

Expert Opinion

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Monoclonal antibodies and antibody fragments: state of the art and future perspectives in the treatment of non-haematological tumors

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Introduction: The use of monoclonal antibodies is one of the strategies for targeting the specific key points of the main pathways of cancer growth and survival, but only a few antibodies have offered a clear clinical benefit in the treatment of non-haematological malignancies.

Areas covered: This review summarizes the general properties of monoclonal antibodies, including structure, nomenclature and production techniques. The antibodies approved for use in clinical practice for the treatment of non-haematological tumors and those antibodies still being developed in this setting are briefly described. The types of antibody fragments are also reported.

Expert opinion: Monoclonal antibodies were initially developed in order to avoid the cytotoxic effects of chemotherapy on healthy tissues. However antibodies have not yet replaced chemotherapy agents, since the combination of both kinds of drugs have usually appeared to achieve higher benefit compared with chemotherapy alone. The research for the development of new monoclonal antibodies aims to identify further targets and to provide innovative antibody constructs.

Keywords: antibody fragments, anti-EGFR, anti-HER2, anti-VEGF, monoclonal antibodies

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1. Introduction

IgG antibodies are large heterodimeric molecules, approximately 150 kDa and are composed of two different kinds of polypeptide chains, known respectively as the heavy (~ 50 kDa) and the light chain (~ 25 kDa). There are two types of light chains, kappa (κ) and lambda (λ). By cleavage with enzyme papain, the Fab (fragment-antigen binding) part can be separated from the Fc (fragment crystalline) part of the molecule. The Fab fragments contain the variable domains, which consist of three hypervariable amino acid domains responsible for the antibody specificity embedded in constant regions.

Antibodies can target tumors by various mechanisms: i) opsonization, which triggers killing by immune cells, ii) modification of innate biological processes such as growth and apoptosis, and iii) delivery of a cytotoxic payload such as a chemotherapy drug, catalytic toxin, radioisotope or enzyme. However an antibody, which has a specific target expressed in tumor cells, must gain access to all viable cells within tumors at sufficient concentrations in order to produce a maximal change in the tumor [1,2].

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Article highlights.

- Monoclonal antibodies were developed for targeting proteins involved in tumor biology.
- Monoclonal antibodies have provided tools for the improvement of survival and quality of life in the management of a great many types of cancer.
- Hybridoma technology was developed to obtain monoclonal antibodies more efficiently.
- The clinical toxicities observed for the murine monoclonal antibodies are secondary to the interaction with the target antigen.
- The production of chimeric and humanized monoclonal antibodies is an attempt to provide drugs with minimal adverse reactions.
- Antibody fragments might overcome the structural limits of solid tumor tissues for penetration by conventional antibodies.

This box summarizes key points contained in the article.

1.1 Monoclonal antibody nomenclature

The nomenclature of monoclonal antibodies is defined by the guidelines of The American Medical Association together with the United States Adopted Names Council [3].

The suffix used for monoclonal antibodies or fragments is -mab. The -mab suffix stem is preceded by source identifiers: -u- human, -o- mouse, -a- rat, -zu- humanized, -e- hamster, -i- primate, -xi- chimera, -axo- rat/mouse and -xizu- indicating a combination of humanized and chimeric chains.

Furthermore, the name of the antibody includes the indication of the disease, target organ system or tumor subclass against which the antibody is used: -vir- viral, -bac- bacterial, -lim- immune system, -les- infectious lesions, -cir- cardiovascular, -fung- antifungal, -ner- neurological system, -kin- interleukin, -mul- musculoskeletal system, -os- bone, -toxa- toxin target, -col- colon, -mel- melanoma, -mar- mammary, -got- testis, -gov- ovary, -pr(o)- prostate and -tum- miscellaneous tumor.

The whole name, however, is made up of three stem elements: -target-source-mab. Drug companies may, however, place a naming prefix at the start of the name of the agent.

1.2 Monoclonal antibody production

Different techniques have been developed for the production of monoclonal antibodies. These methods may be grouped as tissue cultures and mouse ascitis fluid. Their choice depends on cost and time consumption. Hybridoma technology was developed by Köhler and Milstein in order to obtain monoclonal antibodies more efficiently [4,5]. They engineered tumor cells to be immortalized and produce the required antibodies. Mice are immunized with the specific antigen to target by the monoclonal antibody. The hybrid cells are obtained by the fusion of mouse spleen cells and mouse myeloma cells, which therefore contain genetic material from both the parent cells. This feature allows them to produce a specific antibody and to grow in culture indefinitely [6]. Subsequently the researchers

applied recombinant DNA technology to the development of monoclonal antibodies with greater safety and efficacy through chimerization and humanization [7].

Antibody production is checked by its serum titer levels in immunized mice. Spleen cells are withdrawn as soon as the expected level has been reached. These mouse-derived cells are fused with the immortal myeloma cells cultured using growth factors. The hybridoma cells are harvested and cloned for the production of specific antibodies. The term ‘monoclonal’ implies that these clones derive from a single hybridoma cell type.

2. Monoclonal antibodies in cancer treatment

The research into targeted drugs in cancer treatment was prompted because chemotherapy agents lacked specificity for the cancer cell. Monoclonal antibodies were developed for targeting proteins involved in tumor biology. However they are not always specific for the cancer cell because such proteins may also be expressed in healthy tissues. Thus, although the antibody has consistent specificity towards the antigen, the specificity of the antibody or immunoconjugate for cancer was not absolute.

Fever, chills, flushing, rash, nausea and vomiting, bronchospasm, etc. were the clinical toxicities observed for the murine monoclonal antibodies, which are secondary to the interaction with the target antigen. Another limitation is their high immunogenicity. After only a few infusions of these murine proteins, human anti-mouse antibody production occurs. Furthermore, some of the target antigens on the surface of the cancer cells and in circulation are quickly downregulated. This phenomenon could be mediated by internalization of the antibody-antigen complex. It has been exploited for the activity of immunoconjugates, which are monoclonal antibodies conjugated with drugs, toxins and radioisotopes.

2.1 Approved agents

Monoclonal antibodies and immunoconjugates have shown high applicability in hematological malignancies. They have led to significant progress in the availability of effective treatments for the management of such diseases. In this review we discuss the use and development of monoclonal antibodies and antibody fragments in solid tumors. So far, only a few monoclonal antibodies have been approved for their treatment: trastuzumab, bevacizumab, cetuximab, panitumumab (Table 1).

2.1.1 Anti-VEGF

VEGF is the main mediator of neoangiogenesis. This is a mechanism that allows tumors to grow. In fact, as soon as the diameter of a tumor exceeds 2 mm, VEGF together with other growth factors stimulates the formation of new vessels. The production of these growth factors by cancer cells is induced through the hypoxia-inducible transcription factor (HIF-1) [8,9]. VEGF plays an important role in a number of

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Table 1. Approved monoclonal antibodies with indications for non-haematological malignancies and related warnings for their use.

