; Talbot, D.et al. 30-day mortality after systemic anticancer treatment for breast and lung cancer in England: a population-based, observational study. *The Lancet. Oncology* **2016,** *17* (9), 1203.

(973) Alexander, J. L.; Wilson, I. D.; Teare, J.; Marchesi, J. R.; Nicholson, J. K.; Kinross, J. M. Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nature reviews. Gastroenterology & hepatology* **2017,** *14* (6), 356.

(974) Lehouritis, P.; Cummins, J.; Stanton, M.; Murphy, C. T.; McCarthy, F. O.; Reid, G.; Urbaniak, C.; Byrne, W. L.; Tangney, M. Local bacteria affect the efficacy of chemotherapeutic drugs. *Sci Rep* **2015,** *5*, 14554.
(975) Perez-Chanona, E.; Trinchieri, G. The role of microbiota in cancer therapy. *Curr Opin Immunol* **2016,** *39*, 75.
(976) Samet, A.; Sledzinska, A.; Krawczyk, B.; Hellmann, A.; Nowicki, S.; Kur, J.; Nowicki, B. Leukemia and risk of recurrent Escherichia coli bacteremia: genotyping implicates E. coli translocation from the colon to the bloodstream. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* **2013,** *32* (11), 1393.
(977) Viaud, S.; Saccheri, F.; Mignot, G.; Yamazaki, T.; Daillere, R.; Hannani, D.; Enot, D. P.; Pfirschke, C.; Engblom, C.; Pittet, M. J.et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* **2013,** *342* (6161), 971.
(978) Daillere, R.; Vetizou, M.; Waldschmitt, N.; Yamazaki, T.; Isnard, C.; Poirier-Colame, V.; Duong, C. P. M.; Flament, C.; Lepage, P.; Roberti, M. P.et al. Enterococcus hirae and Barnesiella intestinihominis Facilitate Cyclophosphamide-Induced Therapeutic Immunomodulatory Effects. *Immunity* **2016,** *45* (4), 931.
(979) Iida, N.; Dzutsev, A.; Stewart, C. A.; Smith, L.; Bouladoux, N.; Weingarten, R. A.; Molina, D. A.; Salcedo, R.; Back, T.; Cramer, S.et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* **2013,** *342* (6161), 967.
(980) Gui, Q. F.; Lu, H. F.; Zhang, C. X.; Xu, Z. R.; Yang, Y. H. Well-balanced commensal microbiota contributes to anti-cancer response in a lung cancer mouse model. *Genetics and molecular research : GMR* **2015,** *14* (2), 5642.
(981) Shen, S.; Lim, G.; You, Z.; Ding, W.; Huang, P.; Ran, C.; Doheny, J.; Caravan, P.; Tate, S.; Hu, K.et al. Gut microbiota is critical for the induction of chemotherapy-induced pain. *Nature neuroscience* **2017,** *20* (9), 1213.
(982) Signat, B.; Roques, C.; Poulet, P.; Duffaut, D. Fusobacterium nucleatum in periodontal health and disease. *Current issues in molecular biology* **2011,** *13* (2), 25.
(983) Castellarin, M.; Warren, R. L.; Freeman, J. D.; Dreolini, L.; Krzywinski, M.; Strauss, J.; Barnes, R.; Watson, P.; Allen-Vercoe, E.; Moore, R. A.et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome research* **2012,** *22* (2), 299.
(984) Mehta, R. S.; Nishihara, R.; Cao, Y.; Song, M.; Mima, K.; Qian, Z. R.; Nowak, J. A.; Kosumi, K.; Hamada, T.; Masugi, Y.et al. Association of Dietary Patterns With Risk of Colorectal Cancer Subtypes Classified by Fusobacterium nucleatum in Tumor Tissue. *JAMA oncology* **2017,** *3* (7), 921.
(985) Mima, K.; Nishihara, R.; Qian, Z. R.; Cao, Y.; Sukawa, Y.; Nowak, J. A.; Yang, J.; Dou, R.; Masugi, Y.; Song, M.et al. Fusobacterium nucleatum in colorectal carcinoma tissue and patient prognosis. *Gut* **2016,** *65* (12), 1973.
(986) Yu, T.; Guo, F.; Yu, Y.; Sun, T.; Ma, D.; Han, J.; Qian, Y.; Kryczek, I.; Sun, D.; Nagarsheth, N.et al. Fusobacterium nucleatum Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy. *Cell* **2017,** *170* (3), 548.
(987) Taur, Y.; Pamer, E. G. Microbiome mediation of infections in the cancer setting. *Genome medicine* **2016,** *8* (1), 40.
(988) Chitapanarux, I.; Chitapanarux, T.; Traisathit, P.; Kudumpee, S.; Tharavichitkul, E.; Lorvidhaya, V. Randomized controlled trial of live lactobacillus acidophilus plus bifidobacterium bifidum in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. *Radiation oncology* **2010,** *5*, 31.
(989) Perales-Puchalt, A.; Perez-Sanz, J.; Payne, K. K.; Svoronos, N.; Allegrezza, M. J.; Chaurio, R. A.; Anadon, C.; Calmette, J.; Biswas, S.; Mine, J. A.et al. Frontline Science: Microbiota reconstitution restores intestinal integrity after cisplatin therapy. *Journal of leukocyte biology* **2018,** *103* (5), 799.
(990) Sonis, S. T. A biological approach to mucositis. *The journal of supportive oncology* **2004,** *2* (1), 21.

(991) van Vliet, M. J.; Harmsen, H. J.; de Bont, E. S.; Tissing, W. J. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. *PLoS pathogens* **2010,** *6* (5), e1000879.
(992) Das, S. K.; Eder, S.; Schauer, S.; Diwoky, C.; Temmel, H.; Guertl, B.; Gorkiewicz, G.; Tamilarasan, K. P.; Kumari, P.; Trauner, M.et al. Adipose triglyceride lipase contributes to cancer-associated cachexia. *Science* **2011,** *333* (6039), 233.
(993) Ruud, J.; Wilhelms, D. B.; Nilsson, A.; Eskilsson, A.; Tang, Y. J.; Strohle, P.; Caesar, R.; Schwaninger, M.; Wunderlich, T.; Backhed, F.et al. Inflammation- and tumor-induced anorexia and weight loss require MyD88 in hematopoietic/myeloid cells but not in brain endothelial or neural cells. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2013,** *27* (5), 1973.
(994) Suarez-Zamorano, N.; Fabbiano, S.; Chevalier, C.; Stojanovic, O.; Colin, D. J.; Stevanovic, A.; Veyrat-Durebex, C.; Tarallo, V.; Rigo, D.; Germain, S.et al. Microbiota depletion promotes browning of white adipose tissue and reduces obesity. *Nature medicine* **2015,** *21* (12), 1497.
(995) de Matos-Neto, E. M.; Lima, J. D.; de Pereira, W. O.; Figueredo, R. G.; Riccardi, D. M.; Radloff, K.; das Neves, R. X.; Camargo, R. G.; Maximiano, L. F.; Tokeshi, F.et al. Systemic Inflammation in Cachexia - Is Tumor Cytokine Expression Profile the Culprit? *Frontiers in immunology* **2015,** *6*, 629.
(996) Conte, E.; Camerino, G. M.; Mele, A.; De Bellis, M.; Pierno, S.; Rana, F.; Fonzino, A.; Caloiero, R.; Rizzi, L.; Bresciani, E.et al. Growth hormone secretagogues prevent dysregulation of skeletal muscle calcium homeostasis in a rat model of cisplatin-induced cachexia. *Journal of cachexia, sarcopenia and muscle* **2017,** *8* (3), 386.
(997) Garcia, J. M.; Cata, J. P.; Dougherty, P. M.; Smith, R. G. Ghrelin prevents cisplatin-induced mechanical hyperalgesia and cachexia. *Endocrinology* **2008,** *149* (2), 455.
(998) Bindels, L. B.; Delzenne, N. M. Muscle wasting: the gut microbiota as a new therapeutic target? *Int J Biochem Cell Biol* **2013,** *45* (10), 2186.
(999) Klein, G. L.; Petschow, B. W.; Shaw, A. L.; Weaver, E. Gut barrier dysfunction and microbial translocation in cancer cachexia: a new therapeutic target. *Current opinion in supportive and palliative care* **2013,** *7* (4), 361.
(1000) Yeh, K. Y.; Wang, H. M.; Chang, J. W.; Huang, J. S.; Lai, C. H.; Lan, Y. J.; Wu, T. H.; Chang, P. H.; Wang, H.; Wu, C. J.et al. Omega-3 fatty acid-, micronutrient-, and probiotic-enriched nutrition helps body weight stabilization in head and neck cancer cachexia. *Oral surgery, oral medicine, oral pathology and oral radiology* **2013,** *116* (1), 41.
(1001) Varian, B. J.; Goureshetti, S.; Poutahidis, T.; Lakritz, J. R.; Levkovich, T.; Kwok, C.; Teliousis, K.; Ibrahim, Y. M.; Mirabal, S.; Erdman, S. E. Beneficial bacteria inhibit cachexia. *Oncotarget* **2016,** *7* (11), 11803.
(1002) Hartmann, J. T.; Lipp, H. P. Toxicity of platinum compounds. *Expert Opin Pharmacother* **2003,** *4* (6), 889.
(1003) Lanvers-Kaminsky, C.; Zehnhoff-Dinnesen, A. A.; Parfitt, R.; Ciarimboli, G. Drug-induced ototoxicity: Mechanisms, Pharmacogenetics, and protective strategies. *Clin Pharmacol Ther* **2017,** *101* (4), 491.
(1004) Cassidy, J.; Misset, J. L. Oxaliplatin-related side effects: characteristics and management. *Seminars in oncology* **2002,** *29* (5 Suppl 15), 11.
(1005) Ruggiero, A.; Trombatore, G.; Triarico, S.; Arena, R.; Ferrara, P.; Scalzone, M.; Pierri, F.; Riccardi, R. Platinum compounds in children with cancer: toxicity and clinical management. *Anticancer Drugs* **2013,** *24* (10), 1007.
(1006) Yan, M.; Jurasz, P. The role of platelets in the tumor microenvironment: From solid tumors to leukemia. *Biochim Biophys Acta* **2016,** *1863* (3), 392.
(1007) Rachidi, S.; Metelli, A.; Riesenberg, B.; Wu, B. X.; Nelson, M. H.; Wallace, C.; Paulos, C. M.; Rubinstein, M. P.; Garrett-Mayer, E.; Hennig, M.et al. Platelets subvert T cell immunity against cancer via GARP-TGFbeta axis. *Sci Immunol* **2017,** *2* (11).

