Current Topics in Pharmacology
Vol. 16, 2012

Review

Current status of recombinant antibodies in cancer therapy

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ABSTRACT

Antibodies have come a long way from those first isolated by hybridoma over 30 years ago to modern engineered fragments, constructed by rational design. The use of antibodies in cancer therapy is increasing rapidly, with 11 antibodies approved over the past decade and more than 500 ongoing clinical trials involving monoclonal antibodies. The combination of the antibody's inherent characteristics with the growing pool of tumour-specific antigens has generated a wide array of antibody-derived tools that are specifically designed to suppress and eliminate cancer cells. This review will examine some of the many novel antibodies and antibody-based approaches that are currently being developed for clinical application as the new generation of anti-cancer agent therapies.

KEYWORDS: antibodies, antibody engineering, bi-specific antibodies, cancer immunotherapy, immuno-conjugates, immunotoxins, recombinant antibodies

INTRODUCTION

The last decade has seen a burgeoning use of antibodies (Abs) in cancer therapy. The inherent characteristics of these molecules - high specificity and affinity, low molecular weight and high clearance rate - are harnessed for the development of new therapeutic agents that are tailored for a specific subset of patients, according to the molecular phenotype of the malignancy, a concept which has come to be regarded as 'personalized medicine'. To date, 11 antibodies are approved for the treatment of cancer by the FDA, and two are pending approval after conclusion of Phase III trials (Catumaxomab, Pertuzumab). These include Abs targeting epitopes of haematological as well as solid cancers. In addition, a multitude of Ab-fragments or derivatives are in various stages of development: of 495 in Phase III clinical trials with monoclonal antibodies (mAbs), 259 (52.3%) are intended for cancer therapy (from search results for 'monoclonal antibody', 'interventional studies', 'Phase III' on 21 September 2011).

Antibodies consist of three distinct domains: two antigen (Ag)-binding domains (Fab) and a single constant fragment (Fc). Epitope selectivity is maintained through the Fab fragments, where three complementary determining regions (CDRs) - which are hyper variable segments - are located. The Fc portion is responsible for the elimination of the Ag by several mechanisms: antibody-dependent cell cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). ADCC is mediated through the binding of the Fc to Fc receptors, particularly FcγRIII, expressed by NK, DC, macrophages and mast cells. Binding the Ag-bound Ab to the Fc receptor (FcR) activates the immune cells and promotes target-cell lysis. CDC is mediated by the binding of the Fc to C1q protein and onset of subsequent protein interactions leading to the generation of membrane attack complexes (MACs) on the target cell's membrane, and to its lysis. These mechanisms were shown to govern the activity mediated by a number of FDA approved Abs (rituximab, alemtuzumab, ofatumumab, and trastuzumab) [1-3].

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Target antigens

The application of antibodies in the clinic is dependent upon the specific binding of these molecules to their target epitope, and the selectivity of this binding to the desired cells, and to these cells alone. This selectivity is achieved by the isolation and generation of antibodies and their derivatives towards Ags whose expression is localized to tumour cells. These tumour-associated Ags (TAA) can be divided into three groups, based on their expression profiles:

1. Ags derived from differentiation Ags expressed in a specific stage in the cell's life, such as the cancer-testis family of epitope, including, among others, NY-ESO-1 and the MAGE-1. Other attractive epitopes are those specific to a certain cell lineage, such as the melanoma-specific gp100, MART-1, and Tyrosinase Ags, which are uniquely expressed in melanocytes.
2. Epitopes derived of mutated intracellular proteins, such as β-catenin in colon cancer.
3. Ags derived from gene overexpression/ amplification (e.g., HER-2/neu).

Over-expressed antigens

Over expressed molecules in cancer cells include several distinct families: the EGFR family, VEGF molecules, and distinct upregulated molecules, such as the carcino-embryonic Ag (CEA) and the epithelial cell adhesion molecules (EpCAM).

Growth factors and their trans-membranal receptor tyrosine kinases (RTKs) play an important role in cell proliferation, survival, migration and differentiation, and thus have an active role in the development of tumours. The EGFR family, which includes EGFR, HER2/neu, HER3 (ERBB3) and HER4, is overexpressed, dysregulated or mutated in many malignancies: lung, brain, kidney, breast, colorectal and ovarian cancers, for instance. Cetuximab (Erbitux) and Panitumumab (vectibix) are two Abs approved by the FDA which target EGFR and share certain traits. Both act as antagonists of the receptor, and in addition prevent dimerization and RTK activation. In addition, Cetuximab also facilitates ADCC. Two other Abs targeting EGFR are currently undergoing clinical evaluation: Necitumumab (fully human IgG1 Ab, ImClone) is currently in Phase III trial with patients with non-squamous or squamous non-small cell lung carcinoma (NSCLC). Zalutumumab (human IgG1, Genmab) is an anti-EGFR Abbeing tested in Phase III trials against head and neck squamous cell carcinoma. Although overall survival was not significantly prolonged in Zalutumumab-treated patients, progression-free survival favoured this treatment [4].