Approved drug	Indications (FDA)	Warnings
Bevacizumab	Metastatic colorectal cancer, with intravenous 5-fluorouracil-based chemotherapy for first- or second-line treatment Non-squamous NSCLC with carboplatin and paclitaxel for first line treatment of unresectable, locally advanced, recurrent or metastatic disease Metastatic breast cancer, with paclitaxel for treatment of patients who have not received chemotherapy for metastatic human EGFR2 (HER2)-negative breast cancer Glioblastoma, as a single agent for patients with progressive disease following prior therapy Metastatic renal cell carcinoma with IFN- α	Gastrointestinal perforations Surgery and wound healing complications Hemorrhage
Trastuzumab	Adjuvant treatment of HER2 overexpressing early breast cancer Treatment of HER2 overexpressing metastatic breast cancer Treatment of HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma	Cardiomyopathy Infusion reactions Embryo-fetal toxicity Pulmonary toxicity
Cetuximab	Locally or regionally advanced squamous cell carcinoma of the head and neck in combination with radiation therapy Recurrent or metastatic squamous cell carcinoma of the head and neck progressing after platinum-based therapy As a single agent, EGFR-expressing, KRAS wild-type, metastatic colorectal cancer after failure of both irinotecan- and oxaliplatin-based regimens or in patients who are intolerant to irinotecan-based regimens In combination with chemotherapy, EGFR-expressing, KRAS wild-type, metastatic colorectal carcinoma in patients who are refractory to irinotecan-based chemotherapy	Infusion reactions Cardiopulmonary arrest
Panitumumab	As a single agent for the treatment of KRAS wild-type metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine, oxaliplatin and irinotecan chemotherapy regimens	Infusion reactions Dermatologic toxicity

physiological processes, such as embryogenesis, skeletal growth and wound healing [10,11]. In humans, different isoforms of VEGF have been identified: VEGF-A, VEGF-B, VEGF-C, VEGF-D and placenta growth factor (PlGF). However, VEGF-A is generally referred to as VEGF. Its functions are mediated by binding to the VEGF receptors (VEGFR1 or Flt-1, and VEGFR2 or KDR) [12].

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody developed from the murine anti-VEGF monoclonal antibody A4.6.1 and binds to all isoforms of VEGF (Avastin; Genentech.) [13]. Its binding with the receptors VEGFR1 and VEGFR2 prevents their interaction with VEGF. Subsequently the tyrosine kinase activity of these receptors cannot be triggered.

The inhibition of VEGF by bevacizumab leads to the regression of the existing abnormal micro-vessels within the tumor mass, the normalization of tumor vasculature and the inhibition of new vessel formation [14,15]. These effects are useful for helping chemotherapy agents reach the cancer cells more easily. As observed in preclinical models, it is sufficient to withdraw bevacizumab to obtain a rapid regrowth of tumor vasculature. This phenomenon posed the rationale for the continuation of bevacizumab use up to disease progression or beyond [16].

Between 2004 and 2009 the FDA approved bevacizumab with fluoropyrimidines for first-line and second-line

treatment for metastatic colorectal cancer, with carboplatin and paclitaxel for first-line treatment of patients with advanced non-squamous non-small-cell lung cancer, with paclitaxel for first-line treatment of patients with metastatic human EGFR 2(HER2)-negative breast cancer, as monotherapy for recurrent glioblastoma, with interferon for first-line therapy in metastatic renal cell carcinoma.

Two Phase III clinical trials have demonstrated improved survival in patients with advanced colorectal cancer when bevacizumab was added to standard 5-fluorouracil (5-FU)-based chemotherapy regimens, which incorporate irinotecan (irinotecan, 5-FU, leucovorin (IFL)) and oxaliplatin (5-FU, leucovorin, oxaliplatin (FOLFOX4)) [17,18]. Bevacizumab in combination with 5-FU-based chemotherapy has been shown to be effective in both first- and second-line treatment of advanced colorectal cancer. Moreover it is reasonable to believe, as supported by two large observational registry trials in first-line metastatic colorectal cancer (the Bevacizumab Regimens: Investigation of Treatment Effects and Safety (BriTE) trial in the USA and the Bevacizumab Expanded Access Trial (BEAT) trial conducted in Europe and Canada), that bevacizumab in combination with any fluoropyrimidine-based chemotherapy is more effective than any fluoropyrimidine-based chemotherapy alone [19,20].

Achievement of overall survival (OS) and progression-free survival (PFS) benefit from the addition of bevacizumab to

chemotherapy is at the cost of a significant increase in toxicity. Commonly observed adverse effects in clinical trials with bevacizumab included bleeding, thrombosis, hypertension, and proteinuria. Hypertension observed in clinical trials has been manageable with oral antihypertensives; however, frequent monitoring of blood pressure is necessary. Some conditions should be considered as contraindications to the use of bevacizumab, for example, cerebral metastases, advanced atherosclerotic disease or proteinuria. Gastrointestinal perforation and poor wound healing are more frequent with bevacizumab, but rare [21].

There is no evidence to support the use of bevacizumab as monotherapy in advanced colorectal cancer. There is no available evidence to answer the question of whether bevacizumab, when used as a part of first-line therapy, should be continued upon progression as part of a second-line regimen. Further randomized clinical trials are required to answer this question.

A Phase III clinical trial (E4599) in patients with advanced or recurrent stage IIIB/IV NSCLC compared chemotherapy (carboplatin and paclitaxel) alone and the same regimen plus bevacizumab. After completion of six cycles of treatment, patients receiving bevacizumab with chemotherapy continued on bevacizumab as a single agent until disease progression or intolerable toxicities occurred. Adding bevacizumab to paclitaxel and carboplatin chemotherapy significantly increased not only PFS and response rate (RR) but also OS [22]. Recently published data from the Avastin in Lung (AVAIL) trial confirmed clinical efficacy of bevacizumab in combination with a different platinum-doublet chemotherapy (cisplatin plus gemcitabine) in patients with stage IIIB/IV NSCLC; however, the OS benefit favoring bevacizumab did not reach statistical significance [23].

VEGF plays its role in the process of metastatic disease development for breast cancer patients. The angiogenic pathways are poorly active in the early stages of cancer [24]. For this reason it is essential to target the VEGF-associated angiogenesis for adjuvant treatment.

In an open-label Phase III trial (E2100) of paclitaxel plus bevacizumab versus paclitaxel alone as first-line therapy of metastatic breast cancer, 722 patients were studied and showed benefit for PFS and overall response rate (ORR) but not for OS [25].