(1008) Ward, Y.; Lake, R.; Faraji, F.; Sperger, J.; Martin, P.; Gilliard, C.; Ku, K. P.; Rodems, T.; Niles, D.; Tillman, H.et al. Platelets Promote Metastasis via Binding Tumor CD97 Leading to Bidirectional Signaling that Coordinates Transendothelial Migration. *Cell Rep* **2018,** *23* (3), 808.
(1009) Miyamoto, S.; Okada, R.; Ando, K. Platinum hypersensitivity and desensitization. *Jpn J Clin Oncol* **2015,** *45* (9), 795.
(1010) Aroldi, F.; Prochilo, T.; Bertocchi, P.; Zaniboni, A. Oxaliplatin-induced hypersensitivity reaction: underlying mechanisms and management. *J Chemother* **2015,** *27* (2), 63.
(1011) Caiado, J.; Castells, M. Presentation and Diagnosis of Hypersensitivity to Platinum Drugs. *Curr Allergy Asthma Rep* **2015,** *15* (4), 15.
(1012) Otani, I. M.; Wong, J.; Banerji, A. Platinum Chemotherapy Hypersensitivity: Prevalence and Management. *Immunol Allergy Clin North Am* **2017,** *37* (4), 663.
(1013) El-Shitany, N. A.; Eid, B. Proanthocyanidin protects against cisplatin-induced oxidative liver damage through inhibition of inflammation and NF-kappabeta/TLR-4 pathway. *Environ Toxicol* **2017,** *32* (7), 1952.
(1014) Soliman, A. F.; Anees, L. M.; Ibrahim, D. M. Cardioprotective effect of zingerone against oxidative stress, inflammation, and apoptosis induced by cisplatin or gamma radiation in rats. *Naunyn-Schmiedeberg's archives of pharmacology* **2018**.
(1015) Dugbartey, G. J.; Peppone, L. J.; de Graaf, I. A. An integrative view of cisplatin-induced renal and cardiac toxicities: Molecular mechanisms, current treatment challenges and potential protective measures. *Toxicology* **2016,** *371*, 58.
(1016) Manohar, S.; Leung, N. Cisplatin nephrotoxicity: a review of the literature. *Journal of nephrology* **2018,** *31* (1), 15.
(1017) Ishida, S.; Lee, J.; Thiele, D. J.; Herskowitz, I. Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. *Proc Natl Acad Sci U S A* **2002,** *99* (22), 14298.
(1018) Pabla, N.; Murphy, R. F.; Liu, K.; Dong, Z. The copper transporter Ctr1 contributes to cisplatin uptake by renal tubular cells during cisplatin nephrotoxicity. *American journal of physiology. Renal physiology* **2009,** *296* (3), F505.
(1019) Gluba, A.; Banach, M.; Hannam, S.; Mikhailidis, D. P.; Sakowicz, A.; Rysz, J. The role of Toll-like receptors in renal diseases. *Nature reviews. Nephrology* **2010,** *6* (4), 224.
(1020) Kono, H.; Rock, K. L. How dying cells alert the immune system to danger. *Nat Rev Immunol* **2008,** *8* (4), 279.
(1021) Zhang, B.; Ramesh, G.; Uematsu, S.; Akira, S.; Reeves, W. B. TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. *Journal of the American Society of Nephrology : JASN* **2008,** *19* (5), 923.
(1022) Tadagavadi, R. K.; Reeves, W. B. Endogenous IL-10 attenuates cisplatin nephrotoxicity: role of dendritic cells. *J Immunol* **2010,** *185* (8), 4904.
(1023) Deng, J.; Kohda, Y.; Chiao, H.; Wang, Y.; Hu, X.; Hewitt, S. M.; Miyaji, T.; McLeroy, P.; Nibhanupudy, B.; Li, S.et al. Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. *Kidney international* **2001,** *60* (6), 2118.
(1024) Inoue, T. M1 macrophage triggered by Mincle leads to a deterioration of acute kidney injury. *Kidney international* **2017,** *91* (3), 526.
(1025) Lv, L. L.; Tang, P. M.; Li, C. J.; You, Y. K.; Li, J.; Huang, X. R.; Ni, J.; Feng, M.; Liu, B. C.; Lan, H. Y. The pattern recognition receptor, Mincle, is essential for maintaining the M1 macrophage phenotype in acute renal inflammation. *Kidney international* **2017,** *91* (3), 587.
(1026) Alikhan, M. A.; Summers, S. A.; Gan, P. Y.; Chan, A. J.; Khouri, M. B.; Ooi, J. D.; Ghali, J. R.; Odobasic, D.; Hickey, M. J.; Kitching, A. R.et al. Endogenous Toll-Like Receptor 9 Regulates AKI by Promoting Regulatory T Cell Recruitment. *Journal of the American Society of Nephrology : JASN* **2016,** *27* (3), 706.

(1027) Kinsey, G. R.; Sharma, R.; Huang, L.; Li, L.; Vergis, A. L.; Ye, H.; Ju, S. T.; Okusa, M. D. Regulatory T cells suppress innate immunity in kidney ischemia-reperfusion injury. *Journal of the American Society of Nephrology : JASN* **2009,** *20* (8), 1744.
(1028) Kinsey, G. R.; Sharma, R.; Okusa, M. D. Regulatory T cells in AKI. *Journal of the American Society of Nephrology : JASN* **2013,** *24* (11), 1720.
(1029) Martina, M. N.; Noel, S.; Bandapalle, S.; Hamad, A. R.; Rabb, H. T lymphocytes and acute kidney injury: update. *Nephron. Clinical practice* **2014,** *127* (1-4), 51.
(1030) Tadagavadi, R. K.; Reeves, W. B. Renal dendritic cells ameliorate nephrotoxic acute kidney injury. *Journal of the American Society of Nephrology : JASN* **2010,** *21* (1), 53.
(1031) Liu, M.; Chien, C. C.; Burne-Taney, M.; Molls, R. R.; Racusen, L. C.; Colvin, R. B.; Rabb, H. A pathophysiologic role for T lymphocytes in murine acute cisplatin nephrotoxicity. *Journal of the American Society of Nephrology : JASN* **2006,** *17* (3), 765.
(1032) Yamate, J.; Machida, Y.; Ide, M.; Kuwamura, M.; Kotani, T.; Sawamoto, O.; LaMarre, J. Cisplatin-induced renal interstitial fibrosis in neonatal rats, developing as solitary nephron unit lesions. *Toxicologic pathology* **2005,** *33* (2), 207.
(1033) Yamate, J.; Machida, Y.; Ide, M.; Kuwamura, M.; Sawamoto, O.; LaMarre, J. Effects of lipopolysaccharide on the appearance of macrophage populations and fibrogenesis in cisplatin-induced rat renal injury. *Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie* **2004,** *56* (1-2), 13.
(1034) Ramesh, G.; Reeves, W. B. Salicylate reduces cisplatin nephrotoxicity by inhibition of tumor necrosis factor-alpha. *Kidney international* **2004,** *65* (2), 490.
(1035) Kang, K. P.; Kim, D. H.; Jung, Y. J.; Lee, A. S.; Lee, S.; Lee, S. Y.; Jang, K. Y.; Sung, M. J.; Park, S. K.; Kim, W. Alpha-lipoic acid attenuates cisplatin-induced acute kidney injury in mice by suppressing renal inflammation. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **2009,** *24* (10), 3012.
(1036) Ramesh, G.; Reeves, W. B. p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice. *American journal of physiology. Renal physiology* **2005,** *289* (1), F166.
(1037) Ramesh, G.; Reeves, W. B. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *The Journal of clinical investigation* **2002,** *110* (6), 835.
(1038) Li, S.; Wu, P.; Yarlagadda, P.; Vadjunec, N. M.; Proia, A. D.; Harris, R. A.; Portilla, D. PPAR alpha ligand protects during cisplatin-induced acute renal failure by preventing inhibition of renal FAO and PDC activity. *American journal of physiology. Renal physiology* **2004,** *286* (3), F572.
(1039) Li, S.; Gokden, N.; Okusa, M. D.; Bhatt, R.; Portilla, D. Anti-inflammatory effect of fibrate protects from cisplatin-induced ARF. *American journal of physiology. Renal physiology* **2005,** *289* (2), F469.
(1040) Rani, N.; Bharti, S.; Tomar, A.; Dinda, A. K.; Arya, D. S.; Bhatia, J. Inhibition of PARP activation by enalapril is crucial for its renoprotective effect in cisplatin-induced nephrotoxicity in rats. *Free Radic Res* **2016,** *50* (11), 1226.
(1041) Kabel, A. M.; Elkhoely, A. A. Ameliorative Effect of Coenzyme Q10 and/or Candesartan on Carboplatin-Induced Nephrotoxicity: Roles of Apoptosis, Transforming Growth Factor-Beta1, Nuclear Factor Kappa-B And The Nrf2/HO-1 Pathway. *Asian Pac J Cancer Prev* **2017,** *18* (6), 1629.
(1042) Stremska, M. E.; Jose, S.; Sabapathy, V.; Huang, L.; Bajwa, A.; Kinsey, G. R.; Sharma, P. R.; Mohammad, S.; Rosin, D. L.; Okusa, M. D.et al. IL233, A Novel IL-2 and IL-33 Hybrid Cytokine, Ameliorates Renal Injury. *Journal of the American Society of Nephrology : JASN* **2017,** *28* (9), 2681.
(1043) Ju, S. T.; Sharma, R.; Gaskin, F.; Fu, S. M. IL-2 controls trafficking receptor gene expression and Th2 response for skin and lung inflammation. *Clinical immunology* **2012,** *145* (1), 82.
(1044) Sharma, R.; Sharma, P. R.; Kim, Y. C.; Leitinger, N.; Lee, J. K.; Fu, S. M.; Ju, S. T. IL-2-controlled expression of multiple T cell trafficking genes and Th2 cytokines in the regulatory T cell-deficient scurfy mice: implication to multiorgan inflammation and control of skin and lung inflammation. *J Immunol* **2011,** *186* (2), 1268.