HER-2 (human EGF receptor 2) is overexpressed in 25-30% of invasive breast cancers and is associated with poor disease-free survival. Herceptin (Trastuzumab), the FDA-approved humanized IgG1 mAb, recognizes an epitope on the extracellular domain of HER-2 and is used for the treatment of metastatic breast cancer. It induces receptor-internalization and apoptosis. Herceptine has showed a 15-35% response rate as a sole agent [5]. Pertuzumab, another antibody targeting HER-2, acts in a similar manner to Trastuzumab by targeting a different epitope upon the HER-2 molecules. In recent Phase III trials in metastatic breast cancer patients, Pertuzumab was tested, in combination with Trastuzumab and Docetaxel, on previously untreated patients. Results showed an increase of six months in median-free survival in patients treated with Herceptine, Pertuzumab and chemotherapy without additive toxicity, compared to the control group [6]. MM-111, a bi-specific Ab targeting HER2xHER3, and Trastuzumab-DM1, a drug-conjugated form of Herceptine, both target HER-2 and will be discussed later.

Angiogenesis is a physiological process in embryogenesis and development. This process is a hallmark of almost all solid tumour development. In tumour cell growth, the production of pro-angiogenic compounds, such as vascular endothelial growth factor (VEGF), angiopoietins, placental growth factor, basic fibroblast growth factor and various cytokines, is upregulated [7]. These tumour vessels display aberrant growth patterns, and are usually irregular, heterogeneous and leaky. By targeting the vascular compartment with specific angiogenesis inhibitors, tumour growth can be inhibited and sometimes tumour regression can be achieved [8]. To date, Bevacizumab (Avastin) is the only anti-angiogenic mAb approved by the FDA for the treatment of breast, colorectal and

NSCLC in combination with chemotherapy. There is, however, another molecule, Ramucirumab (Imclone), which is currently in use in advanced clinical trials. This human IgG1 mAb targets VEGFR2 and is being used to treat patients with gastric, hepatocellular, breast, colorectal and NSCL carcinomas.

Carcinoembryonic antigen (CEA) is a tumour-associated glycoprotein normally expressed on the luminal surface of the gut. However, it is overexpressed in a number of epithelial-derived tumours, including colorectal carcinoma, ovarian carcinoma and lung adenocarcinoma. Overexpression of this Ag is accompanied by its shedding from the membrane into the circulation. CEA, which has been targeted by mAbs in several clinical trials in a variety of applications, including radioimmunotherapy (RIT), ADEPT and radioimmuno-guided surgery, will be discussed further under the subsections.

Differentiation antigens

Differentiation Ags that are expressed in a restricted, well-defined manner in a specific cell lineage, but not on other tissues of a different histological origin, are promising candidates for targeted therapy. The most widely studied examples of differentiation Ags currently being used for targeted therapy are expressed by haematopoietic malignancies, including CD19, CD20, CD22 and CD30 on B-cell lymphomas; CD33, CD38, CD45 and CD64 on acute myeloid leukemias (AML); and the upregulated IL-2 receptor on T-cell leukemias [8-10].

T-cell receptor-like Abs

MHC class I plays a central role in the immune response against a variety of cells that have undergone malignant transformation. Endogenous proteins are processed and loaded on MHC-I molecules, which then migrate to the membrane. This presentation allows immune cells such as CD8 T-cells to survey the intracellular state of protein expression. Malignant transformation of the cell (as well as viral infection) is manifested in a shift in the normal expression pattern of intracellular proteins, and this shift is made detectable by the change in the pattern of peptides presented. These MHC-peptide complexes can be targeted by recombinant T-cell receptors (TCRs). However, the affinities of most TCRs produced until now are too low to enable detection of target structures under normal assay conditions. In addition, most of the recombinant TCRs that have been generated, mainly in a single-chain version, have been found to have limited stability [11-12].

In light of this, the new generation of Abs, which possess both the unique specificity of T-cells and the high affinity of an Ab, have great therapeutic potential. These soluble antibodies can be produced in large quantities, penetrate tumours better and exert biological activity that is similar to T-cells. They are also more efficient and less difficult to use in a therapeutic modality. TCR-like Abs have many advantages: they combine the features of both the cellular and humoral arms of the immune system; as antibodies, they can be produced as soluble, high-affinity, high-specificity molecules; and they have the ability of T-cells to inspect the intracellular state of cells, and help to detect and eliminate virus-compromised or transformed cells.