Bevacizumab was approved for use as first-line therapy in advanced breast cancer according to these findings. Recently, the FDA voted unanimously against this licensed indication in the product's labeling, since the Phase III Avastin and docetaxel (AVADO) (docetaxel plus placebo versus docetaxel plus low-dose bevacizumab or high-dose bevacizumab) and Regimens in Bevacizumab for Breast Oncology (RIBBON)-1 (bevacizumab plus chemotherapy or placebo plus chemotherapy) trials showed a statistically significant improvement in progression-free survival, although of a much smaller magnitude than that observed in the E2100 study [26,27].

The approval of bevacizumab for colon cancer treatment by the FDA prompted attempts by several neuro-oncologists to

use it for recurrent malignant glioma. The first publication of these data reported 66% radiographic responses for patients treated with the combination of bevacizumab and irinotecan [28]. This rate of antitumor activity is far higher than response rates achieved with treatment with temozolomide (5 – 8%). These results were also confirmed in other retrospective analyses. Bevacizumab leads to rapid reductions in peritumoral edema, with subsequent reduction or cessation of corticosteroid use. This anti-VEGF monoclonal antibody also seems to be well-tolerated, with low risk of intracranial hemorrhage [29].

Bevacizumab for recurrent glioblastoma has been evaluated in various prospective Phase II clinical trials as monotherapy or in combination with irinotecan. The obtained findings accelerated its approval by the FDA. The response rates were 28 – 38% for the combination and 35% for monotherapy. The 6-months PFS rates were 43 – 50% and 29%, respectively. The tolerability profile was similar to that previously reported [30,31]. Ongoing Phase III trials are evaluating the combination of bevacizumab with temozolomide and radiotherapy.

The majority of renal cell cancers (RCCs) overexpress the HIF protein with the subsequent upregulation of the VEGF gene [14]. A Phase III trial studied the patients with metastatic clear-cell RCC comparing IFN- α combined with either bevacizumab or placebo. The patients treated with the bevacizumab combination had a significantly longer PFS (10.2 months versus 5.4 months) and higher objective tumor response rate (30.6 versus 12.4%). In an interim analysis, there was no significant survival advantage. Common toxicities seen in this and previous trials were hypertension, proteinuria and a tendency to bleeding and thrombotic events [32].

2.1.2 Anti-HER2

Trastuzumab is a humanized monoclonal antibody, which binds the extracellular domain of the membrane receptor HER2. This antibody is able to block the homodimerization and the cleavage of this HER family receptor. Subsequently the intracellular signal transduction cascade can be arrested. Moreover other mechanisms seem to be involved in explaining the molecular effects of trastuzumab. Antibody-dependent cell-mediated toxicity is an immunological phenomenon, which has been documented for trastuzumab as well as for other monoclonal antibodies [33].

The HER2 gene is over-expressed in about 15 – 20% of breast tumors. Its expression can be detected by both the immunohistochemical HER2 protein levels and fluorescent *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) HER2 gene amplification. Breast cancer is defined as HER2-positive, if the HER2 protein expression by immunohistochemistry (IHC) has a 3+ score or if HER2 gene amplification has a ratio of HER2 : centromere signals $\geq 2,2$. HER2 positivity is associated with higher aggressiveness of the breast cancer, as indicated by a higher

proliferation rate, the metastatic potential and angiogenesis. HER2-positive breast cancer patients show a worse prognosis, with shorter median survival, about half of that for HER2-negative patients.

Trastuzumab has been studied in combination with chemotherapeutic agents, such as anthracyclines, taxanes, platinum compounds and vinorelbine. A synergistic effect has been observed, and in particular an enhanced cytotoxic effect, more evident apoptosis induction and proliferation rate reduction.

In HER2-positive untreated patients with advanced breast cancer, trastuzumab has shown a clear benefit, when it is added to chemotherapeutic agents, such as docetaxel, paclitaxel or doxorubicin-cyclophosphamide combination. These patients experienced higher RR, time to progression (TTP) and OS [34,35]. Subsequently trastuzumab was studied in combination with other drugs. Usually trastuzumab-treated patients develop acquired resistance, as highlighted by disease progression within one year following the start of delivery [36]. When the patient experiences a progression during treatment with a trastuzumab-containing regimen, the continuation of trastuzumab is suggested with a concomitant change of chemotherapy, based on the findings from both retrospective and Phase III trials [37,38].

The main limitation for the clinical application of trastuzumab is represented by its potential cardiotoxicity, above all when it is combined with anthracyclines. For this reason this combination is not recommended. Recently some Phase II trials reported the feasibility of the combination of trastuzumab with liposomal doxorubicin [39,40].

For the adjuvant treatment of early breast cancer, trastuzumab has been tested in several studies involving more than 10,000 women. The available data, considered in their entirety, suggest that one year of treatment with trastuzumab is able to reduce the risk of relapse by about 50% [41]. The duration of treatment for one year has been chosen empirically, arguing that trastuzumab needs to be delivered for a long time to be efficacious. Up till now this duration is considered standard, even though some studies have highlighted similar benefit for shorter periods of administration [42,43]. A longer delivery of trastuzumab for 2 years is under evaluation. However the real goal is to demonstrate the equivalent benefit deriving from a shorter duration, since it might lead to less cardiotoxicity. Adjuvant trastuzumab is indicated for all HER2-positive breast tumors. Its use in patients with tumors smaller than 1 cm has not yet been clarified, since they could not be enrolled in the clinical studies for trastuzumab in the adjuvant setting. Retrospective data showed that the overexpression of HER2 implies a worse prognosis independent of tumor dimension. Subsequently further clinical studies should be prompted to shed light on this aspect.

2.1.3 Anti-EGFR

Two anti-EGFR monoclonal antibodies are currently available for the treatment of metastatic colorectal cancer

(mCRC). Cetuximab is a chimeric immunoglobulin G1 antibody, and panitumumab is a fully human immunoglobulin G2 antibody.

Patients with advanced colorectal cancer respond to anti-EGFR antibodies independently of EGFR protein expression in tumor tissue, even though a clear explanation has not yet been provided [44,45]. Other factors seem to be associated with resistance to anti-EGFR monoclonal antibodies and include ligand expression, alterations in downstream signaling pathways, and cross-talk between different members of the HER family [46]. However the most important predictive factor for the resistance to these drugs are somatic mutations in the KRAS oncogene [47-50]. These mutations imply a constitutive activation of the Ras/Raf/MAPK signaling pathway, which is independent of the EGFR activation by ligand binding. KRAS mutations are observed in ~ 38% of colorectal tumors [51,52]. Other molecular predictive factors seem to be correlated with anti-EGFR antibodies efficacy. They include somatic mutations of BRAF and PI3K catalytic, alpha polypeptide (PI3KCA) genes, the amplification of the EGFR gene and the impaired expression of the phosphatase and tensin homolog (PTEN) protein [53-57]. However these are still under evaluation. The European Medicines Agency has restricted the use of these antibodies in patients with wild-type KRAS tumors [58].