(1045) Simovic Markovic, B.; Gazdic, M.; Arsenijevic, A.; Jovicic, N.; Jeremic, J.; Djonov, V.; Arsenijevic, N.; Lukic, M. L.; Volarevic, V. Mesenchymal Stem Cells Attenuate Cisplatin-Induced Nephrotoxicity in iNOS-Dependent Manner. *Stem cells international* **2017,** *2017*, 1315378.
(1046) Holman, L. L.; Ren, Y.; Westin, S. N. Status epilepticus associated with platinum chemotherapy in a patient with cervical cancer: a case report. *BMC Cancer* **2015,** *15*, 728.
(1047) Park, S. B.; Goldstein, D.; Krishnan, A. V.; Lin, C. S.; Friedlander, M. L.; Cassidy, J.; Koltzenburg, M.; Kiernan, M. C. Chemotherapy-induced peripheral neurotoxicity: a critical analysis. *CA Cancer J Clin* **2013,** *63* (6), 419.
(1048) Starobova, H.; Vetter, I. Pathophysiology of Chemotherapy-Induced Peripheral Neuropathy. *Front Mol Neurosci* **2017,** *10*, 174.
(1049) Avan, A.; Postma, T. J.; Ceresa, C.; Avan, A.; Cavaletti, G.; Giovannetti, E.; Peters, G. J. Platinum-induced neurotoxicity and preventive strategies: past, present, and future. *Oncologist* **2015,** *20* (4), 411.
(1050) Seretny, M.; Currie, G. L.; Sena, E. S.; Ramnarine, S.; Grant, R.; MacLeod, M. R.; Colvin, L. A.; Fallon, M. Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis. *Pain* **2014,** *155* (12), 2461.
(1051) Chiorazzi, A.; Semperboni, S.; Marmiroli, P. Current View in Platinum Drug Mechanisms of Peripheral Neurotoxicity. *Toxics* **2015,** *3* (3), 304.
(1052) Kanat, O.; Ertas, H.; Caner, B. Platinum-induced neurotoxicity: A review of possible mechanisms. *World journal of clinical oncology* **2017,** *8* (4), 329.
(1053) Argyriou, A. A. Updates on Oxaliplatin-Induced Peripheral Neurotoxicity (OXAIPN). *Toxics* **2015,** *3* (2), 187.
(1054) Lees, J. G.; Makker, P. G.; Tonkin, R. S.; Abdulla, M.; Park, S. B.; Goldstein, D.; Moalem-Taylor, G. Immune-mediated processes implicated in chemotherapy-induced peripheral neuropathy. *European journal of cancer* **2017,** *73*, 22.
(1055) Vichaya, E. G.; Chiu, G. S.; Krukowski, K.; Lacourt, T. E.; Kavelaars, A.; Dantzer, R.; Heijnen, C. J.; Walker, A. K. Mechanisms of chemotherapy-induced behavioral toxicities. *Front Neurosci* **2015,** *9*, 131.
(1056) Deuis, J. R.; Zimmermann, K.; Romanovsky, A. A.; Possani, L. D.; Cabot, P. J.; Lewis, R. J.; Vetter, I. An animal model of oxaliplatin-induced cold allodynia reveals a crucial role for Nav1.6 in peripheral pain pathways. *Pain* **2013,** *154* (9), 1749.
(1057) Fukuda, Y.; Li, Y.; Segal, R. A. A Mechanistic Understanding of Axon Degeneration in Chemotherapy-Induced Peripheral Neuropathy. *Front Neurosci* **2017,** *11*, 481.
(1058) Khrunin, A. V.; Moisseev, A.; Gorbunova, V.; Limborska, S. Genetic polymorphisms and the efficacy and toxicity of cisplatin-based chemotherapy in ovarian cancer patients. *Pharmacogenomics J* **2010,** *10* (1), 54.
(1059) Lecomte, T.; Landi, B.; Beaune, P.; Laurent-Puig, P.; Loriot, M. A. Glutathione S-transferase P1 polymorphism (Ile105Val) predicts cumulative neuropathy in patients receiving oxaliplatin-based chemotherapy. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2006,** *12* (10), 3050.
(1060) Alles, S. R. A.; Smith, P. A. Etiology and Pharmacology of Neuropathic Pain. *Pharmacological reviews* **2018,** *70* (2), 315.
(1061) Clark, A. K.; Old, E. A.; Malcangio, M. Neuropathic pain and cytokines: current perspectives. *J Pain Res* **2013,** *6*, 803.
(1062) Starkweather, A. Increased interleukin-6 activity associated with painful chemotherapy-induced peripheral neuropathy in women after breast cancer treatment. *Nurs Res Pract* **2010,** *2010*, 281531.
(1063) Ma, J.; Kavelaars, A.; Dougherty, P. M.; Heijnen, C. J. Beyond symptomatic relief for chemotherapy-induced peripheral neuropathy: Targeting the source. *Cancer* **2018**.

(1064) Park, H. J.; Stokes, J. A.; Corr, M.; Yaksh, T. L. Toll-like receptor signaling regulates cisplatin-induced mechanical allodynia in mice. *Cancer Chemother Pharmacol* **2014,** *73* (1), 25.
(1065) Das, N.; Dewan, V.; Grace, P. M.; Gunn, R. J.; Tamura, R.; Tzarum, N.; Watkins, L. R.; Wilson, I. A.; Yin, H. HMGB1 Activates Proinflammatory Signaling via TLR5 Leading to Allodynia. *Cell Rep* **2016,** *17* (4), 1128.
(1066) Warwick, R. A.; Hanani, M. The contribution of satellite glial cells to chemotherapy-induced neuropathic pain. *Eur J Pain* **2013,** *17* (4), 571.
(1067) Krarup-Hansen, A.; Rietz, B.; Krarup, C.; Heydorn, K.; Rorth, M.; Schmalbruch, H. Histology and platinum content of sensory ganglia and sural nerves in patients treated with cisplatin and carboplatin: an autopsy study. *Neuropathol Appl Neurobiol* **1999,** *25* (1), 29.
(1068) Li, D.; Kim, W.; Shin, D.; Jung, Y.; Bae, H.; Kim, S. K. Preventive Effects of Bee Venom Derived Phospholipase A(2) on Oxaliplatin-Induced Neuropathic Pain in Mice. *Toxins* **2016,** *8* (1).
(1069) Makker, P. G.; Duffy, S. S.; Lees, J. G.; Perera, C. J.; Tonkin, R. S.; Butovsky, O.; Park, S. B.; Goldstein, D.; Moalem-Taylor, G. Characterisation of Immune and Neuroinflammatory Changes Associated with Chemotherapy-Induced Peripheral Neuropathy. *PloS one* **2017,** *12* (1), e0170814.
(1070) Sakamoto, A.; Andoh, T.; Kuraishi, Y. Involvement of mast cells and proteinase-activated receptor 2 in oxaliplatin-induced mechanical allodynia in mice. *Pharmacological research* **2016,** *105*, 84.
(1071) Janes, K.; Wahlman, C.; Little, J. W.; Doyle, T.; Tosh, D. K.; Jacobson, K. A.; Salvemini, D. Spinal neuroimmune activation is independent of T-cell infiltration and attenuated by A3 adenosine receptor agonists in a model of oxaliplatin-induced peripheral neuropathy. *Brain Behav Immun* **2015,** *44*, 91.
(1072) Jung, Y.; Lee, J. H.; Kim, W.; Yoon, S. H.; Kim, S. K. Anti-allodynic effect of Buja in a rat model of oxaliplatin-induced peripheral neuropathy via spinal astrocytes and pro-inflammatory cytokines suppression. *BMC Complement Altern Med* **2017,** *17* (1), 48.
(1073) Kim, C.; Lee, J. H.; Kim, W.; Li, D.; Kim, Y.; Lee, K.; Kim, S. K. The Suppressive Effects of Cinnamomi Cortex and Its Phytocompound Coumarin on Oxaliplatin-Induced Neuropathic Cold Allodynia in Rats. *Molecules* **2016,** *21* (9).
(1074) Di Cesare Mannelli, L.; Pacini, A.; Micheli, L.; Tani, A.; Zanardelli, M.; Ghelardini, C. Glial role in oxaliplatin-induced neuropathic pain. *Exp Neurol* **2014,** *261*, 22.
(1075) Pacini, A.; Micheli, L.; Maresca, M.; Branca, J. J.; McIntosh, J. M.; Ghelardini, C.; Di Cesare Mannelli, L. The alpha9alpha10 nicotinic receptor antagonist alpha-conotoxin RgIA prevents neuropathic pain induced by oxaliplatin treatment. *Exp Neurol* **2016,** *282*, 37.
(1076) Antonioli, L.; Fornai, M.; Blandizzi, C.; Pacher, P.; Hasko, G. Adenosine signalling and the immune system: when a lot could be too much. *Immunol Lett* **2018**.
(1077) Sheth, S.; Brito, R.; Mukherjea, D.; Rybak, L. P.; Ramkumar, V. Adenosine receptors: expression, function and regulation. *International journal of molecular sciences* **2014,** *15* (2), 2024.
(1078) Hu, L. Y.; Zhou, Y.; Cui, W. Q.; Hu, X. M.; Du, L. X.; Mi, W. L.; Chu, Y. X.; Wu, G. C.; Wang, Y. Q.; Mao-Ying, Q. L. Triggering receptor expressed on myeloid cells 2 (TREM2) dependent microglial activation promotes cisplatin-induced peripheral neuropathy in mice. *Brain Behav Immun* **2018,** *68*, 132.
(1079) Hijri, F. Z.; Arifi, S.; Ouattassi, N.; Mellas, N.; El Mesbahi, O. Oxaliplatin-induced ototoxicity in adjuvant setting for colorectal cancer: unusual side effect. *J Gastrointest Cancer* **2014,** *45* (1), 106.
(1080) Yuce, S.; Seker, M. M.; Koc, S.; Uysal, I. O.; Kacan, T.; Dogan, M.; Dogan, M.; Babacan, N. A.; Kilickap, S. Oxaliplatin and ototoxicity: is it really safe for hearing? *Turk J Med Sci* **2014,** *44* (4), 586.
(1081) Sheth, S.; Mukherjea, D.; Rybak, L. P.; Ramkumar, V. Mechanisms of Cisplatin-Induced Ototoxicity and Otoprotection. *Front Cell Neurosci* **2017,** *11*, 338.
(1082) Wood, M. B.; Zuo, J. The Contribution of Immune Infiltrates to Ototoxicity and Cochlear Hair Cell Loss. *Front Cell Neurosci* **2017,** *11*, 106.