TCR-like Abs have been used in research to study the nature of Ag presentation. The availability of a selection of TCR-like Abs targeting various melanoma epitopes have revealed that presentation of epitopes on cancer cells is not uniform. In one study, Michaeli *et al.* have found that, while MART-1 and gp100-derived peptides are expressed in moderate numbers on melanoma cells obtained from patients, HLA-A2/tyrosinase-$_{369\text{-}277}$ complexes were presented in approximately 4000 complexes/ cell. This high presentation might be the root of the difficulty in detecting tyrosinase-specific T-cell clones in patients, while MART-1 and gp100 are among the more immunodominant epitopes [13]. Thus, TCR-like Abs serve as an important epitope selection tool for the clinic.

Similar to mAbs that target specific tumour Ag, TCR-like antibodies are being used to mediate ADCC and CDC. In xenograft *in-vivo* models, Weidanz and colleagues generated a mouse IgG2a mAb RL4B specific to human chorionic gonadotropin (hCG)-β presented by HLA-A2 [14] and demonstrated that this mAb prevented and suppressed breast tumour growth. Another use of TCR-like Abs is in the generation of immunotoxins: in our lab, a TCR-like Ab targeting the melanoma differentiation antigen gp100, as

well as MART-1 and TRAP, was linked to a truncated Pseudomonas exotoxin (PE) molecule. These immunotoxins exhibited cytotoxic activity in an Ag-specific and HLA-A2-restricted manner, and were found to prevent specific tumour growth at nontoxic doses using xenograft models [15-17].

TCR-like antibodies may be used in therapies and in diagnosis of HLA-matched patients. When tumour biopsies are available, the tumour Ag presentation hierarchy of a certain patient can be evaluated using TCR-like Abs, leading to personalized treatment. The availability of TCR-like antibodies directed to several epitopes associated with a certain tumour type raises the possibility of a cocktail-like treatment composed of antibodies recognizing different Ags. However, further development of this approach needs to be tested experimentally.

Bi-specific antibodies

As suggested by their name, bi-specific antibodies (BsAbs) combine two Ab-targeting moieties specific to different epitopes. BsAbs have been developed over the past two decades, but only in the last five years has this class of Abs really taken off, with three antibodies currently in Phase III clinical trials pending approval. BsAbs have several advantages over mAbs: the ability to physically attract effector cells to the target (cell retargeting, see BiTE), thereby causing a cytotoxic effect and killing off target cells; secondly, the development, characterization, efficacy and safety testing of a single antibody are significantly less costly than the validation of two or even three separate Abs. To date a single BsAb has been approved by the European Union (catumaxomab, 2009). Three BsAbs are in advanced Phase II clinical trials: FBTA05 (Trion/Fresenius's), which targets CD20 on lymphoma cells and CD3 on T-cells as B-cell lymphoma treatment; blinatumomab (Micromet) for acute lymphoblastic leukaemia; and MM-111 (Merimack), which targets HER-2 and HER-3.

Initially, BsAbs were generated using hybridoma technology [18]. In this method, two hybridoma cells which generate Abs with different specificities are fused, yielding a hybrid-hybridoma cell [19-20]. This quadroma generates antibodies by random pairing of the heavy and light chains contributed by each of the parent-hybridoma cells. This method suffers from several limitations; only a tenth of the molecules are bi-specific, and sequential purifications of these Abs are needed in order to obtain homogenous preparation. In addition, immunogenicity arising from rat or mouse IgG molecules may inhibit repeated administrations of the BsAb. Despite its limitations, several Abs generated in this way have gone to clinical trial with one being approved [21]. Other methods of BsAbs generation include chemical crosslinking of either whole IgG molecules or Fab fragments [22-24]. The aforementioned limitations were solved by the production of recombinant BsAbs. The genetic engineering of BsAbs generated several types of such molecules, with two main forms: tandem single-chain Fv (TaScFv) and diabodies. These formats are the result of the recombination of the antibody's variable domain. TaScFv are two ScFv molecules connected by a linker [25-28]. Another example is MM-111, which is the molecule produced from two ScFv Ab fragments, targeting ErbB2 and ErbB3, linked to human serum albumin by a polypeptide linker [29]. This molecule is currently under Phase I trials in patients with HER-2 positive cancer. In contrast to TaScFv, which are monomeric molecules, diabodies are heterodimeric molecules joined together by a linker too short to allow assembly of the variable domains located on the same chain, thus favouring an inter-chain binding [30].

Bispecific T-cell engager molecules (BiTEs) represent a class of bispecific single-chain antibodies that enable polyclonal activation, redirection of cytotoxic T-cells against target cells and formation of lytic immunological synapse. One of the ScFv molecules targets the tumour surface expression marker, and the other targets either the CD3 molecule (component of T-cell receptor complex in T-cells), or CD16 (surface marker of NK cells). BiTEs achieve activation of T-cells at very low doses and effector-target ratios, without the conventional requirements of MHC-I ligation to the TCR [31-33]. Epitope distance to the target cell membrane and Ag size were identified as factors that determine the BiTE efficiency [34]. BiTE Abs are currently being tested for a solid tumour (EGFR, Her2, CEA, EpCAM, EphA2, MCSP) as well as for leukemic

epitopes (CD19, CD33) [35-40]. The use of BsAbs harbors potential activation of regulatory T-cells and the attenuation of the effector response [41].