At first, cetuximab was studied for the treatment of patients with chemo-refractory advanced colorectal cancer. In particular cetuximab combined with irinotecan achieved a higher response rate than cetuximab alone in a randomized Phase II trial in patients resistant to irinotecan (22.9 versus 10.8%, $p = 0.007$) [45]. Median PFS and response rate were also improved by the same combination of cetuximab and irinotecan when compared with irinotecan alone (PFS: 4.0 versus 2.6 months, $p \leq 0.0001$; RR: 16.4 versus 4.2%, $p < 0.0001$), even though a non-significant difference was observed for the median OS (10.7 versus 10.0 months, $p = 0.71$), which was the primary endpoint of this study [59]. However this result may be a consequence of the crossover to cetuximab, which a large number of patients in the control arm experienced. Cetuximab showed benefit also when administered alone as compared with best supportive care in chemotherapy-refractory patients. In this study the median OS was 6.1 months for cetuximab and 4.6 months for best supportive care ($p = 0.005$) [60].

More recently an advantage for the use of cetuximab in first-line treatment was demonstrated. The addition of cetuximab to flinic acid, 5 FU and irinotecan (FOLFIRI) versus FOLFIRI alone in the Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (CRYSTAL) trial resulted in a modest but statistically significant increase in the median PFS (8.9 versus 8.0 months, $p = 0.048$) [61]. In the large randomized Phase II study for chemonaïve patients with metastatic colorectal cancer, they were treated with either FOLFOX plus cetuximab or FOLFOX alone. No statistically significant differences in median PFS

(7.2 months in both arms) and response rate (46 versus 36%, $p = 0.064$) were observed [62]. In any case, a significantly wider difference for RR was achieved in subjects with good performance status (49 versus 37%, $p = 0.032$).

A subgroup analysis of these two randomized trials according to KRAS status was performed [63,64]. Patients with wild-type KRAS obtained a better outcome when cetuximab was added to FOLFIRI or FOLFOX. The median PFS proved to be significantly different in the two treatment arms in KRAS-wild-type patients, but not in the KRAS-mutated ones. Moreover the combination of FOLFOX with cetuximab appeared harmful in the patients with KRAS mutations.

Cetuximab was also approved for the treatment of head and neck cancer, in combination with radiotherapy for treatment of locally advanced disease and as a single agent in patients with advanced platinum resistant disease.

The first application was prompted by the results of a Phase III randomized trial. A total of 424 patients were divided into two arms: radiation therapy alone versus supplementary treatment with cetuximab. At a median follow-up of 54 months, a doubling of the survival rate was observed in patients receiving cetuximab compared with patients receiving radiation therapy alone (49 versus 29 months; $p = 0.03$). Statistically significant increases in loco-regional control and PFS were also reported for the group receiving cetuximab. This is the first study to demonstrate a statistically significant survival benefit rate for patients treated with curative intent using an anti-EGFR antibody [65]. These findings led to the achievement of the combination of cetuximab with radiotherapy as an alternative to chemo-radiotherapy in unsuitable patients.

The pivotal role of cetuximab, alone or in combination with chemotherapy, was also studied in the treatment of recurrent or metastatic squamous-cell carcinoma of head and neck cancer. Addition of cetuximab to cisplatin significantly improved RR, although PFS and OS were not significantly improved [66]. Cetuximab plus platinum-fluorouracil chemotherapy was compared with the same chemotherapy regimen alone in the Erbitux in First-Line Treatment of Recurrent or Metastatic Head and Neck Cancer (EXTREME) study, a Phase III trial for patients with recurrent or metastatic squamous-cell carcinoma of the head and neck. The first-line treatment with combination improved overall survival (hazard ratio (HR) for death, 0.80; $p = 0.04$) and all the other endpoints [67].

A Phase III trial evaluated single-agent panitumumab versus best supportive care (BSC) in patients with chemorefractory (100 and 37% of the patients had received two and three lines of previous treatment, respectively) advanced colorectal cancer, which expressed EGFR regardless of the KRAS mutational status. Panitumumab reduced the risk of disease progression compared with BSC alone by almost half (HR = 0.54, 95% CI: 0.44 – 0.66, $p < 0.0001$) [68]. The group of patients bearing a KRAS mutation achieved no clinical responses when treated with panitumumab, whereas those with wild-type KRAS achieved a 17% ORR, as a result of

the treatment with panitumumab [47]. It is interesting to note that among patients in the control arm who crossed over to receive panitumumab after progression, RR was 11% with an additional 33% of patients achieving stable disease (SD) [69].

2.2 Agents in development

During the last year about ten monoclonal antibodies have been under evaluation in Phase III trials for the treatment of solid tumors. Among these, unmodified, conjugated and radiolabeled antibodies are included. They target various antigens: HER family receptors (pertuzumab, zalutumumab), IGF-1R (dalotuzumab, figitumumab), VEGF-R2 (ramucirumab), cytotoxic T lymphocyte antigen 4 (CTLA-4) (ipilimumab), etc. [70].

In this review we only report three antibodies under development for cancer treatment, which prove to be more interesting for this discussion.

2.2.1 Edrecolomab

Edrecolomab is a murine monoclonal antibody directed against the transmembrane glycoprotein epithelial cell adhesion molecule (EpCAM). This antigen is normally expressed on many human epithelia and overexpressed in many malignancies, including colorectal cancer.

Preclinical data have demonstrated its effect on antibody-dependent cell-mediated cytotoxicity. Early clinical data demonstrated antitumor activity, even in patients with advanced disease [71]. However this antibody was included in a small Phase III trial in patients with resected stage III colorectal cancer, before efficacy in patients with advanced disease was formally demonstrated [72].

The authors of this study concluded that edrecolomab yielded a significant improvement of relapse-free and overall survival, similar to that observed for FU plus leucovorin. Based on these findings, edrecolomab was approved for adjuvant therapy in colon cancer in Germany. Four large prospective randomized trials were prompted in patients with stage II and III colon cancer to confirm the results of this pivotal trial [73-76].

Two of these trials compared edrecolomab alone with no treatment in stage II disease and the others edrecolomab alone versus chemotherapy with 5-FU plus leucovorin or the combination of chemotherapy and edrecolomab in stage III colon cancer. These studies showed a lack of efficacy of edrecolomab in the adjuvant setting of colon cancer patients.

2.2.2 Pertuzumab

Pertuzumab is the first in a class of HER2 dimerization inhibitors. Binding to HER-2 inhibits its dimerization with other HER receptors and this is thought to result in slower tumor growth [77]. This antibody is under evaluation for the 'trastuzumab beyond progression' approach to extend as much as possible the efficacy of trastuzumab-based treatment.