(1083) Liu, W.; Molnar, M.; Garnham, C.; Benav, H.; Rask-Andersen, H. Macrophages in the Human Cochlea: Saviors or Predators-A Study Using Super-Resolution Immunohistochemistry. *Frontiers in immunology* **2018,** *9*, 223.
(1084) Ishiyama, G.; Lopez, I. A.; Ishiyama, P.; Vinters, H. V.; Ishiyama, A. The blood labyrinthine barrier in the human normal and Meniere's disease macula utricle. *Sci Rep* **2017,** *7* (1), 253.
(1085) Juhn, S. K.; Hunter, B. A.; Odland, R. M. Blood-labyrinth barrier and fluid dynamics of the inner ear. *Int Tinnitus J* **2001,** *7* (2), 72.
(1086) Kaur, T.; Zamani, D.; Tong, L.; Rubel, E. W.; Ohlemiller, K. K.; Hirose, K.; Warchol, M. E. Fractalkine Signaling Regulates Macrophage Recruitment into the Cochlea and Promotes the Survival of Spiral Ganglion Neurons after Selective Hair Cell Lesion. *J Neurosci* **2015,** *35* (45), 15050.
(1087) Oh, G. S.; Kim, H. J.; Choi, J. H.; Shen, A.; Kim, C. H.; Kim, S. J.; Shin, S. R.; Hong, S. H.; Kim, Y.; Park, C.et al. Activation of lipopolysaccharide-TLR4 signaling accelerates the ototoxic potential of cisplatin in mice. *J Immunol* **2011,** *186* (2), 1140.
(1088) Paken, J.; Govender, C. D.; Pillay, M.; Sewram, V. Cisplatin-Associated Ototoxicity: A Review for the Health Professional. *J Toxicol* **2016,** *2016*, 1809394.
(1089) Waissbluth, S.; Chuang, A.; Del Valle, A.; Cordova, M. Long term platinum-induced ototoxicity in pediatric patients. *Int J Pediatr Otorhinolaryngol* **2018,** *107*, 75.
(1090) Callejo, A.; Sedo-Cabezon, L.; Juan, I. D.; Llorens, J. Cisplatin-Induced Ototoxicity: Effects, Mechanisms and Protection Strategies. *Toxics* **2015,** *3* (3), 268.
(1091) Mujica-Mota, M. A.; Schermbrucker, J.; Daniel, S. J. Eye color as a risk factor for acquired sensorineural hearing loss: a review. *Hear Res* **2015,** *320*, 1.
(1092) Jeong, H. J.; Kim, S. J.; Moon, P. D.; Kim, N. H.; Kim, J. S.; Park, R. K.; Kim, M. S.; Park, B. R.; Jeong, S.; Um, J. Y.et al. Antiapoptotic mechanism of cannabinoid receptor 2 agonist on cisplatin-induced apoptosis in the HEI-OC1 auditory cell line. *J Neurosci Res* **2007,** *85* (4), 896.
(1093) So, H.; Kim, H.; Kim, Y.; Kim, E.; Pae, H. O.; Chung, H. T.; Kim, H. J.; Kwon, K. B.; Lee, K. M.; Lee, H. Y.et al. Evidence that cisplatin-induced auditory damage is attenuated by downregulation of pro-inflammatory cytokines via Nrf2/HO-1. *J Assoc Res Otolaryngol* **2008,** *9* (3), 290.
(1094) So, H.; Kim, H.; Lee, J. H.; Park, C.; Kim, Y.; Kim, E.; Kim, J. K.; Yun, K. J.; Lee, K. M.; Lee, H. Y.et al. Cisplatin cytotoxicity of auditory cells requires secretions of proinflammatory cytokines via activation of ERK and NF-kappaB. *J Assoc Res Otolaryngol* **2007,** *8* (3), 338.
(1095) Kim, H. J.; Oh, G. S.; Lee, J. H.; Lyu, A. R.; Ji, H. M.; Lee, S. H.; Song, J.; Park, S. J.; You, Y. O.; Sul, J. D.et al. Cisplatin ototoxicity involves cytokines and STAT6 signaling network. *Cell Res* **2011,** *21* (6), 944.
(1096) Chung, W. H.; Boo, S. H.; Chung, M. K.; Lee, H. S.; Cho, Y. S.; Hong, S. H. Proapoptotic effects of NF-kappaB on cisplatin-induced cell death in auditory cell line. *Acta Otolaryngol* **2008,** *128* (10), 1063.
(1097) Mukherjea, D.; Jajoo, S.; Whitworth, C.; Bunch, J. R.; Turner, J. G.; Rybak, L. P.; Ramkumar, V. Short interfering RNA against transient receptor potential vanilloid 1 attenuates cisplatin-induced hearing loss in the rat. *J Neurosci* **2008,** *28* (49), 13056.
(1098) Mukherjea, D.; Jajoo, S.; Kaur, T.; Sheehan, K. E.; Ramkumar, V.; Rybak, L. P. Transtympanic administration of short interfering (si)RNA for the NOX3 isoform of NADPH oxidase protects against cisplatin-induced hearing loss in the rat. *Antioxid Redox Signal* **2010,** *13* (5), 589.
(1099) Kaur, T.; Mukherjea, D.; Sheehan, K.; Jajoo, S.; Rybak, L. P.; Ramkumar, V. Short interfering RNA against STAT1 attenuates cisplatin-induced ototoxicity in the rat by suppressing inflammation. *Cell Death Dis* **2011,** *2*, e180.
(1100) Schmitt, N. C.; Rubel, E. W.; Nathanson, N. M. Cisplatin-induced hair cell death requires STAT1 and is attenuated by epigallocatechin gallate. *J Neurosci* **2009,** *29* (12), 3843.
(1101) Borse, V.; Al Aameri, R. F. H.; Sheehan, K.; Sheth, S.; Kaur, T.; Mukherjea, D.; Tupal, S.; Lowy, M.; Ghosh, S.; Dhukhwa, A.et al. Epigallocatechin-3-gallate, a prototypic chemopreventative agent for protection against cisplatin-based ototoxicity. *Cell Death Dis* **2017,** *8* (7), e2921.

(1102) Kaur, T.; Borse, V.; Sheth, S.; Sheehan, K.; Ghosh, S.; Tupal, S.; Jajoo, S.; Mukherjea, D.; Rybak, L. P.; Ramkumar, V. Adenosine A1 Receptor Protects Against Cisplatin Ototoxicity by Suppressing the NOX3/STAT1 Inflammatory Pathway in the Cochlea. *J Neurosci* **2016,** *36* (14), 3962.
(1103) Ford, M. S.; Nie, Z.; Whitworth, C.; Rybak, L. P.; Ramkumar, V. Up-regulation of adenosine receptors in the cochlea by cisplatin. *Hear Res* **1997,** *111* (1-2), 143.
(1104) Whitworth, C. A.; Ramkumar, V.; Jones, B.; Tsukasaki, N.; Rybak, L. P. Protection against cisplatin ototoxicity by adenosine agonists. *Biochem Pharmacol* **2004,** *67* (9), 1801.
(1105) Mukherjea, D.; Jajoo, S.; Sheehan, K.; Kaur, T.; Sheth, S.; Bunch, J.; Perro, C.; Rybak, L. P.; Ramkumar, V. NOX3 NADPH oxidase couples transient receptor potential vanilloid 1 to signal transducer and activator of transcription 1-mediated inflammation and hearing loss. *Antioxid Redox Signal* **2011,** *14* (6), 999.
(1106) Ramkumar, V.; Ravi, R.; Wilson, M. C.; Gettys, T. W.; Whitworth, C.; Rybak, L. P. Identification of A1 adenosine receptors in rat cochlea coupled to inhibition of adenylyl cyclase. *Am J Physiol* **1994,** *267* (3 Pt 1), C731.
(1107) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **1997,** *389* (6653), 816.
(1108) Zheng, J.; Dai, C.; Steyger, P. S.; Kim, Y.; Vass, Z.; Ren, T.; Nuttall, A. L. Vanilloid receptors in hearing: altered cochlear sensitivity by vanilloids and expression of TRPV1 in the organ of corti. *J Neurophysiol* **2003,** *90* (1), 444.
(1109) Boussios, S.; Pentheroudakis, G.; Katsanos, K.; Pavlidis, N. Systemic treatment-induced gastrointestinal toxicity: incidence, clinical presentation and management. *Ann Gastroenterol* **2012,** *25* (2), 106.
(1110) Denlinger, C. S.; Barsevick, A. M. The challenges of colorectal cancer survivorship. *J Natl Compr Canc Netw* **2009,** *7* (8), 883.
(1111) Vera, G.; Castillo, M.; Cabezos, P. A.; Chiarlone, A.; Martin, M. I.; Gori, A.; Pasquinelli, G.; Barbara, G.; Stanghellini, V.; Corinaldesi, R.et al. Enteric neuropathy evoked by repeated cisplatin in the rat. *Neurogastroenterol Motil* **2011,** *23* (4), 370.
(1112) Stojanovska, V.; McQuade, R. M.; Miller, S.; Nurgali, K. Effects of Oxaliplatin Treatment on the Myenteric Plexus Innervation and Glia in the Murine Distal Colon. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **2018**, 22155418774755.
(1113) McQuade, R. M.; Carbone, S. E.; Stojanovska, V.; Rahman, A.; Gwynne, R. M.; Robinson, A. M.; Goodman, C. A.; Bornstein, J. C.; Nurgali, K. Role of oxidative stress in oxaliplatin-induced enteric neuropathy and colonic dysmotility in mice. *Br J Pharmacol* **2016,** *173* (24), 3502.
(1114) Kraneveld, A. D.; Rijnierse, A.; Nijkamp, F. P.; Garssen, J. Neuro-immune interactions in inflammatory bowel disease and irritable bowel syndrome: future therapeutic targets. *Eur J Pharmacol* **2008,** *585* (2-3), 361.
(1115) Escudero, M. C.; Lassaletta, A.; Sevilla, J.; Fernandez-Plaza, S.; Perez, A.; Diaz, M. A.; Madero, L. Chemotherapy-related secondary acute myeloid leukemia in patients diagnosed with osteosarcoma. *J Pediatr Hematol Oncol* **2004,** *26* (7), 454.
(1116) Travis, L. B.; Holowaty, E. J.; Bergfeldt, K.; Lynch, C. F.; Kohler, B. A.; Wiklund, T.; Curtis, R. E.; Hall, P.; Andersson, M.; Pukkala, E.et al. Risk of leukemia after platinum-based chemotherapy for ovarian cancer. *N Engl J Med* **1999,** *340* (5), 351.
(1117) Samanta, D.; Senapati, S.; Sharma, P.; Mohanty, A.; Samantaray, M. Acute myelogenous leukemia following treatment of invasive cervix carcinoma: A case report and a review of the literature. *Journal of Cancer Research and Therapeutics* **2009,** *5* (4), 302.
(1118) Rund, D.; Ben-Yehuda, D. Therapy-related leukemia and myelodysplasia: evolving concepts of pathogenesis and treatment. *Hematology (Amsterdam, Netherlands)* **2004,** *9* (3), 179.
(1119) Takeyama, K.; Seto, M.; Uike, N.; Hamajima, N.; Ino, T.; Mikuni, C.; Kobayashi, T.; Maruta, A.; Muto, Y.; Maseki, N.et al. Therapy-related leukemia and myelodysplastic syndrome: a large-scale