To date, several BiTE Abs are in ongoing clinical trials. MT110 (CD3xEpCAM), has been shown to target primary colorectal cancer stem cells and inhibit tumour formation in cell lines [42], as well as in freshly isolated samples [43]. Blinatumomab (MT103, CD3xCD19) has shown favourable indication in treatment of B-cell non-Hodgkin's lymphoma and B-ALL in Phase I studies, with a total of 11 major responses (4 complete responses (CR) and 7 partial regressions (PR)) [44-45]. In a Phase II study in ALL patients with persistent minimal residual disease (MRD) after CR, an 80% response rate was achieved [46]. In a separate study in three children with B-precursor ALL and recurrence after an allo-matched unrelated donor, CR was achieved. Currently Phase II studies are in progress in patients with B-ALL and NHL. FBTA05 (anti-CD20×anti-CD3) has shown to induce higher cytotoxic reactions than Rituximab and Alemtuzumab *in vitro* [47-48], and is currently in Phase II trials in patients with B-cell lymphoma. Catumaxomab, a mouse/rat-derived, trifunctional Ab, targets the epithelial cell-adhesion molecule (EpCAM) on tumour cells and CD3 on T-cells [49-50]. This Ab activates T-cell lysis of target cells in addition to ADCC mediated through its Fc region. This BsAb is approved by the EU, and recently concluded a Phase III trial in patients with malignant ascites due to epithelial cancers.

In addition to their functional advantages, the small size of BsAb molecules (~60 kD) enables them to penetrate solid tumours, thus reducing the recommended treatment dosage up to 15,000 times that of monoclonal Abs [44]. It also, however, promotes their rapid clearance from the serum. Current clinical protocols use long-term intravenous injections to maintain effective dosage in the bloodstream [51].

Antibody conjugates and fusions

Apart from using Abs in their native or fragmented form, where antibodies may produce anti-cancer effects via a variety of mechanisms such as ADCC and CDC, apoptosis via blockage of signals and neutralization of circulating ligands, Abs are also modified into creating what was envisioned a century ago by Paul Ehrlich as 'magic bullets'. These modifications, whether through genetic alterations or chemical conjugation, are harnessing Ab properties such as specificity, favourable pharmacokinetics, selectivity, biodistribution and the functional activity of the Ab to a potent drug or toxin molecule. Abs are conjugated to toxins, drugs, HLA molecules, radioisotopes and prodrugs, to name just a few, and are used in anti-cancer therapy as well as in imaging. To date, three immuno-conjugates have been approved by the FDA - Tositumomab-131I (Bexxar), Ibritumomab tiuxetan (Zevalin), Brentuximab vedotin (Adcetris) - and another two are at advanced stages of clinical trialing - Naptumomab estafenatox, and Trastuzumab emtansine.

Ab cytokine/chemokine conjugates (Immunocytokines)

Studies have shown that the tumour's inability to appropriately activate the immune system, rather than the absence of tumour Ags, is the reason for impaired immune responses to tumours. Thus, immunotherapy evolved rapidly as a concomitant treatment with chemotherapy and radiotherapy, improving anti-tumour responses. However, systematic administration of these factors caused severe adverse effects (AE) due to the necessity to concentrate cytokines locally within the tumour area [52-53]. Moreover, advanced neoplasms are often dispersed and inaccessible to localized intratumour cytokine injections. The conjugation of cytokines to Ab/Ab fragments targeting tumour epitopes solves this problem in patients with advanced stages. These immunocytokine conjugates exhibit rapid clearance rates (2-3 hours) which enables the administration of highly effective cytokine concentration without AE.

Several cytokines are used in this fusion method, such as GM-CSF, IL-12 and TNFα, with the most important cytokine being IL-2. IL-2 is a proinflammatory cytokine, which upon binding to its receptor on T-cells, stimulates the growth, differentiation, and survival of Ag-selected T-cells via the activation of the expression of specific genes [54-55]. Several IL-2-conjugated Ab-targeting moieties are currently in clinical trials, both in Phase I and II. huKS-IL2 (EMD 273066)

and hu14.18-IL2(EMD 273063) are humanized anti-EpCAM (huKS) or humanized anti-disialoganglioside GD2 antibodies, respectively, in which a human IL-2 molecule is genetically linked to the carboxy terminus of each IgG-heavy chain, making the intact molecule bivalent with respect to IL-2 [56-57]. Following a Phase I study [58], EMD 273063 was used in Phase II trial on patients with relapsed or refractory neuroblastoma. Of 36 patients, minimal disease was only found in 23 patients using [I^{123}] metaiodobenzylguanidine (MIBG) scintigraphy and/or bone marrow (BM) histology. Five had CR and none of the others responded (including 13 with advanced stage cancers) [59]. A Phase II study is being conducted on patients with stage III+ melanoma. EMD 273066 was used in a Phase Ib trial in combination with MTX on patients with ovarian, lung, colon, and prostate cancer, as well as one each with melanoma, acinic cell carcinoma and an unspecified adenocarcinoma. EMD 521873 is another form of immunocytokine in which the Ab is conjugated to IL-2. In this molecule the IL-2 is mutated (D20T), causing it to be 20 times less toxic than a wild-type in its natural state when conjugated with IL-2 [60]. EMD 521873 is in Phase I trials as sole treatment or in combination with radiotherapy.