A recent Phase II trial revealed that half the patients who had progressed on trastuzumab achieved a clear benefit from

the combination of trastuzumab with pertuzumab; RR of 24.2% (complete response rate, 7.6%; partial response rate, 16.7%) and a median PFS duration of 24 weeks [78]. The combination appeared to be well tolerated, and no patients were withdrawn as a result of toxicities.

Clinical Evaluation Of Pertuzumab And Trastuzumab (CLEOPATRA) is an ongoing Phase III clinical trial for the evaluation of trastuzumab plus chemotherapy with or without pertuzumab for untreated HER2-positive advanced breast cancer patients. It will provide important information about the efficacy and safety of adding pertuzumab to one current standard of care in patients with HER2-positive metastatic breast cancer [79].

Pertuzumab was evaluated in almost 400 patients with ovarian cancer who had been treated in three large Phase II studies. It showed the enhancement of gemcitabine activity in platinum-resistant ovarian cancer and of carboplatin activity in platinum-sensitive disease. Those patients whose cancer had activated HER2 or low HER3 mRNA expression appeared to benefit from pertuzumab [80].

2.2.3 Catumaxomab

Catumaxomab is a hybrid, trifunctional and bispecific monoclonal antibody. It combines two half antibodies of mouse anti-EpCAM IgG2a and rat anti-CD3 IgG2b. Catumaxomab is defined as bispecific because it can bind two different antigens and trifunctional because it is active through three different events. Preclinical studies have, in fact, shown that one antigen binding site recognizes the EpCAM on tumor cells, the other antigen binding site binds to CD3, a component of the T cell receptor complex, and the Fc-fragment binds to FcγR Type I and III-positive cells, including macrophages, dendritic cells and NK cells [81-83].

Catumaxomab has been studied for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive epithelial tumors, when standard therapy is not available or no longer feasible. Treatment consists of four constant-rate intraperitoneal infusions via intraperitoneal catheter at doses of 10, 20, 50 and 150 μg of catumaxomab on days 0, 3, 7 and 10 as proposed at the result of a Phase I/II trial [84]. This treatment was compared with paracentesis alone in a pivotal Phase II/III study [85]. Catumaxomab is able to prolong puncture-free survival in patients with malignant ascites requiring symptomatic therapeutic paracentesis. Side effects are explained by the cytokine release induced by the drug and are usually reversible. These commonly include fever, chills, nausea and vomiting.

3. Antibody fragments

The efficacy of treatments in solid tumors is different from that in hematological malignancies because of different anatomical and physiological properties. Solid tumors differ from normal tissue with regard to vasculature, interstitial fluid pressure, cell density, tissue structure and composition and

extracellular matrix components [86]. In fact, monoclonal antibodies prove to have difficulty in penetrating solid tumors. Experimentally the tumor penetration of antibodies is generally around 0.01% of the injected dose [87]. The aim of present-day research is the production of antibody-based molecules with optimized molecular size, valence, charge and affinity, able to overcome the barriers in solid tumors. The most relevant blocks of an antibody are Fab fragments (55 kDa) and single-chain Fv (scFv; 25 kDa). These could be used alone or as parts of larger protein constructs (Figure 1) [88,89].

Fab fragments, obtained by proteolytic digestion of IgG, include a single antibody light chain linked by a disulfide bond to a heavy chain fragment consisting of the variable region and the first heavy chain constant region. It represents a single binding site for the antigen. (Fab)₂ fragments retain the heavy chain hinge region and are bivalent.

Single-chain Fv (scFv) are single polypeptide chains incorporating a heavy chain variable and a light chain variable region, with linker polypeptidic antigens. scFv molecules can also be engineered to incorporate a carboxy-terminal cysteine residue, thereby enabling formation of a (scFv)₂ fragment by virtue of disulfide bridging.

A 'diabody' is a bivalent molecule. It is formed by two scFvs linked noncovalently. The variable regions of one heavy chain and the variable regions of the other light chains form two antigen-binding sites with extensive noncovalent interaction. Diabodies have rigid, compact structures, which allow a lower separation of the two binding sites. For this reason they prove to be ideal for bridging between cells [90].

A further possibility is a 'minibody' in which two scFv fragments are linked by a component of the heavy-chain region (for example CH3), resulting in a bivalent molecule [91].

In general, lower molecular weight constructs penetrate more quickly into tumors but have shorter and lower overall retention in tumor tissue. Increased valence generally increases tumor uptake and specificity.

4. Conclusions

Monoclonal antibodies represent one of the most important technological applications for the treatment of various diseases by the binding to specific molecules. The application of these agents for the therapy of malignancies exploits the possibility of arresting specific pathways of cancer biology, which are mediated by the targeted antigens. Another mode of action of monoclonal antibodies is their role as vectors for cytotoxic drugs or radioactive isotopes.

In solid tumors four monoclonal antibodies have already been approved for clinical use. These have provided valid tools for the improvement of survival and quality of life in the management of a great many types of cancer. Very often the benefit deriving from their delivery requires the combination with chemotherapy. For some of these monoclonal antibodies the indication is restricted to specific subgroups

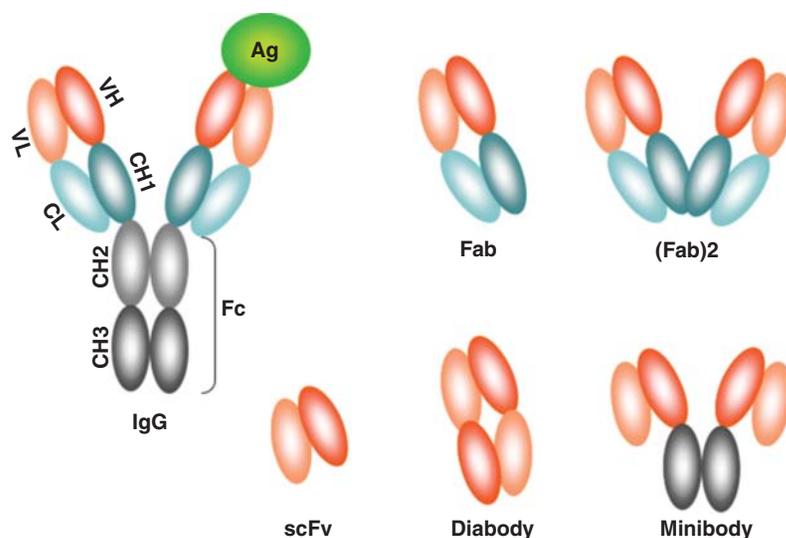


Figure 1. Antibody construct structures are related to IgG antibody structure. The different components of the molecules are distinguished by variation of the colours of each portion.

Ag: Antigen; CH1, CH2, CH3: Constant regions of heavy chain; CL: Constant region of light chain; Fab: Fragment-antigen binding; Fc: Fragment crystalline; VH: Variable region of heavy chain; VL: Variable region of light chain.

of patients, which present particular molecular patterns. For example, trastuzumab requires the overexpression of HER2 to be effective and the anti-EGFR antibodies show no efficacy in patients with KRAS or BRAF mutations.