Japanese study of clinical and cytogenetic features as well as prognostic factors. *International journal of hematology* **2000,** *71* (2), 144.
(1120) Merlin, F.; Prochilo, T.; Kildani, B.; Tucci, A.; Ferrari, S.; Rossi, G.; D'Adda, P.; Beretta, G. D. Secondary acute lymphoblastic leukaemia following oxaliplatin for adjuvant chemotherapy in colon cancer. *Acta oncologica (Stockholm, Sweden)* **2008,** *47* (3), 464.
(1121) Pedersen-Bjergaard, J. Insights into Leukemogenesis from Therapy-Related Leukemia. *New England Journal of Medicine* **2005,** *352* (15), 1591.
(1122) Aul, C.; Gattermann, N.; Schneider, W. Age-related incidence and other epidemiological aspects of myelodysplastic syndromes. *Br J Haematol* **1992,** *82* (2), 358.
(1123) Sekeres, M. A.; Schoonen, W. M.; Kantarjian, H.; List, A.; Fryzek, J.; Paquette, R.; Maciejewski, J. P. Characteristics of US patients with myelodysplastic syndromes: results of six cross-sectional physician surveys. *Journal of the National Cancer Institute* **2008,** *100* (21), 1542.
(1124) Pedersen-Bjergaard, J. Therapy-related myelodysplasia and acute leukemia. *Leuk Lymphoma* **1995,** *15 Suppl 1*, 11.
(1125) Pedersen-Bjergaard, J.; Pedersen, M.; Roulston, D.; Philip, P. Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia. *Blood* **1995,** *86* (9), 3542.
(1126) Friedberg, J. W.; Neuberg, D.; Stone, R. M.; Alyea, E.; Jallow, H.; LaCasce, A.; Mauch, P. M.; Gribben, J. G.; Ritz, J.; Nadler, L. M.et al. Outcome in patients with myelodysplastic syndrome after autologous bone marrow transplantation for non-Hodgkin's lymphoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1999,** *17* (10), 3128.
(1127) Kalaycio, M.; Rybicki, L.; Pohlman, B.; Sobecks, R.; Andresen, S.; Kuczkowski, E.; Bolwell, B. Risk factors before autologous stem-cell transplantation for lymphoma predict for secondary myelodysplasia and acute myelogenous leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2006,** *24* (22), 3604.
(1128) Josting, A.; Wiedenmann, S.; Franklin, J.; May, M.; Sieber, M.; Wolf, J.; Engert, A.; Diehl, V.; German Hodgkin's Lymphoma Study, G. Secondary myeloid leukemia and myelodysplastic syndromes in patients treated for Hodgkin's disease: a report from the German Hodgkin's Lymphoma Study Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2003,** *21* (18), 3440.
(1129) Smith, S. M.; Le Beau, M. M.; Huo, D.; Karrison, T.; Sobecks, R. M.; Anastasi, J.; Vardiman, J. W.; Rowley, J. D.; Larson, R. A. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood* **2003,** *102* (1), 43.
(1130) Smith, R. E.; Bryant, J.; DeCillis, A.; Anderson, S.; National Surgical Adjuvant, B.; Bowel Project, E. Acute myeloid leukemia and myelodysplastic syndrome after doxorubicin-cyclophosphamide adjuvant therapy for operable breast cancer: the National Surgical Adjuvant Breast and Bowel Project Experience. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2003,** *21* (7), 1195.
(1131) Giles, F. J.; Koeffler, H. P. Secondary myelodysplastic syndromes and leukemias. *Current opinion in hematology* **1994,** *1* (4), 256.
(1132) Allodji, R. S.; Schwartz, B.; Veres, C.; Haddy, N.; Rubino, C.; Le Deley, M. C.; Labbe, M.; Diop, F.; Jackson, A.; Dayet, F.et al. Risk of Subsequent Leukemia After a Solid Tumor in Childhood: Impact of Bone Marrow Radiation Therapy and Chemotherapy. *Int J Radiat Oncol Biol Phys* **2015,** *93* (3), 658.
(1133) Kaatsch, P.; Reinisch, I.; Spix, C.; Berthold, F.; Janka-Schaub, G.; Mergenthaler, A.; Michaelis, J.; Blettner, M. Case-control study on the therapy of childhood cancer and the occurrence of second malignant neoplasms in Germany. *Cancer Causes Control* **2009,** *20* (6), 965.
(1134) Dertinger, S. D.; Avlasevich, S. L.; Torous, D. K.; Bemis, J. C.; Phonethepswath, S.; Labash, C.; Carlson, K.; Mereness, J.; Cottom, J.; Palis, J.et al. Persistence of cisplatin-induced mutagenicity in

hematopoietic stem cells: implications for secondary cancer risk following chemotherapy. *Toxicological sciences : an official journal of the Society of Toxicology* **2014,** *140* (2), 307.

(1135) Shimada, T.; Saito, T.; Okadome, M.; Shimamoto, K.; Ariyoshi, K.; Eto, T.; Tomita, Y.; Kodama, K. Secondary leukemia after chemotherapy and/or radiotherapy for gynecologic neoplasia. *Int J Gynecol Cancer* **2014,** *24* (2), 178.

(1136) Martin, J.; Majumdar, G. Platinum compounds and therapy related acute promyelocytic leukemia. *The hematology journal : the official journal of the European Haematology Association* **2002,** *3* (6), 321.

(1137) See, H. T.; Thomas, D. A.; Bueso-Ramos, C.; Kavanagh, J. Secondary leukemia after treatment with paclitaxel and carboplatin in a patient with recurrent ovarian cancer. *Int J Gynecol Cancer* **2006,** *16 Suppl 1*, 236.

(1138) Carneiro, B. A.; Kaminer, L.; Eldibany, M.; Sreekantaiah, C.; Kaul, K.; Locker, G. Y. Oxaliplatin-related acute myelogenous leukemia. *The oncologist* **2006,** *11* (3), 261.

(1139) Merrouche, Y.; Mugneret, F.; Cahn, J. Y. Secondary acute promyelocytic leukemia following irinotecan and oxaliplatin for advanced colon cancer. *Annals of oncology : official journal of the European Society for Medical Oncology* **2006,** *17* (6), 1025.

(1140) Zhang, Y.-C.; Zhou, Y.-Q.; Yan, B.; Shi, J.; Xiu, L.-J.; Sun, Y.-W.; Liu, X.; Qin, Z.-F.; Wei, P.-K.; Li, Y.-J. Secondary acute promyelocytic leukemia following chemotherapy for gastric cancer: A case report. *World Journal of Gastroenterology* **2015,** *21* (14), 4402.

(1141) Damodaran, S.; Bellavia, T.; Sait, S. N. J.; Wang, E. S.; Wetzler, M.; Khushalani, N. I. Acute myeloid leukemia secondary to oxaliplatin treatment for esophageal cancer. *Clinical colorectal cancer* **2012,** *11* (2), 151.

(1142) Mimura, N.; Tsujimura, H.; Ise, M.; Sakai, C.; Takagi, T.; Nagata, M.; Kumagai, K. Therapy-related leukemia following chemoradiotherapy for esophageal cancer. *Eur J Haematol* **2010,** *85* (4), 353.

(1143) Cağlar, K.; Varan, A.; Akyüz, C.; Selek, U.; Kutluk, T.; Yalçin, B.; Atahan, I. L.; Büyükpamukçu, M. Second neoplasms in pediatric patients treated for cancer: a center's 30-year experience. *Journal of pediatric hematology/oncology* **2006,** *28* (6), 374.

(1144) Crump, M.; Tu, D.; Shepherd, L.; Levine, M.; Bramwell, V.; Pritchard, K. Risk of acute leukemia following epirubicin-based adjuvant chemotherapy: a report from the National Cancer Institute of Canada Clinical Trials Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2003,** *21* (16), 3066.

(1145) Ishizawa, S.; Slovak, M. L.; Popplewell, L.; Bedell, V.; Wrede, J. E.; Carter, N. H.; Snyder, D. S.; Arber, D. A. High frequency of pro-B acute lymphoblastic leukemia in adults with secondary leukemia with 11q23 abnormalities. *Leukemia* **2003,** *17* (6), 1091.

(1146) Auxenfants, E.; Morel, P.; Lai, J. L.; Sartiaux, C.; Detourmignies, L.; Bauters, F.; Fenaux, P. Secondary acute lymphoblastic leukemia with t (4;11): report on two cases and review of the literature. *Annals of hematology* **1992,** *65* (3), 143.

