resolution and can track specific cells during blood circulation to facilitate diagnosis.

Material and methods: Near-infrared persistent luminescence nanoparticles (NIR PLNs) reveal a special nonlinear radiation process, involving the absorption and retention of photons for several hours, followed by, the emission of long-term luminescence lasting for, one to two hours. Persistent luminescence is an optical phenomenon whereby luminescence lasts for an appreciable time after the ceasing of excitation. In the past few decades, persistent phosphors have been used in important resources for our daily life.

Results: For the biological applications, NIR persistent luminescence materials have recently attracted increasing academic attention because of their potential as novel optical contrast agents for *in vivo* bioimaging within the NIR biological window. NIR persistent luminescence within the tissue transparency window (650 – 1350 nm) will be seen for hours after the termination of excitation.

Conclusions: It is expected that sample injections will be performed using intratracheal, subcutaneous, and intravenous injections to evaluate the response of mice to different conditions of drug administration.

No conflict of interest.

POSTER SESSION

Cytotoxics (including Antimetabolites, Anthracyclin, Alkylating agents, Aurora kinases, Polo-like kinase, Topos-isomerase inhibitors, Tubulin-binding compounds)

132 Poster

Phase IIa/IIb clinical trial of NC-6004 (Nanoparticle Cisplatin) plus Pembrolizumab in patients with head and neck cancer (HNSCC) who have failed platinum or a platinum-containing regimen

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Background: Immune checkpoint inhibitors (ICI) have revolutionized advanced cancer treatment. Nevertheless, less than 20% of cancer patients currently benefit. In order to enhance antitumor activity of ICI, combination trials are under investigation and chemotherapy combination is the most popular. It is known that cisplatin (CDDP), one of most widely used chemotherapeutic agent, induces immunogenic cell death resulting in boosting ICI activity. In fact, ICI in combination with platinum agents have been approved in several cancers. NC-6004 is a polymeric micelle exhibiting sustained release of CDDP and selective distribution to tumors due to the enhanced permeability and retention (EPR) effect. Previous data showed (1) NC-6004 could overcome multiple drug resistance and (2) NC-6004 has equivalent activity to conventional CDDP and better safety profile. Accrual of the Phase IIa portion has been completed but the study is ongoing. Phase IIb will be initiated later in 2020.

Material and methods: In the Phase IIa portion, patients were enrolled at 5 sites in Europe. NC-6004 at 90, 105, 120 or 135 mg/m^2 IV was given over 1 hr on day 1 with Pembrolizumab at 200 mg IV over 30 mins on day 1 every 3 wks. Patient enrollment was done by traditional 3+3 design. Primary endpoint was to determine MTD and Recommended Phase 2 Dose (RP2D). Safety, antitumor activity as well as pharmacokinetics were evaluated as secondary endpoints.

Results: Overall 16 pts (14 M, 2 F) with HNSCC enrolled. All subjects were ICI naïve and CDDP pre-treated. 7 patients (44%) had prior cetuximab treatment. Two patients (13%) had high PD-L1 expression (TPS≥50%). No DLT was observed, therefore MTD was not identified. RP2D was determined to be 135 mg/m^2. Neither Grade 3/4 toxicity nor serious adverse event was observed in Cycle 1 at each dose level. The most common AE was hypomagnesaemia (31%). No Grade 3 or greater eGFR reduction was observed. All AEs were manageable and no unexpected AE was identified. Activity was observed: tumor shrinkage in 8/14 (57%), PR in 3/14 (21%, 1 confirmed, 2 unconfirmed).

Conclusions: Although MTD and RP2D of 135 mg/m^2 are higher than conventional CDDP doses usually used in HNSCC treatment, no clinically significant neuro- oto- or nephrotoxicity was observed owing to the nanoparticle formulation. Thus, unlike the toxicity of CDDP, NC-6004 combination didn't jeopardize the ability to deliver ICI, resulting in a combination regimen with a good safety profile. Overall, this data demonstrates promising activity and excellent tolerability of NC-6004 in conjunction with pembrolizumab in a platinum pre-treated population.

Conflict of interest:
Ownership: N/A.
Advisory Board: N/A.
Board of Directors: N/A.
Corporate-sponsored Research: Atsushi Osada is a full-time employee of NanoCarrier.
Other Substantive Relationships: N/A.

133 Poster

Evaluation of [1,2]oxazolo[5,4-e]isoindoles in lymphoma cells

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Background: Anti-tubulin agents are important chemotherapeutics. Combretastatin A-4 (CA-4) emerged as lead compound for the design of new tubulin-binding agents. Its analogues 4,5-diarylisoxazoles, containing the [1,2]oxazole ring as linker of two aryl moieties, displayed higher anti-tubulin activity than CA-4. [1,2]oxazolo[5,4-*e*]isoindoles also gave excellent results reducing cell growth of NCI-60 tumor cell lines and diffuse malignant peritoneal mesothelioma (DMPM) cells. Selected derivatives showed *in vivo* antitumor activity at well-tolerated doses in a DMPM xenograft model.