The aim of the research in this field of drug development is to find new antibodies for other targets and to produce different antibody constructs. The production of chimeric and humanized monoclonal antibodies is an attempt to provide drugs with minimal adverse reactions. Antibody fragments might represent an interesting way to overcome the structural limits in solid tumor tissues for penetration by macromolecules such as conventional antibodies.

5. Expert opinion

The idea for the application of monoclonal antibodies in cancer treatment was originally based on the assumption that an antibody may bind to an antigen on the cell surface of tumor cells through its Fab and to the receptor for Fc on the immune cells. The consequence of this binding should be the activation of the antibody-dependent cell-mediated cytotoxicity (ADCC). The efficacy of these agents was initially explained through the elimination of cancer cells by ADCC.

An important number of both preclinical and clinical studies has led to the awareness that other mechanisms might also explain the antitumor activity of monoclonal antibodies. In fact, together with ADCC, these molecules might also block specific pathways mediated by a specific antigen. The targets for the tumor-directed antibodies are usually products of oncogenes; we might therefore suppose that a monoclonal antibody is really effective against cancer when it exploits

the phenomenon of oncogene addiction. The survival of cancer cells may be impaired by the inactivation of a single oncogene, even if they contain multiple genetic and epigenetic abnormalities. Monoclonal antibodies inhibit cancer cell growth if they bind to oncogenic proteins expressed on the cell surface or in the extracellular space. This finding reduced the role of monoclonal antibodies as immunotherapy and enhanced their role in target therapy. For example bevacizumab, which binds to soluble VEGF, should not prompt immunological phenomena against cancer cells, but this binding blocks the function of neoangiogenesis. Moreover, several preclinical studies have highlighted the fact that bevacizumab achieves its effect through the amelioration of tumor vasculature. This mechanism allows a better penetration of cytotoxic agents within the tumor tissue. It also explains why bevacizumab has no clinical effect when it is delivered without chemotherapy [92,93].

ADCC remains one of the modes of action for other monoclonal antibodies, including trastuzumab, cetuximab and panitumumab [94-96]. This consideration is based on both direct preclinical evidence and indirect clinical deductions. However one of the most relevant problems, resulting from the clinical research performed on monoclonal antibodies, is the difficulty in identifying those which are truly active in early phase trials such as those involving single-agent treatments. This is a problem regarding the use of targeted drugs compared with cytotoxic agents. The latter are usually studied in Phase III trials if they show antitumor activity, evaluated through tumor shrinkage as observed by imaging techniques. This method may not be valid for monoclonal antibodies and other targeted drugs. For this reason,

widespread efforts are required to investigate the biological effect of these agents through appropriate preclinical models, in order to understand if their efficacy might be achieved by single agents or by combined treatments.

The research also involves a further problem. Several drugs prove to be ineffective in an unselected population of patients. The identification of predictive factors for sensitivity/resistance to these agents will help clinicians to avoid useless treatment in those patients who would not respond because of particular clinical or molecular characteristics. The clinical application of these factors would lead to a more economical use of public resources and help to spare patients from certain side effects.

In this paper we have chosen to report as examples the development of three new monoclonal antibodies. The choice is based on their peculiarity.

Edrecolomab, an anti-EpCAM antibody, was studied for a considerable time in the adjuvant setting of colorectal cancer patients just after it was approved for use in Germany about ten years ago. This approval was obtained on the basis of the positive results deriving from a small clinical trial [72]. The unsuitability of that approval is now clear, but it should lead to reflection regarding the need for extended clinical evaluation before the inclusion of a drug in clinical practice.

Pertuzumab has been studied up till now to extend the efficacy of trastuzumab beyond the tumor progression developed during a trastuzumab-containing treatment. Undoubtedly trastuzumab is a hinge of breast cancer therapy. It is, however, inconceivable that a great deal of money should be spent in order to develop an antibody that might merely help another

antibody to extend its own efficacy. It would be more reasonable to search for new active monoclonal antibodies.

Catumaxomab is interesting because it is bivalent and trifunctional. It may represent the first of a series of monoclonal antibodies with these characteristics. However, its efficacy has been demonstrated for loco-regional treatment only. An evaluation of its real usefulness should be made when it becomes included in clinical practice.

The production of antibody fragments is a valid option for developing new drugs. The pharmacokinetics and pharmacodynamics of these agents should be carefully investigated, since their molecular structure cannot be compared with those of conventional monoclonal antibodies. Their typical penetration into tumor tissue, together with their whole-body distribution and systemic clearance might limit the clinical application of these antibody constructs.

Finally, in our opinion, it is essential to align the development of new monoclonal antibodies and constructs with research involving the biological processes of tumor growth and the mechanisms of acquired resistance induced by various treatments.

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G Di Fede & G Bronte contributed equally to this work.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (●) or of considerable interest (●●) to readers.

1. Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. *Nat Biotechnol* 2005;23:1147-57
- **A relevant review of the most useful clinical applications for monoclonal antibodies in cancer therapy.**
2. Schaedel O, Reiter Y. Antibodies and their fragments as anti-cancer agents. *Curr Pharm Des* 2006;12:363-78
3. General policies for monoclonal antibodies. Geneva, Switzerland: World Health Organization (WHO) 2009. Available from: <http://www.who.int/medicines/services/inn/Generalpoliciesformonoclonalantibodies2009.pdf> [Last accessed 4 June 2011]
4. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495-7
5. Milstein C. With the benefit of hindsight. *Immunol Today* 2000;21:359-64
6. Carter P. Improving the efficacy of antibody-based cancer therapies. *Nat Rev* 2001;1:118-29
7. Hudson PJ, Souriau C. Engineered antibodies. *Nat Med* 2003;9:129-34
8. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;285:1182-6
9. Kaelin WG Jr. The von Hippel-Lindau protein, HIF hydroxylation, and oxygen sensing. *Biochem Biophys Res Commun* 2005;338:627-38
10. Ferrara N, Chen H, Davis-Smyth T, et al. Vascular endothelial growth factor is essential for corpus luteum angiogenesis. *Nat Med* 1998;4:336-40
11. Bloch W, Huggel K, Sasaki T, et al. The angiogenesis inhibitor endostatin impairs blood vessel maturation during wound healing. *FASEB J* 2000;14:2373-6
12. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003;9:669-76
13. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov* 2004;3:391-400
14. Presta LG, Chen H, O'Connor SJ, et al. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res* 1997;57:4593-9
15. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004;10:145-7
16. Mancuso MR, Davis R, Norberg SM, et al. Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J Clin Invest* 2006;116:2610-21
17. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335-42
- **The first Phase III trial, to our knowledge, which showed the advantage of bevacizumab addition to chemotherapy regimens for colon cancer.**
18. Giantonio BJ, Catalano PJ, Meropol NJ, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* 2007;25:1539-44
19. Kozloff M, Hainswrth J, Badarinarath S, et al. Efficacy of bevacizumab plus chemotherapy as first-line treatment of patients with metastatic colorectal cancer: updated results from a large observational registry in the US (BRiTE) [abstract 3537]. *J Clin Oncol* 2006;2006:155
20. Van Cutsem E, Rivera F, Berry S, et al. Safety and efficacy of first-line bevacizumab with FOLFOX, XELOX, FOLIRI and fluoropyrimidines in metastatic colorectal cancer: the BEAT study. *Ann Oncol* 2009;20:1842-7
21. Saif MW, Elfiky A, Salem RR. Gastrointestinal perforation due to bevacizumab in colorectal cancer. *Ann Surg Oncol* 2007;14:1860-9
22. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006;355:2542-50
- **One of the clinical studies to demonstrate the efficacy of bevacizumab in combination with chemotherapy for lung cancer treatment.**
23. Reck M, von Pawel J, Zatlouk P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAIL. *J Clin Oncol* 2009;27:1227-34
24. Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. *Nat Med* 2006;12:895-904
25. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 2007;357:2666-76
26. Miles DW, Chan A, Dirix LY, et al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol* 2010;28:3239-47
27. Robert NJ, Dieras V, Glaspy J. RIBBON-1: randomized, double-blind, placebo-controlled, Phase III trial of chemotherapy with or without bevacizumab (B) for first-line treatment of HER2-negative locally recurrent or metastatic breast cancer (MBC). *J Clin Oncol* 2009;abstract 1005
28. Stark Vance V. Bevacizumab and CPT-11 in the treatment of relapsed malignant glioma. World Federation of Neuro-Oncology Second Quadrennial Meeting and the Sixth Meeting of the European Association for Neuro-Oncology; 5 – 8 May 2005; Neuro-Oncology, Edinburgh; United Kingdom; 2005. p. 369
29. Van Meir EG, Hadjipanayis CG, Norden AD, et al. Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. *CA Cancer J Clin* 2010;60:166-93
30. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol* 2009;27:4733-40
31. Kreisl TN, Kim L, Moore K, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J Clin Oncol* 2009;27:740-5

Expert Opin. Biol. Ther. Downloaded from informahealthcare.com by Universita Delgli Studi Di Palermo on 06/12/11 For personal use only.

32. Escudier B, Pluzanska A, Koralewski P, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet* 2007;370:2103-11
33. Musolino A, Naldi N, Bortesi B, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol* 2008;26:1789-96
34. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783-92
- **A consistent demonstration of trastuzumab efficacy in metastatic breast cancer patients that overexpress HER2.**
35. Marty M, Cognetti F, Maraninchi D, et al. Randomized Phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* 2005;23:4265-74
36. Nahta R, Yu D, Hung MC, et al. Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. *Nat Clin Pract* 2006;3:269-80
37. Montemurro F, Redana S, Viale G, et al. Retrospective evaluation of clinical outcomes in patients with HER2-positive advanced breast cancer progressing on trastuzumab-based therapy in the pre-lapatinib era. *Clin Breast Cancer* 2008;8:436-42
38. Pirvulescu C, Uhlig M, von Minckwitz G. Trastuzumab improves the efficacy of chemotherapy in breast cancer treatment beyond progression. *Breast Care (Basel, Switzerland)* 2008;3:364-5
39. Guarneri V, Lenihan DJ, Valero V, et al. Long-term cardiac tolerability of trastuzumab in metastatic breast cancer: the M.D. Anderson Cancer Center experience. *J Clin Oncol* 2006;24:4107-15
40. Rayson D, Richel D, Chia S, et al. Anthracycline-trastuzumab regimens for HER2/neu-overexpressing breast cancer: current experience and future strategies. *Ann Oncol* 2008;19:1530-9
41. Slamon DJ, Mackey J, Robert N, et al. Role of anthracycline-based therapy in the adjuvant treatment of breast cancer: Efficacy analyses determined by molecular subtypes of the disease [abstract 13]. San Antonio Breast Cancer Symposium; 2007. *Br Cancer Res Treat* 2007;106:112
42. Sledge G, O' Neill A, Thor A, et al. Adjuvant trastuzumab: Long term results of E2198. San Antonio Breast Cancer Symposium; 2006. *Br Cancer Res Treat* 2006;95(Suppl 1): abstract 2075
43. Joensuu H, Kellokumpu-Lehtinen PL, et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N Engl J Med* 2006;354:809-20
44. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol* 2005;23:1803-10
45. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;351:337-45
- **Efficacy demonstration for a Cetuximab-based regimen in metastatic colorectal cancer patients.**
46. Vitoria-Petit AM, Kerbel RS. Acquired resistance to EGFR inhibitors: mechanisms and prevention strategies. *Int J Radiat Oncol Biol Phys* 2004;58:914-26
47. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;26:1626-34
48. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008;19:508-15
49. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. *Br J Cancer* 2007;96:1166-9
50. Lievre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008;26:374-9
51. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst* 1998;90:675-84
52. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res* 2007;67:2643-8
53. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008;26:5705-12
54. Moroni M, Veronese S, Benvenuti S, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol* 2005;6:279-86
55. Sartore-Bianchi A, Moroni M, Veronese S, et al. Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. *J Clin Oncol* 2007;25:3238-45
56. Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res* 2009;69:1851-7
57. Loupakis F, Pollina L, Stasi I, et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. *J Clin Oncol* 2009;27:2622-269
58. Committee for Medicinal Products for Human Use Post-authorisation Summary of Positive Opinion for Erbitux. London, UK: European Medicines Agency, 2008. Available from: http://www.emea.europa.eu/pdfs/human/opinion/Erbitux_28040208en.pdf [Last accessed 5 June 2011]
59. Sobrero AF, Maurel J, Fehrenbacher L, et al. EPIC: Phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with