(1147) Bigoni, R.; Cuneo, A.; Roberti, M. G.; Moretti, S.; De Angeli, C.; Dabusti, M.; Campioni, D.; del Senno, L.; Biondi, A.; Chaplin, T.et al. Therapy-related adult acute lymphoblastic leukemia with t(4;11)(q21; q23): MLL rearrangement, p53 mutation and multilineage involvement. *Leukemia* **1999,** *13* (5), 704.

(1148) Kadikoylu, G.; Yavasoglu, I.; Barutca, S.; Meydan, N.; Bolaman, Z. Chronic myeloid leukemia following the treatment of rectal adenocarcinoma. *Medical oncology (Northwood, London, England)* **2008,** *25* (4), 467.

(1149) Rogers, J. E.; Yang, D. Differentiation syndrome in patients with acute promyelocytic leukemia. *J Oncol Pharm Pract* **2012,** *18* (1), 109.

(1150) Lou, Y.; Lu, Y.; Zhu, Z.; Ma, Y.; Suo, S.; Wang, Y.; Chen, D.; Tong, H.; Qian, W.; Meng, H.et al. Improved long-term survival in all Sanz risk patients of newly diagnosed acute promyelocytic

leukemia treated with a combination of retinoic acid and arsenic trioxide-based front-line therapy. *Hematol Oncol* **2018**.

(1151) Eghtedar, A.; Rodriguez, I.; Kantarjian, H.; O'Brien, S.; Daver, N.; Garcia-Manero, G.; Ferrajoli, A.; Kadia, T.; Pierce, S.; Cortes, J.et al. Incidence of secondary neoplasms in patients with acute promyelocytic leukemia treated with all-trans retinoic acid plus chemotherapy or with all-trans retinoic acid plus arsenic trioxide. *Leuk Lymphoma* **2015,** *56* (5), 1342.

(1152) Epperla, N.; Pham, A. Q.; Burnette, B. L.; Wiseman, G. A.; Habermann, T. M.; Macon, W. R.; Ansell, S. M.; Inwards, D. J.; Micallef, I. N.; Johnston, P. B.et al. Risk of histological transformation and therapy-related myelodysplasia/acute myeloid leukaemia in patients receiving radioimmunotherapy for follicular lymphoma. *British journal of haematology* **2017,** *178* (3), 427.

(1153) Casadei, B.; Pellegrini, C.; Pulsoni, A.; Annechini, G.; De Renzo, A.; Stefoni, V.; Broccoli, A.; Gandolfi, L.; Quirini, F.; Tonialini, L.et al. 90-yttrium-ibritumomab tiuxetan consolidation of fludarabine, mitoxantrone, rituximab in intermediate/high-risk follicular lymphoma: updated long-term results after a median follow-up of 7 years. *Cancer Medicine* **2016,** *5* (6), 1093.

(1154) Zinzani, P. L.; Derenzini, E.; Pellegrini, C.; Rigacci, L.; Fabbri, A.; Gandolfi, L.; Argnani, L.; Casadei, B.; Pulsoni, A.; Gobbi, M.et al. Long-term efficacy and toxicity results of the FLUMIZ trial (fludarabine and mitoxantrone followed by yttrium-90 ibritumomab tiuxetan in untreated follicular lymphoma). *Annals of oncology : official journal of the European Society for Medical Oncology* **2012,** *23* (3), 805.

(1155) Ibatici, A.; Pica, G. M.; Nati, S.; Vitolo, U.; Botto, B.; Ciochetto, C.; Petrini, M.; Galimberti, S.; Ciabatti, E.; Orciuolo, E.et al. Safety and efficacy of (90) yttrium-ibritumomab-tiuxetan for untreated follicular lymphoma patients. An Italian cooperative study. *British journal of haematology* **2014,** *164* (5), 710.

(1156) Kossman, S. E.; Weiss, M. A. Acute myelogenous leukemia after exposure to strontium-89 for the treatment of adenocarcinoma of the prostate. *Cancer* **2000,** *88* (3), 620.

(1157) Jacene, H.; Gomella, L.; Yu, E. Y.; Rohren, E. M. Hematologic Toxicity From Radium-223 Therapy for Bone Metastases in Castration-Resistant Prostate Cancer: Risk Factors and Practical Considerations. *Clin Genitourin Cancer* **2018**.

(1158) Varkaris, A.; Gunturu, K.; Kewalramani, T.; Tretter, C. Acute Myeloid Leukemia After Radium-223 Therapy: Case Report. *Clin Genitourin Cancer* **2017,** *15* (4), e723.

(1159) Odo, U.; Vasudevamurthy, A. K.; Sartor, O. Acute Promyelocytic Leukemia After Treatment of Metastatic Castration-Resistant Prostate Cancer With Radium-223. *Clin Genitourin Cancer* **2017,** *15* (3), e501.

(1160) Sridhar, S. S.; Freedland, S. J.; Gleave, M. E.; Higano, C.; Mulders, P.; Parker, C.; Sartor, O.; Saad, F. Castration-resistant prostate cancer: from new pathophysiology to new treatment. *Eur Urol* **2014,** *65* (2), 289.

(1161) Chaudhary, U. B.; Haldas, J. R. Long-term complications of chemotherapy for germ cell tumours. *Drugs* **2003,** *63* (15), 1565.

(1162) Dorr, M.; Meggers, E. Metal complexes as structural templates for targeting proteins. *Curr Opin Chem Biol* **2014,** *19*, 76.

(1163) Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer Statistics, 2017. *CA Cancer J Clin* **2017,** *67* (1), 7.

(1164) Fournier, C.; Rivera Vargas, T.; Martin, T.; Melis, A.; Apetoh, L. Immunotherapeutic properties of chemotherapy. *Current opinion in pharmacology* **2017,** *35*, 83.

(1165) Galon, J.; Costes, A.; Sanchez-Cabo, F.; Kirilovsky, A.; Mlecnik, B.; Lagorce-Pages, C.; Tosolini, M.; Camus, M.; Berger, A.; Wind, P.et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **2006,** *313* (5795), 1960.

(1166) Pages, F.; Mlecnik, B.; Marliot, F.; Bindea, G.; Ou, F. S.; Bifulco, C.; Lugli, A.; Zlobec, I.; Rau, T. T.; Berger, M. D.et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet* **2018**.

(1167) Emile, J. F.; Julie, C.; Le Malicot, K.; Lepage, C.; Tabernero, J.; Mini, E.; Folprecht, G.; Van Laethem, J. L.; Dimet, S.; Boulagnon-Rombi, C.et al. Prospective validation of a lymphocyte infiltration prognostic test in stage III colon cancer patients treated with adjuvant FOLFOX. *European journal of cancer* **2017,** *82*, 16.
(1168) Liu, H.; Zhang, T.; Ye, J.; Li, H.; Huang, J.; Li, X.; Wu, B.; Huang, X.; Hou, J. Tumor-infiltrating lymphocytes predict response to chemotherapy in patients with advance non-small cell lung cancer. *Cancer Immunol Immunother* **2012,** *61* (10), 1849.
(1169) Pircher, A.; Gamerith, G.; Amann, A.; Reinold, S.; Popper, H.; Gachter, A.; Pall, G.; Woll, E.; Jamnig, H.; Gastl, G.et al. Neoadjuvant chemo-immunotherapy modifies CD4(+)CD25(+) regulatory T cells (Treg) in non-small cell lung cancer (NSCLC) patients. *Lung Cancer* **2014,** *85* (1), 81.
(1170) Roselli, M.; Cereda, V.; di Bari, M. G.; Formica, V.; Spila, A.; Jochems, C.; Farsaci, B.; Donahue, R.; Gulley, J. L.; Schlom, J.et al. Effects of conventional therapeutic interventions on the number and function of regulatory T cells. *Oncoimmunology* **2013,** *2* (10), e27025.
(1171) Ganesan, A. P.; Johansson, M.; Ruffell, B.; Yagui-Beltran, A.; Lau, J.; Jablons, D. M.; Coussens, L. M. Tumor-infiltrating regulatory T cells inhibit endogenous cytotoxic T cell responses to lung adenocarcinoma. *J Immunol* **2013,** *191* (4), 2009.
(1172) Baras, A. S.; Drake, C.; Liu, J. J.; Gandhi, N.; Kates, M.; Hoque, M. O.; Meeker, A.; Hahn, N.; Taube, J. M.; Schoenberg, M. P.et al. The ratio of CD8 to Treg tumor-infiltrating lymphocytes is associated with response to cisplatin-based neoadjuvant chemotherapy in patients with muscle invasive urothelial carcinoma of the bladder. *Oncoimmunology* **2016,** *5* (5), e1134412.
(1173) Fritzsching, B.; Fellenberg, J.; Moskovszky, L.; Sapi, Z.; Krenacs, T.; Machado, I.; Poeschl, J.; Lehner, B.; Szendroi, M.; Bosch, A. L.et al. CD8(+)/FOXP3(+)-ratio in osteosarcoma microenvironment separates survivors from non-survivors: a multicenter validated retrospective study. *Oncoimmunology* **2015,** *4* (3), e990800.
(1174) Bosmuller, H.; Haitchi-Petnehazy, S.; Webersinke, G.; Marschon, R.; Roithmeier, F.; Stummvoll, W.; Fehm, T.; Klier-Richter, M.; Bonzheim, I.; Staebler, A.et al. Intratumoral lymphocyte density in serous ovarian carcinoma is superior to ERCC1 expression for predicting response to platinum-based therapy. *Virchows Archiv : an international journal of pathology* **2011,** *459* (2), 183.
(1175) Sato, E.; Olson, S. H.; Ahn, J.; Bundy, B.; Nishikawa, H.; Qian, F.; Jungbluth, A. A.; Frosina, D.; Gnjatic, S.; Ambrosone, C.et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci U S A* **2005,** *102* (51), 18538.
(1176) Bamias, A.; Tsiatas, M. L.; Kafantari, E.; Liakou, C.; Rodolakis, A.; Voulgaris, Z.; Vlahos, G.; Papageorgiou, T.; Tsitsilonis, O.; Bamia, C.et al. Significant differences of lymphocytes isolated from ascites of patients with ovarian cancer compared to blood and tumor lymphocytes. Association of CD3+CD56+ cells with platinum resistance. *Gynecol Oncol* **2007,** *106* (1), 75.
(1177) Wu, X.; Feng, Q. M.; Wang, Y.; Shi, J.; Ge, H. L.; Di, W. The immunologic aspects in advanced ovarian cancer patients treated with paclitaxel and carboplatin chemotherapy. *Cancer Immunol Immunother* **2010,** *59* (2), 279.
(1178) Tamura, K.; Hazama, S.; Yamaguchi, R.; Imoto, S.; Takenouchi, H.; Inoue, Y.; Kanekiyo, S.; Shindo, Y.; Miyano, S.; Nakamura, Y.et al. Characterization of the T cell repertoire by deep T cell receptor sequencing in tissues and blood from patients with advanced colorectal cancer. *Oncol Lett* **2016,** *11* (6), 3643.
(1179) Kaneko, M.; Nozawa, H.; Sasaki, K.; Hongo, K.; Hiyoshi, M.; Tada, N.; Murono, K.; Nirei, T.; Kawai, K.; Sunami, E.et al. Elevated neutrophil to lymphocyte ratio predicts poor prognosis in advanced colorectal cancer patients receiving oxaliplatin-based chemotherapy. *Oncology* **2012,** *82* (5), 261.
(1180) Wood, G.; Grenader, T.; Nash, S.; Adams, R.; Kaplan, R.; Fisher, D.; Maughan, T.; Bridgewater, J. Derived neutrophil to lymphocyte ratio as a prognostic factor in patients with advanced colorectal