By applying phage-display technology, the Neri group has developed three Abs specifically targeting the alternatively-spliced extra domain A (EDA) and B (EDB) of Fibronectin and A1 domain of Tenascin-C (F8, L19 and F16, respectively) [61-63]. These Abs were conjugated to several cytokines and are used in Phase I and Phase II trials. L19-IL2 (ScFv antibody targeting EDB-FN, [62]) was used in a Phase I trial, in which RD of IL19-IL2 was determined. AE in this study included fever with chills, nausea, vomiting, asthenia, oedema, skin rash, pruritus, elevated serum creatinine levels and pain at tumour site [64]. Currently other Phase I trials are performed in pancreatic cancer patients where L19-IL2 is given in combination with Gemcitabine. Phase II studies are currently underway with L19-IL2 administered either as a sole treatment or in combination with other agents - Dacarbazine in patients with advanced melanoma and Gemcitabine in patients with pancreatic cancer. Multiple-Phase I/II studies are performed with F16-IL2 immunocytokine in breast cancer patients, where F16-IL2 is given in combination with doxorubicin or paclitaxel. A third derivative, L19-TNFα immunocytokine, has recently completed a Phase I study as a sole treatment in colorectal cancer patients.

ADEPT

The non-specific toxic side-effect of chemotherapy on normal tissues is a major issue that severely limits the application of chemotherapeutic drugs in the clinic. As stated earlier, antibody conjugates can deliver anti-tumour payload to specific tumour tissues, thus reducing systemic toxicity and increasing efficacy. Antibody-directed enzyme prodrug therapy (ADEPT) is a dual-stage treatment, where the antibody and the effector molecule are administered separately. In this method, an enzyme is conjugated to an Ab targeting the TAA and administered to the patient. Enzymes should have high catalytic activity and not have any active human counterpart to avoid endogenous activation of the prodrug in normal tissues [65]. In the second stage of the treatment, after the Ab has cleared from circulation, a prodrug is administered. Once the prodrug has reached the tumour site, it is cleaved to an active mature form, and a cytotoxic effect is elicited. Due to the high concentration and localization of the enzyme at the tumour site, a high dosage of the active drug is concentrated in this location and the majority of the effect is elicited on Ab-enzyme targeted cells. A variety of enzymes and prodrugs have been used. The ideal enzyme should be easily accessible, easy to link to an antibody while retaining its own functionality, and capable of being used with minimum immunogenicity. The ideal prodrug should be converted to a toxic drug by the appropriate enzyme, with minimum toxicity when not activated, and upon activation should have a steep dose-activity curve. Several enzymes are used in this method, such as CarboxypeptidaseG2 [66-68] and Human β-glucuronidase [69], for instance. In a Phase I clinical study conducted by Mayer *et al.* anti-CEA Ab conjugated to a bacterial carboxypeptidase G2 derived fusion protein was administered to patients with colorectal, breast, peritoneal, or esophageal cancer. When the Ab has cleared the serum (<0.005 units/mL as measured by HPLC), a

bis-iodo phenol mustard prodrug was administered. The enzyme dimerizes to become functional, forming a stable glycosylated fusion protein, and converts BIP prodrug to an active drug by cleavage of a glutamate moiety. There was evidence of tumour response, and some patients exhibited stable disease. Most importantly, this trial improved the toxicity of prodrugs observed in earlier Phase I trials [70].

ADEPT has demonstrated promising activities in preclinical cancer models. One of the main advantages of this approach is in the use of a non-active agent, which is transformed to an active one in the vicinity of the tumour, thus reducing systemic toxicity and side effects. However, issues of immunogenicity and specificity in targeting of tumour cells need to be significantly improved. Furthermore, a wider selection of prodrugs and enzyme-conjugated Abs needs to be developed in addition to those currently available. Nevertheless, this strategy heralds great clinical potential.

Immunotoxins and antibody-drug conjugates (ADCs)

The standard treatment for cancer patients has long been the administration of compounds which target the various stages of cell proliferation. The inherent property of cancer cells to rapidly divide renders them susceptible to these drugs. However, such drugs are not cell-specific and therefore have limited treatment concentration due to the non-specific damage to other proliferating cells, such as bone-marrow and intestinal cells. The conjugation of such toxins and drugs to Abs enhances their specificity to the intended target and increases their therapeutic window.