Monoclonal antibodies and antibody fragments

- metastatic colorectal cancer. *J Clin Oncol* 2008;26:2311-19
60. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007;357:2040-8
61. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;360:1408-17
62. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009;27:663-71
63. Van Cutsem E, Rougier P, Kohne C, et al. A meta-analysis of the CRYSTAL and OPUS studies combining cetuximab with chemotherapy (CT) as 1st-line treatment for patients (pts) with metastatic colorectal cancer (mCRC): results according to KRAS and BRAF mutation status. The joint ECCO 15-34th ESMO Multidisciplinary Congress September 20-24, 2009; Berlin [abstract P-6077]. *Eur J Cancer Suppl* 2009;7:345
64. Bokemeyer C, Bondarenko I, Hartmann JT, et al. Overall survival of patients with KRAS wild-type tumours treated with FOLFOX4 ± cetuximab as 1st-line treatment for metastatic colorectal cancer: the OPUS study. The joint ECCO 15-34th ESMO Multidisciplinary Congress September 20-24, 2009; Berlin [abstract 6079]. *Eur J Cancer* 7(Suppl):S346
65. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006;354:567-78
66. Burtneß B, Goldwasser MA, Flood W, et al. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an Eastern Cooperative Oncology Group study. *J Clin Oncol* 2005;23:8646-54
67. Vermorken JB, Mesia R, Rivera F, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med* 2008;359:1116-27
68. Van Cutsem E, Peeters M, Siena S, et al. Open-label Phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol* 2007;25:1658-64
69. Van Cutsem E, Siena S, Humblet Y, et al. An open-label, single-arm study assessing safety and efficacy of panitumumab in patients with metastatic colorectal cancer refractory to standard chemotherapy. *Ann Oncol* 2008;19:92-8
70. Reichert JM. Antibodies to watch in 2010. *mAbs* 2:84-100
71. Herlyn D, Wettendorff M, Schmolle E, et al. Anti-idiotypic immunization of cancer patients: modulation of the immune response. *Proc Natl Acad Sci USA* 1987;84:8055-9
72. Riethmuller G, Schneider-Gadicke E, Schlimok G, et al. Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. German Cancer Aid 17-1A Study Group. *Lancet* 1994;343:1177-83
73. Hartung G, Hofheinz RD, Dencausse Y, et al. Adjuvant therapy with edrecolomab versus observation in stage II colon cancer: a multicenter randomized Phase III study. *Onkologie* 2005;28:347-50
74. Colacchio TA, Niedzwiecki D, Compton C, et al. Phase III trial of adjuvant immunotherapy with MOAb 17-1A following resection for stage II adenocarcinoma of the colon (CALGB 9581). ASCO Annual Meeting; 2004: 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). *J Clin Oncol* 2004;22(15 Suppl):3522
75. Punt CJ, Nagy A, Douillard JY, et al. Edrecolomab alone or in combination with fluorouracil and folinic acid in the adjuvant treatment of stage III colon cancer: a randomised study. *Lancet* 2002;360:671-7
76. Fields AL, Keller A, Schwartzberg L, et al. Adjuvant therapy with the monoclonal antibody Edrecolomab plus fluorouracil-based therapy does not improve overall survival of patients with stage III colon cancer. *J Clin Oncol* 2009;27:1941-7
77. Gelmon KA, Fumoleau P, Verma S, et al. Results of a phase II trial of trastuzumab (H) and pertuzumab (P) in patients (pts) with HER2-positive metastatic breast cancer (MBC) who had progressed during trastuzumab therapy. ASCO Annual Meeting; 2008 May 30-June 3; Chicago. *J Clin Oncol* 2008;26(20 Suppl):abstract 1026
78. Baselga J, Imadlou K, Paton V, et al. Efficacy, safety and tolerability of dual monoclonal antibody therapy with pertuzumab + trastuzumab in HER2+ metastatic breast cancer patients previously treated with trastuzumab. CTRC-AACR San Antonio Breast Cancer Symposium; 2008.San Antonio, Texas: Cancer Research 2008. Abstract 3138. Available at: <http://www.posters2view.com/sabcs08/viewp.php?nu=3138>
79. Baselga J, Swain SM. CLEOPATRA: a Phase III evaluation of pertuzumab and trastuzumab for HER2-positive metastatic breast cancer. *Clin Breast Cancer* 2010;10:489-91
80. Langdon SP, Faratian D, Nagumo Y, et al. Pertuzumab for the treatment of ovarian cancer. *Expert Opin Biol Ther* 2010;10:1113-20
81. Schmitt M, Schmitt A, Reinhardt P, et al. Opsonization with a trifunctional bispecific (alphaCD3 × alphaEpCAM) antibody results in efficient lysis in vitro and in vivo of EpCAM positive tumor cells by cytotoxic T lymphocytes. *Int J Oncol* 2004;25:841-8
82. Ruf P, Gires O, Jager M, et al. Characterisation of the new EpCAM-specific antibody HO-3: implications for trifunctional antibody immunotherapy of cancer. *Br J Cancer* 2007;97:315-21
83. Zeidler R, Mysliwicz J, Csanady M, et al. The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumour cells. *Br J Cancer* 2000;83:261-6
84. Burges A, Wimberger P, Kumper C, et al. Effective relief of malignant ascites in patients with advanced ovarian cancer by a trifunctional anti-EpCAM × anti-CD3 antibody: a Phase I/II study. *Clin Cancer Res* 2007;13:3899-905
85. Heiss MM, Murawa P, Koralewski P, et al. The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: Results of a prospective randomized phase II/III trial. *Int J Cancer* 2010;127:2209-21
86. Jang SH, Wientjes MG, Lu D, Au JL. Drug delivery and transport to solid tumors. *Pharm Res* 2003;20:1337-50

87. Adams GP, Schier R, McCall AM, et al. Prolonged in vivo tumour retention of a human diabody targeting the extracellular domain of human HER2/neu. *Br J Cancer* 1998;77:1405-12
88. Albrecht H, Burke PA, Natarajan A, et al. Production of soluble ScFvs with C-terminal-free thiol for site-specific conjugation or stable dimeric ScFvs on demand. *Bioconjug Chem* 2004;15:16-26
89. Kim SJ, Park Y, Hong HJ. Antibody engineering for the development of therapeutic antibodies. *Mol Cells* 2005;20:17-29
90. Perisic O, Webb PA, Holliger P, et al. Crystal structure of a diabody, a bivalent antibody fragment. *Structure* 1994;2:1217-26
91. Hu S, Shively L, Raubitschek A, et al. Minibody: a novel engineered anti-carcinoembryonic antigen antibody fragment (single-chain Fv-CH3) which exhibits rapid, high-level targeting of xenografts. *Cancer Res* 1996;56:3055-61
92. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med* 2001;7:987-9
- **An explanation of the biological effect of bevacizumab, which clarifies also its clinical applicability.**
93. Inai T, Mancuso M, Hashizume H, et al. Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. *Am J Pathol* 2004;165:35-52
94. Sliwkowski MX, LoSance JA, Lewis GD, et al. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin Oncol* 1999;26(4 Suppl 12):60-70
95. Kimura H, Sakai K, Arao T, et al. Antibody-dependent cellular cytotoxicity of cetuximab against tumor cells with wild-type or mutant epidermal growth factor receptor. *Cancer Sci* 2007;98:1275-80
96. Schneider-Merck T, Lammerts van Bueren JJ, Berger S, et al. Human IgG2 antibodies against epidermal growth factor receptor effectively trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG1, only by cells of myeloid lineage. *J Immunol* 2010;184:512-20

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