cancer according to RAS and BRAF status: a post-hoc analysis of the MRC COIN study. *Anticancer Drugs* **2017,** *28* (5), 546.

(1181) Grenader, T.; Nash, S.; Adams, R.; Kaplan, R.; Fisher, D.; Maughan, T.; Bridgewater, J. Derived neutrophil lymphocyte ratio is predictive of survival from intermittent therapy in advanced colorectal cancer: a post hoc analysis of the MRC COIN study. *Br J Cancer* **2016,** *114* (6), 612.

(1182) Yao, Y.; Yuan, D.; Liu, H.; Gu, X.; Song, Y. Pretreatment neutrophil to lymphocyte ratio is associated with response to therapy and prognosis of advanced non-small cell lung cancer patients treated with first-line platinum-based chemotherapy. *Cancer Immunol Immunother* **2013,** *62* (3), 471.

(1183) Ohtake, S.; Kawahara, T.; Kasahara, R.; Ito, H.; Osaka, K.; Hattori, Y.; Teranishi, J. I.; Makiyama, K.; Mizuno, N.; Umemoto, S.et al. Pretreatment Neutrophil-to-Lymphocyte Ratio Can Predict the Prognosis in Bladder Cancer Patients Who Receive Gemcitabine and Nedaplatin Therapy. *BioMed research international* **2016,** *2016*, 9846823.

(1184) Hutterer, G. C.; Sobolev, N.; Ehrlich, G. C.; Gutschi, T.; Stojakovic, T.; Mannweiler, S.; Pummer, K.; Zigeuner, R.; Pichler, M.; Dalpiaz, O. Pretreatment lymphocyte-monocyte ratio as a potential prognostic factor in a cohort of patients with upper tract urothelial carcinoma. *J Clin Pathol* **2015,** *68* (5), 351.

(1185) Karpathiou, G.; Giroult, J. B.; Forest, F.; Fournel, P.; Monaya, A.; Froudarakis, M.; Dumollard, J. M.; Prades, J. M.; Gavid, M.; Peoc'h, M. Clinical and Histologic Predictive Factors of Response to Induction Chemotherapy in Head and Neck Squamous Cell Carcinoma. *Am J Clin Pathol* **2016,** *146* (5), 546.

(1186) Onal, C.; Guler, O. C.; Yildirim, B. A. Prognostic Use of Pretreatment Hematologic Parameters in Patients Receiving Definitive Chemoradiotherapy for Cervical Cancer. *Int J Gynecol Cancer* **2016,** *26* (6), 1169.

(1187) Grenader, T.; Nash, S.; Plotkin, Y.; Furuse, J.; Mizuno, N.; Okusaka, T.; Wasan, H.; Valle, J.; Bridgewater, J. Derived neutrophil lymphocyte ratio may predict benefit from cisplatin in the advanced biliary cancer: the ABC-02 and BT-22 studies. *Annals of oncology : official journal of the European Society for Medical Oncology* **2015,** *26* (9), 1910.

(1188) Formica, V.; Morelli, C.; Ferroni, P.; Nardecchia, A.; Tesauro, M.; Pellicori, S.; Cereda, V.; Russo, A.; Riondino, S.; Guadagni, F.et al. Neutrophil/lymphocyte ratio helps select metastatic pancreatic cancer patients benefitting from oxaliplatin. *Cancer Biomark* **2016,** *17* (3), 335.

(1189) Sharma, P.; Allison, J. P. The future of immune checkpoint therapy. *Science* **2015,** *348* (6230), 56.

(1190) Larkin, J.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J. J.; Cowey, C. L.; Lao, C. D.; Schadendorf, D.; Dummer, R.; Smylie, M.; Rutkowski, P.et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* **2015,** *373* (1), 23.

(1191) Robert, C.; Schachter, J.; Long, G. V.; Arance, A.; Grob, J. J.; Mortier, L.; Daud, A.; Carlino, M. S.; McNeil, C.; Lotem, M.et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* **2015,** *372* (26), 2521.

(1192) Borghaei, H.; Paz-Ares, L.; Horn, L.; Spigel, D. R.; Steins, M.; Ready, N. E.; Chow, L. Q.; Vokes, E. E.; Felip, E.; Holgado, E.et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* **2015,** *373* (17), 1627.

(1193) Brahmer, J.; Reckamp, K. L.; Baas, P.; Crino, L.; Eberhardt, W. E.; Poddubskaya, E.; Antonia, S.; Pluzanski, A.; Vokes, E. E.; Holgado, E.et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *N Engl J Med* **2015,** *373* (2), 123.

(1194) Siefker-Radtke, A.; Curti, B. Immunotherapy in metastatic urothelial carcinoma: focus on immune checkpoint inhibition. *Nat Rev Urol* **2018,** *15* (2), 112.

(1195) Motzer, R. J.; Escudier, B.; McDermott, D. F.; George, S.; Hammers, H. J.; Srinivas, S.; Tykodi, S. S.; Sosman, J. A.; Procopio, G.; Plimack, E. R.et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med* **2015,** *373* (19), 1803.

(1196) Cavalieri, S.; Rivoltini, L.; Bergamini, C.; Locati, L. D.; Licitra, L.; Bossi, P. Immuno-oncology in head and neck squamous cell cancers: News from clinical trials, emerging predictive factors and unmet needs. *Cancer Treat Rev* **2018,** *65*, 78.
(1197) Lazzari, C.; Karachaliou, N.; Bulotta, A.; Vigano, M.; Mirabile, A.; Brioschi, E.; Santarpia, M.; Gianni, L.; Rosell, R.; Gregorc, V. Combination of immunotherapy with chemotherapy and radiotherapy in lung cancer: is this the beginning of the end for cancer? *Ther Adv Med Oncol* **2018,** *10*, 1758835918762094.
(1198) Kim, J. H.; Kim, H. S.; Kim, B. J. Prognostic value of smoking status in non-small-cell lung cancer patients treated with immune checkpoint inhibitors: a meta-analysis. *Oncotarget* **2017,** *8* (54), 93149.
(1199) Lu, J.; Ramirez, R. A. The Role of Checkpoint Inhibition in Non-Small Cell Lung Cancer. *Ochsner J* **2017,** *17* (4), 379.
(1200) Aguiar, P. N., Jr.; De Mello, R. A.; Barreto, C. M. N.; Perry, L. A.; Penny-Dimri, J.; Tadokoro, H.; Lopes, G. L., Jr. Immune checkpoint inhibitors for advanced non-small cell lung cancer: emerging sequencing for new treatment targets. *ESMO Open* **2017,** *2* (3), e000200.
(1201) Herbst, R. S.; Baas, P.; Kim, D. W.; Felip, E.; Perez-Gracia, J. L.; Han, J. Y.; Molina, J.; Kim, J. H.; Arvis, C. D.; Ahn, M. J.et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* **2016,** *387* (10027), 1540.
(1202) Reck, M.; Rodriguez-Abreu, D.; Robinson, A. G.; Hui, R.; Csoszi, T.; Fulop, A.; Gottfried, M.; Peled, N.; Tafreshi, A.; Cuffe, S.et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* **2016,** *375* (19), 1823.
(1203) Pai-Scherf, L.; Blumenthal, G. M.; Li, H.; Subramaniam, S.; Mishra-Kalyani, P. S.; He, K.; Zhao, H.; Yu, J.; Paciga, M.; Goldberg, K. B.et al. FDA Approval Summary: Pembrolizumab for Treatment of Metastatic Non-Small Cell Lung Cancer: First-Line Therapy and Beyond. *Oncologist* **2017,** *22* (11), 1392.
(1204) Govindan, R.; Szczesna, A.; Ahn, M. J.; Schneider, C. P.; Gonzalez Mella, P. F.; Barlesi, F.; Han, B.; Ganea, D. E.; Von Pawel, J.; Vladimirov, V.et al. Phase III Trial of Ipilimumab Combined With Paclitaxel and Carboplatin in Advanced Squamous Non-Small-Cell Lung Cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2017,** *35* (30), 3449.
(1205) Socinski, M. A.; Jotte, R. M.; Cappuzzo, F.; Orlandi, F.; Stroyakovskiy, D.; Nogami, N.; Rodriguez-Abreu, D.; Moro-Sibilot, D.; Thomas, C. A.; Barlesi, F.et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med* **2018**.
(1206) Antonia, S. J.; Villegas, A.; Daniel, D.; Vicente, D.; Murakami, S.; Hui, R.; Yokoi, T.; Chiappori, A.; Lee, K. H.; de Wit, M.et al. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. *N Engl J Med* **2017,** *377* (20), 1919.
(1207) Dwary, A. D.; Master, S.; Patel, A.; Cole, C.; Mansour, R.; Mills, G.; Koshy, N.; Peddi, P.; Burton, G.; Hammoud, D.et al. Excellent response to chemotherapy post immunotherapy. *Oncotarget* **2017,** *8* (53), 91795.
(1208) Simon, A.; Kourie, H. R.; Kerger, J. Is there still a role for cytotoxic chemotherapy after targeted therapy and immunotherapy in metastatic melanoma? A case report and literature review. *Chinese journal of cancer* **2017,** *36* (1), 10.
(1209) Du, L.; Herbst, R. S.; Morgensztern, D. Immunotherapy in Lung Cancer. *Hematology/oncology clinics of North America* **2017,** *31* (1), 131.
(1210) Malhotra, J.; Jabbour, S. K.; Aisner, J. Current state of immunotherapy for non-small cell lung cancer. *Translational lung cancer research* **2017,** *6* (2), 196.
(1211) Giaccone, G.; Bazhenova, L. A.; Nemunaitis, J.; Tan, M.; Juhasz, E.; Ramlau, R.; van den Heuvel, M. M.; Lal, R.; Kloecker, G. H.; Eaton, K. D.et al. A phase III study of belagenpumatucel-L, an allogeneic

tumour cell vaccine, as maintenance therapy for non-small cell lung cancer. *European journal of cancer* **2015,** *51* (16), 2321.