Antibody-drug conjugates

Drugs used in antibody conjugation mainly target either tubulin or DNA. Auristatins and maytansines target tubulin, causing the disruption of cell mitosis through G2/M arrest [71-72]. Calicheamicin binds to the DNA's minor groove and induces double-1strand breaks, thus promoting apoptosis [73]. Due to the high potency and toxicity of these substances, important considerations are to be taken into account when conjugating these substances to an Ab. First, the target to which the Ab specifically binds must be a membrane-expressed epitope which cannot be shed into circulation. This will prevent systemic cytotoxicity and will enable the internalization of the cytotoxic payload into the cell. Both IgG form and a ScFv diabody were evaluated for efficient tumour uptake. The balance between tumour penetration and clearance rate plays a significant role in this comparison, where, although a ScFv Ab exhibits more accumulation intratumour, its clearance rate is substantially faster. Therefore, in order to avoid an increase in drug concentration, IgG1 is the preferred form of Ab conjugation [74-75].

Gemtuzumabozogamicin (Mylotarg), an anti-CD33 mAb conjugated to calicheamicin, was the first Ab-drug conjugate approved by the FDA for treatment for AML [76]. However, it was withdrawn due to failure to show any evidence of improved rates of CR or survival, with a significantly higher rate of fatal induction toxicity (5.8% in the Mylotarg-chemotherapy versus 0.8% in the chemotherapy-alone) [77]. Other Ab-drug conjugates are currently in several stages of clinical trials: Brentuximabvedotin (SGN-35), a chimeric IgG1Ab, targets CD30 and is conjugated to monomethylauristatin E (MMAE) through a valine-citrulline peptide linker. In a Phase I study, 36 out of 42 patients studied (86%) exhibited tumour regression. Of 16 patients with disease-related symptoms at baseline, 13 (81%) patients became symptom free [78]. A Phase III trial is currently recruiting where SGN-35 is given as a single agent (NCT01100502). Another ADC at Phase III is trastuzumab-DM1. Unconjugated trastuzumab (Herceptine) was first approved by the FDA for treatment in metastatic breast cancer in 1998. DM1, a semi-synthetic analogue of maytansine, is conjugated through a succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC) linker. Phase II studies exhibited an objective response rate of 32.7%-47.8%, and clinical benefit (CR, PR or stable disease >6 months) was 46.4%-57.1% [79]. Phase III trials are currently recruiting patients (NCT01120184, NCT01419197, NCT00829166). Inotuzumab Ozogamycin is a third ADC currently recruiting for a Phase III trial. This ADC targets CD22, and is conjugated to the same active derivative as Mylotarg. It is used as treatment in NHL patients in combination with rituximab compared to a drug

regimen of rituximab and gemcitabine or bendamustine (NCT01232556).

Several other drugs are currently in clinical trials. Among them are Lorvotuzumabmertansine (IMGN901, Phase I/II) a humanized IgG1 Ab conjugated to DM1 moiety which targets CD52, Glembatumumab (CDX-011, CR011-vcMMAE, Phase II), fully-human Ab conjugated to MMAE moiety and targets transmembrane glycoprotein NMB, SAR3419 (Phase II), and DM4 conjugated anti-CD19 Ab.

Issues concerning linker optimization and drug potency have shown improvement, and although this field holds great promise, several aspects still require careful consideration: firstly, target cell expression of Pgp, which is responsible for multi-drug resistance, has been shown to suppress the activity of Ab-drug conjugates [80]; secondly, target selection of epitopes, which will not only be cell-specific but also will not be shed to circulation must also be considered.

Immunotoxins

Similar to ADCs, immunotoxins are generated by conjugating bacterial and plant derived protein toxins to a targeting moiety. The targeting domain of the toxins is replaced by a desired ScFv or Fab fragment, thus enabling the specific targeting while retaining the toxin's activity. Following administration, the Ab moiety targets its ligand expressed on the membrane of a cancer cell. Binding triggers endocytosis of the entire molecule, and the cleavage of the catalytic domain in the late endosome. The catalytic subunit of the immunotoxin translocates to the cytosol where it exerts its toxic effect. The toxins used in this application catalyze different types of enzymatic inhibitors of protein synthesis. The most frequently used toxins are Pseudomonas exotoxin (PE), diphtheria toxin (DT), and plant toxins such as ricin, saporin, and gelonin. PE and DT catalyze the ADP ribosylation of elongation factor 2(EF2), halting the elongation of growing peptide chains; plant toxins, on the other hand, inactivate ribosomes via glycosidase activity [81-82].