[1,2]oxazolo[5,4-*e*]isoindoles were screened in four lymphoma histotypes: germinal center B-cell and activated diffuse large B cell lymphoma, marginal zone lymphoma and mantle cell lymphoma.

Material and methods: Cell proliferation was measured with the MTT test after 72 h treatment. Compounds were pre-screened at the dose of 1 μM in SU-DHL-10, HBL1, VL51 and MINO cell lines. Those with percentage of proliferating cells down to 60% proceeded to screenings with a wider range of concentrations.

Results: At 1 μM, 6 out of 13 derivatives determined a reduction in the proliferation rate down to 7–61%. Hence, they were tested at concentrations range of 0.15–10 μM and showed anti-proliferative activity with IC50 values between the low micromolar and the nanomolar range. The most potent derivative, SIX2-G, reached nanomolar activity in the majority of cell lines. Furthermore, the compounds inhibited tubulin assembly and colchicine binding.

Structure-activity relationship suggest that a methoxybenzyl substitution at the pyrrole nitrogen is crucial. In particular, methoxy groups in position 3,4 and/or 5 are relevant.

CPD	VL51	MINO	HBL1	SU-DHL-10	Inhibition of tubulin assembly (IC_{50})	% inhibition of colchicine binding
SIX13	0.27	0.23	0.25	0.28	2.1 ± 0.2	77 ± 0.5
SIX2-A	1	0.75	0.73	0.83	5.2 ± 0.7	69 ± 1
SIX2-G	**0.12**	**0.07**	**0.08**	**0.07**	**2.3 ± 0.3**	**80 ± 0.6**
SIX13-O	0.25	0.23	0.27	0.26	1.7 ± 0.2	72 ± 2
SIX13-S	0.27	0.37	0.47	0.5	3.2 ± 0.1	34 ± 4
SIX13-U	0.1	0.07	0.09	0.1	1.7 ± 0.06	57 ± 2

IC_{50} values are expressed in μM.

Conclusions: [1,2]oxazolo[5,4-*e*]isoindoles are promising anti-tubulin agents with anti-tumor activity in different lymphoma histotypes.

No conflict of interest.

POSTER SESSION

DNA Repair Modulation (including PARP, CHK, ATR, ATM)

134 Poster

Role of AKT3 in the intrinsic radioresistance of lung adenocarcinoma

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Background: Radioresistance poses a critical challenge to effective radiotherapy in the management of lung adenocarcinomas (LUAD). The hyperactivated PI3K-AKT pathway is one of the molecular events by which tumors become radioresistant. The AKT kinase, the downstream effector of PI3K kinase, comprised of three highly homologous isoforms, AKT1, AKT2, and AKT3. Despite several studies on AKT isoforms, the exact role of AKT3 in conferring radioresistance remains elusive.

Methods: To investigate the correlation between AKT3 and lung adenocarcinoma, we analyzed a dataset of LUAD patients from the Cancer Genome Atlas (n = 706), quantified mRNA and protein levels of AKT isoforms in a panel of seven non-small cell lung carcinoma (NSCLC) cell lines. To explore the function of AKT3 in the radioresistance of LUAD, we generated AKT3 shRNA expressing NCI-H1299 and A549 stable LUAD cell lines. We investigated the consequences of AKT3 depletion on the response of these cells to ionizing radiation (IR). Cell proliferation index, clonogenic survival, γ-H2AX foci formation, levels of DNA repair proteins, radiation-induced apoptosis, and spheroid growth kinetics were studied as indices of radiation response of AKT3 knockdown cell lines and to establish the role of AKT3 in radioresistance of LUAD.

Results: Analysis of copy number variation and gene expression levels of AKT isoforms in the TCGA dataset revealed that AKT3 comprises the most frequently amplified and expressed AKT isoform in LUAD patients. The analysis of mRNA and protein levels of all three AKT isoforms in a panel of NSCLS cell lines revealed that AKT3 was expressed in all the NSCLC cell lines we tested in our study, with relatively high levels in LUAD cell lines. The knockdown of AKT3 resulted in a significant reduction in the proliferation index of NCI-H1299 and A549 cells at 2 Gy and 4 Gy dose of IR. Correspondingly, in comparison to scrambled shRNA expressing cells, a loss of clonogenicity to IR was observed in both the cells expressing AKT3 shRNA. Further, AKT3 knockdown significantly increased the residual double-stranded breaks (DSB) after IR as assessed from γ-H2AX foci formation and protein levels of DNA repair pathways proteins. AKT3 knockdown also resulted in a significant increase in radiation-induced cell death, leading to radiosensitization. In addition to that, AKT3 depletion resulted in a significant reduction in 3-dimensional multicellular spheroids volume. Upon irradiation, a significant decrease in the spheroid growth was also observed in NCI-H1299 and A549 cells expressing AKT3 shRNA. Taken together, these findings demonstrate the role of AKT3 in the intrinsic radioresistance of LUAD and highlight the rationale for the development of AKT isoform-selective small molecule inhibitors.

No conflict of interest.