(1212) Butts, C.; Socinski, M. A.; Mitchell, P. L.; Thatcher, N.; Havel, L.; Krzakowski, M.; Nawrocki, S.; Ciuleanu, T. E.; Bosquee, L.; Trigo, J. M.et al. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. *The Lancet. Oncology* **2014,** *15* (1), 59.

(1213) Fennell, D. A.; Kirkpatrick, E.; Cozens, K.; Nye, M.; Lester, J.; Hanna, G.; Steele, N.; Szlosarek, P.; Danson, S.; Lord, J.et al. CONFIRM: a double-blind, placebo-controlled phase III clinical trial investigating the effect of nivolumab in patients with relapsed mesothelioma: study protocol for a randomised controlled trial. *Trials* **2018,** *19* (1), 233.

(1214) Fujimoto, N.; Aoe, K.; Kozuki, T.; Oze, I.; Kato, K.; Kishimoto, T.; Hotta, K. A Phase II Trial of First-Line Combination Chemotherapy With Cisplatin, Pemetrexed, and Nivolumab for Unresectable Malignant Pleural Mesothelioma: A Study Protocol. *Clinical lung cancer* **2018**.

(1215) Fehrenbacher, L.; Spira, A.; Ballinger, M.; Kowanetz, M.; Vansteenkiste, J.; Mazieres, J.; Park, K.; Smith, D.; Artal-Cortes, A.; Lewanski, C.et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet* **2016,** *387* (10030), 1837.

(1216) Rittmeyer, A.; Barlesi, F.; Waterkamp, D.; Park, K.; Ciardiello, F.; von Pawel, J.; Gadgeel, S. M.; Hida, T.; Kowalski, D. M.; Dols, M. C.et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* **2017,** *389* (10066), 255.

(1217) Carbone, D. P.; Reck, M.; Paz-Ares, L.; Creelan, B.; Horn, L.; Steins, M.; Felip, E.; van den Heuvel, M. M.; Ciuleanu, T. E.; Badin, F.et al. First-Line Nivolumab in Stage IV or Recurrent Non-Small-Cell Lung Cancer. *N Engl J Med* **2017,** *376* (25), 2415.

(1218) Hellmann, M. D.; Ciuleanu, T. E.; Pluzanski, A.; Lee, J. S.; Otterson, G. A.; Audigier-Valette, C.; Minenza, E.; Linardou, H.; Burgers, S.; Salman, P.et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. *N Engl J Med* **2018,** *378* (22), 2093.

(1219) Bellmunt, J.; de Wit, R.; Vaughn, D. J.; Fradet, Y.; Lee, J. L.; Fong, L.; Vogelzang, N. J.; Climent, M. A.; Petrylak, D. P.; Choueiri, T. K.et al. Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. *N Engl J Med* **2017,** *376* (11), 1015.

(1220) Powles, T.; Duran, I.; van der Heijden, M. S.; Loriot, Y.; Vogelzang, N. J.; De Giorgi, U.; Oudard, S.; Retz, M. M.; Castellano, D.; Bamias, A.et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* **2018,** *391* (10122), 748.

(1221) Sharma, P.; Retz, M.; Siefker-Radtke, A.; Baron, A.; Necchi, A.; Bedke, J.; Plimack, E. R.; Vaena, D.; Grimm, M. O.; Bracarda, S.et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. *The Lancet. Oncology* **2017,** *18* (3), 312.

(1222) Massard, C.; Gordon, M. S.; Sharma, S.; Rafii, S.; Wainberg, Z. A.; Luke, J.; Curiel, T. J.; Colon-Otero, G.; Hamid, O.; Sanborn, R. E.et al. Safety and Efficacy of Durvalumab (MEDI4736), an Anti-Programmed Cell Death Ligand-1 Immune Checkpoint Inhibitor, in Patients With Advanced Urothelial Bladder Cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2016,** *34* (26), 3119.

(1223) Patel, M. R.; Ellerton, J.; Infante, J. R.; Agrawal, M.; Gordon, M.; Aljumaily, R.; Britten, C. D.; Dirix, L.; Lee, K. W.; Taylor, M.et al. Avelumab in metastatic urothelial carcinoma after platinum failure (JAVELIN Solid Tumor): pooled results from two expansion cohorts of an open-label, phase 1 trial. *The Lancet. Oncology* **2018,** *19* (1), 51.

(1224) Balar, A. V.; Castellano, D.; O'Donnell, P. H.; Grivas, P.; Vuky, J.; Powles, T.; Plimack, E. R.; Hahn, N. M.; de Wit, R.; Pang, L.et al. First-line pembrolizumab in cisplatin-ineligible patients with locally

advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. *The Lancet. Oncology* **2017,** *18* (11), 1483.

(1225) Balar, A. V.; Galsky, M. D.; Rosenberg, J. E.; Powles, T.; Petrylak, D. P.; Bellmunt, J.; Loriot, Y.; Necchi, A.; Hoffman-Censits, J.; Perez-Gracia, J. L.et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* **2017,** *389* (10064), 67.

(1226) Ferris, R. L.; Blumenschein, G., Jr.; Fayette, J.; Guigay, J.; Colevas, A. D.; Licitra, L.; Harrington, K.; Kasper, S.; Vokes, E. E.; Even, C.et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. *N Engl J Med* **2016,** *375* (19), 1856.

(1227) Seiwert, T. Y.; Burtness, B.; Mehra, R.; Weiss, J.; Berger, R.; Eder, J. P.; Heath, K.; McClanahan, T.; Lunceford, J.; Gause, C.et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *The Lancet. Oncology* **2016,** *17* (7), 956.

(1228) Shitara, K.; Ozguroglu, M.; Bang, Y. J.; Bartolomeo, M. D.; Mandala, M.; Ryu, M. H.; Fornaro, L.; Olesinski, T.; Caglevic, C.; Chung, H. C.et al. Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. *Lancet* **2018**.

(1229) Fuchs, C. S.; Doi, T.; Jang, R. W.; Muro, K.; Satoh, T.; Machado, M.; Sun, W.; Jalal, S. I.; Shah, M. A.; Metges, J. P.et al. Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer: Phase 2 Clinical KEYNOTE-059 Trial. *JAMA oncology* **2018,** *4* (5), e180013.

(1230) Taieb, J.; Moehler, M.; Boku, N.; Ajani, J. A.; Yanez Ruiz, E.; Ryu, M. H.; Guenther, S.; Chand, V.; Bang, Y. J. Evolution of checkpoint inhibitors for the treatment of metastatic gastric cancers: Current status and future perspectives. *Cancer Treat Rev* **2018,** *66*, 104.

(1231) Dietrich, B.; Srinivas, S. Urothelial carcinoma: the evolving landscape of immunotherapy for patients with advanced disease. *Research and reports in urology* **2018,** *10*, 7.

(1232) Rothschild, U.; Muller, L.; Lechner, A.; Schlosser, H. A.; Beutner, D.; Laubli, H.; Zippelius, A.; Rothschild, S. I. Immunotherapy in head and neck cancer - scientific rationale, current treatment options and future directions. *Swiss medical weekly* **2018,** *148*, w14625.

(1233) Outh-Gauer, S.; Alt, M.; Le Tourneau, C.; Augustin, J.; Broudin, C.; Gasne, C.; Denize, T.; Mirghani, H.; Fabre, E.; Menard, M.et al. Immunotherapy in head and neck cancers: A new challenge for immunologists, pathologists and clinicians. *Cancer Treat Rev* **2018,** *65*, 54.

(1234) Heong, V.; Ngoi, N.; Tan, D. S. Update on immune checkpoint inhibitors in gynecological cancers. *Journal of gynecologic oncology* **2017,** *28* (2), e20.

(1235) Gadducci, A.; Guerrieri, M. E. Immune Checkpoint Inhibitors in Gynecological Cancers: Update of Literature and Perspectives of Clinical Research. *Anticancer Res* **2017,** *37* (11), 5955.

(1236) Lheureux, S.; Butler, M. O.; Clarke, B.; Cristea, M. C.; Martin, L. P.; Tonkin, K.; Fleming, G. F.; Tinker, A. V.; Hirte, H. W.; Tsoref, D.et al. Association of Ipilimumab With Safety and Antitumor Activity in Women With Metastatic or Recurrent Human Papillomavirus-Related Cervical Carcinoma. *JAMA oncology* **2017**.

(1237) Hamanishi, J.; Mandai, M.; Ikeda, T.; Minami, M.; Kawaguchi, A.; Murayama, T.; Kanai, M.; Mori, Y.; Matsumoto, S.; Chikuma, S.et al. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2015,** *33* (34), 4015.

(1238) Smyth, E. C.; Verheij, M.; Allum, W.; Cunningham, D.; Cervantes, A.; Arnold, D.; Committee, E. G. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of oncology : official journal of the European Society for Medical Oncology* **2016,** *27* (suppl 5), v38.

(1239) Dolcetti, R.; De Re, V.; Canzonieri, V. Immunotherapy for Gastric Cancer: Time for a Personalized Approach? *International journal of molecular sciences* **2018,** *19* (6).

TOC graphic