Several immunotoxins are currently in clinical trials. BL22 is a molecule comprised of a CD22 targeting fragment genetically linked to a PE toxin lacking the targeting domain. CD22 is a sialic acid-binding Ig-like lectin, which inhibits B-cell calcium signalling and is expressed in a variety of B-cell malignancies. BL22 achieved an overall response-rate of 81% in a Phase I trial, mostly of HCL patients [83]. A Phase II trial in HCL patients demonstrated an objective response rate (ORR) of 72% [84-85]. However, further development of this immunotoxin was halted in favour of developing an immunotoxin more effective in CLL, ALL and NHL. Moxetumomab pasudotox is an affinity-matured form of BL22 with a 15-fold increase in binding affinity and a 50-fold increase in cytotoxicity towards HCL and CLL cells [86]. This immunotoxin has shown encouraging effect in Phase I studies both, in patients with HCL and pediatric ALL patients, and currently Phase I/II studies are underway in patients with CLL, NHL, HCL and pediatric ALL.

Other immunotoxins in clinical trials include Imtox-25, and LMB-2. Imtox-25 (RFT5-dgA) is an anti-CD25 targeting moiety conjugated to a chemically deglycosylated form of ricin A chain (dgA), and is tested on patients with refractory or relapsed adult T-cell leukemia or lymphoma [87]. LMB2 (anti-Tac(Fv)-PE38) contains the PE toxic domain and a targeting moiety, in which the variable VH and VL domains of the anti-CD25 mAb anti-Tac were fused together with a peptide linker and VL was fused to PE38 via a short connector [88]. Phase II trials in patients with adult T-cell leukemia, CLL and cutaneous T-cell lymphoma were recently concluded.

Other immunotoxins are currently in Phase I/II trials. These molecules are targeted against haematological as well as solid-tumour epitopes, including CD19 − Combotox (RFB4-dgA + HD37-dgA, Phase I), Lewis Y (LMB-9, Phase I) and SS1(dsFv)-PE38 (SS1P) anti-mesothelin immunotoxin (Phase I).

The use of immunotoxins faces several key hurdles in its clinical application. The first is the most prevalent side effect of capillary leak syndrome (CLS) in the administration of ADC and immunotoxins. This side effect is often not dose-limiting and is associated with the disruption of integrins in endothelial cells [89]. A second issue is the development of human anti-mouse

antibodies (HAMA), as well as anti-drug antibodies. Significant progress has been made in increasing the efficiency of these molecules either by elimination of T- and B-cell epitopes from the toxins, or 'PEGylation' of the molecules. Despite this, these side effects still pose a risk of significant reduction in activity. Further research is required in order to improve the efficiency and safety of toxin-based agents.

Radioisotope conjugates

Radioimmunotherapy (RIT) offers the possibility of treating localized, metastatic, or diffuse tumours by local or systemic administration of the therapeutic agent. The clinical use of radio-immunotoxins is based on the creation of high concentrations of the radioactive isotope on the Ag positive tumour cells, thus enabling their destruction. Radioactive isotopes include primarily α or β emitters, as they emit low or intermediate energy to low or medium distances (<1 μm and 0.05-12.00 mm, respectively). Given their characteristics, these isotopes are the most suitable candidates for the treatment of haematological and small, solid tumours [90-91]. RIT has several clinical applications. It is used for diagnostic applications and localization of tumours by the use of ^{125}I labeled antibodies that, once bound to Ag-positive tumour cells, enables the detection of the tumour by an external gamma-radiation sensor.

A wide range of Abs are in various stages of clinical evaluation, with ibritumomabtiuxetan (Zevalin®), a ^{90}Y-labelled murine anti-CD20 antibody, and tositumomab (Bexxar®) approved for clinical use. These ^{131}I-labelled murine anti-CD20 antibodies are two antibodies approved for non-Hodgkin's lymphoma treatment and are the subject of more than 80 ongoing clinical trials, tested in combination with chemotherapy or as high-dose therapies in conjunction with autologous stem cell transplantation [90, 92, 93]. Several other Abs are currently being evaluated in various stages of clinical trials. BC8, an anti-CD45 Ab conjugated to ^{131}I is currently under Phase I and II studies in patients with haematological malignancies. BC8 antibody is used in combination with chemotherapy or total-body irradiation, and is followed by BM transplantation. In a Phase I study, ^{131}I-BC8 antibody was combined with a standard reduced-intensity conditioning regimen before allogeneic haematopoietic cell transplantation. This treatment produced CR in all patients, and all had 100% donor-derived CD3+ and CD33+ cells in the blood by day 28 after the transplantation [94]. Radio-labelled Abs are administered mainly through IV injections. This method limits the administered dose due to high BM toxicity. MN-14 (Labetuzumab), anti-CEA humanized murine Ab and one of the Abs commonly used in this setting, has given encouraging results in a Phase II trial in which the median survival rate of 58 months and a survival of 42.1% after 5 years were achieved [95]. In spite of this, solid tumours prove a major difficulty in RIT. The radioactive dose delivered by the Ab is not sufficient to eradicate the tumour, and increasing the emitted energy by conjugation of more potent isotopes may increase bone-marrow toxicity. Other targeted epitopes for solid tumour RIT which are currently in clinical trials include: EDB (L19SIP Ab [96]), EpCAM (NR-LU-10-strept and 90Y-DOTA-Biotin [97]), EGFR (^{125}I-425, [98]), MUC1 (^{90}Y-m170, [99]), TAG-72 (^{90}Y-hCC49DCh2(IDEC-159) [100], ^{131}I-CC49 [101]) and TN-C (^{211}At-ch81C6, [102]), to name just a few, all of which are extensively reviewed elsewhere [91].