135 Poster

A CDC7-selective Inhibitor, TAK-931, suppresses homologous recombination repair activity to enhance antiproliferative activity of a PARP inhibitor

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Background: Cell division cycle 7 (CDC7) is a serine/threonine kinase, which plays important roles in initiation of DNA replication. We developed a CDC7-selective inhibitor, TAK-931, as a clinical candidate compound. We previously demonstrated that TAK-931 enhances the biological activity of the DNA damaging agents for antiproliferation in cancer cells. In this study, we aimed to identify novel molecular mechanisms of TAK-931 focusing on homologous recombination repair (HRR) pathways, which would be involved in enhanced antiproliferative effects in combination with TAK-931.

Methods: Stable isotope labeling using amino acids in cell culture (SILAC)-labeled H460 cells in combination treatment with IR and TAK-931 were subjected to quantitative mass spectrometry. For HRR assay, DR-GFP and Sce-I plasmids were co-transfected into 293 T cells in presence or absence of TAK-931. In vivo combination studies of TAK-931 with a PARP inhibitor were conducted in multiple human xenograft models including patient-derived xenograft models (PDXs).

Results: In the phosphoproteomics analysis, informatics analysis revealed that double-strand break (DSB) repair ($p < 0.01$) pathway was significantly enriched in the combination-treated cells with irradiation and TAK-931 (IR+TAK-931) compared with the untreated cells after 24 hours. In the DSB repair pathway, phosphorylations of homologous recombination repair (HRR)-associated proteins, such as BRCA2, ATM, and Rad50, were particularly elevated. The DR-GFP-based HRR reporter assay also revealed that TAK-931 intensively suppresses HRR activity. These findings suggest that TAK-931-mediated insufficient HRR, which appears to exhibit a chemically-induced BRCA defectiveness (termed "BRCAness"), would accumulate more DSBs by the combination treatment, and accordingly prolong the activation of HRR-associated proteins in these cells. We also conducted in vivo combination studies with a PARP inhibitor, which is expected to be more effective for BRCAness tumors. Consistent with our hypothesis, in vivo combination treatments of TAK-931 with PARP inhibitor, also enhanced antitumor activities in multiple human xenograft models, including breast and ovarian PDXs.

Conclusions: Our findings suggest that TAK-931 could chemically induce BRCAness in cancer cells to enhance antiproliferative activity of the PARP inhibitor.

Conflict of interest:
Corporate-sponsored Research: This research was supported by Takeda Pharmaceutical Company, Ltd.
Other Substantive Relationships: YJ, HN, KK, and AO are/were employees of Takeda Pharmaceutical Company.

136 Poster

Investigating synergy between WEE1 and PARP inhibitors in BRCA2 mutant and corrected cells

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Background: The PARP inhibitor (PARPi) rucaparib exploits defective homologous recombination repair (HRR) in *BRCA* mutated cancer cells via synthetic lethality. PARPi causes stalled replication forks resulting in an increase in replication stress (RS). RS activates ATR, which signals to HRR and cell cycle checkpoints via CHK1 and WEE1. Here we investigated the synergy between rucaparib and the WEE1i MK-1775 (AZD1775) and the mechanism underpinning this.

Materials and Methods: The effects of rucaparib and MK-1775 were examined in V-C8 (*BRCA2* mutant Chinese hamster fibroblasts) and V-C8. B2 (*BRCA2*corrected) cell lines. Cytotoxicity was determined using colony formation assays. Endogenous PAR levels and PARP activity was determined by measuring the PAR product by immunoblotting with the 10H Ab. WEE1 activity was measured by CDK1y15phosphorylation, and upstream CHK1 and ATR activity were measured by, CHK1s296and CHK1s345phosphorylation, respectively, by Western blot. DNA damage and repair by HRR was determined by gammaH2AX and RAD51 focus formation, respectively, by immunofluorescence microscopy. Cell cycle analysis was investigated using flow cytometry.

Results: HRR defective V-C8 cells were significantly more sensitive to rucaparib cytotoxicity than HRR competent V-C8.B2 cells (LC50s: <0.01 μM vs. >10 μM, $p < 0.001$). There was no significant difference in sensitivity to MK-1775 cytotoxicity (LC50s: 454.5 nM vs 372.1 nM). MK-1775 (100 nM) sensitised V-C8.B2 cells to rucaparib 7.2-fold ± 1.4. No sensitisation was observed in V-C8 cells. PARP activity was similar in both cell lines but V-C8 cells had 2.3-fold higher endogenous PAR levels than V-C8.B2 cells, indicating a higher level of endogenous DNA breakage. Rucaparib inhibited PARP activity similarly in both cell lines (IC50: 59 nM and 53 nM). Rucaparib (10 μM) increased CDK1y15phosphorylation in both V-C8 and V-C8.B2 cells (1.8 and 1.5-fold, respectively). Upstream ATR and CHK1 were also activated by rucaparib in both cell lines to a similar extent. MK-1775 (100 nM) completely inhibited the increased CDK1y15phosphorylation as well as activating CHK1 and ATR upstream (1.3 to1.4-fold and 3.1to 5.0-fold, respectively). Rucaparib (10 μM) caused a 4.8-fold increase in RAD51 foci in γH2AX positive V-C8.B2 cells. Addition of MK-1775 (100nM) inhibited this