Redirection of T-cells

During the last decade, adoptive cell transfer (ACT) of autologous T-cells has been performed in cancer patients with varying levels of success, especially in melanoma patients. In this method, T-cells are isolated, expanded *in vitro* and are re-infused back into the patients [103]. Melanoma patients treated in this method have shown a 50% objective response (OR) in a Phase II study (10% achieved CR) [104-105]. However, difficulties in isolation and expansion of tumour-specific T-cells have hindered the use of this treatment in other types of cancers. A solution to this problem was offered by the application of genetic modification and the re-direction of T-cells. This was achieved by virally transducing recombinant targeting moiety, either TCR or antibody, to autologous T-cells. In the first approach, Dudley *et al.* first demonstrated the feasibility in 15 melanoma patients transfused with autologous TCR-transduced T-cells [106]. In transduction of an

Ab, the antibody V-region responsible for recognition and activation is attached to a cytotoxic T-cell signaling moiety Fc Receptor γ chain to mediate tumour-specific destruction and cytokine release [107-108]. These T-cells transduced with chimeric antibody receptors (CARs) are therefore independent of MHC-restricted activation and are suitable for use in tumours which have downregulated their HLA expression. Recently, Porter *et al.* have reported a study in a single CLL patient, infused with anti-CD19 transduced T-cells. The anti-CD19 targeting moiety was conjugated to a CD3 zeta chain [109]. The cells expanded 1000-fold and persisted at high levels for six months with continued expression of the CAR. The patient exhibited complete response and ongoing response ten months following treatment, along with complete elimination of CD19-expressing B-cells. Side effects including hypogammaglobulinemia, corrected with infusions of i.v.Ig. Despite this encouraging report, several considerations in re-directing T-cells must be addressed: firstly, the nature of signalling and co-stimulation in these T-cells is different from physiological activation, and might affect activation/exhaustion of the engineered cells. Secondly, there is a risk of developing an immune response against the engineered T-cells [110]. Finally, the high costs associated with this method will prohibit its wide-spread use in the patient population.

CONCLUSION

Antibody therapy offers an attractive approach to cancer treatment, and the increasing number of antibodies and antibody-derivatives coming to clinical trial suggests that this promising field will generate more clinical success in years to come. However, success will depend upon the discovery of new selective markers of tumour cells, which are capable of extending the therapeutic window and minimizing toxicity. Successful cancer treatments are likely to require the manipulation of the immune system. To this end, bi-specific Abs and immuno-conjugates are promising new agents in the battle against cancer.

ACKNOWLEDGEMENTS AND FUNDING

The authors wish to thank Dahan R., Zafir-Lavie I., and Michaeli M. for their support in the creation of this manuscript.

REFERENCES

1. Strome, S. E., Sausville, E. A., and Mann, D. 2007, Oncologist, 12, 1084.
2. Hudis, C. A. 2007, N. Engl. J. Med., 357, 39.
3. Cheson, B. D. and Leonard, J. P. 2008, N. Engl. J. Med., 359, 613.
4. Machiels, J.-P., Subramanian, S., Ruzsa, A., Repassy, G., Lifirenko, I., Flygare, A., Sørensen, P., Nielsen, T., Lisby, S., and Clement, P. M. J. 2011, The Lancet Oncology, 12, 333.
5. Vogel, C. L., Cobleigh, M. A., Tripathy, D., Gutheil, J. C., Harris, L. N., Fehrenbacher, L., Slamon, D. J., Murphy, M., Novotny, W. F., Burchmore, M., Shak, S., Stewart, S. J., and Press, M. 2002, J. Clin. Oncol., 20, 719.
6. Baselga, J., Cortés, J., Kim, S.-B., Im, S.-A., Hegg, R., Im, Y.-H., Roman, L., Pedrini, J. L., Pienkowski, T., Knott, A., Clark, E., Benyunes, M. C., Ross, G., and Swain, S. M. 2012, New England Journal of Medicine, 366, 109.
7. Carmeliet, P. 2003, Nat. Med., 9, 653.
8. Weis, S. M. and Cheresh, D. A. 2011, Nat. Med., 17, 1359.
9. Šmahel, M. 2011, Cancer Immunology, Immunotherapy, 60, 1655.
10. FitzGerald, D. J., Wayne, A. S., Kreitman, R. J., and Pastan, I. 2011, Cancer Res., 71, 6300.
11. Plaksin, D., Polakova, K., McPhie, P., and Margulies, D. H. 1997, J. Immunol., 158, 2218.
12. Wulfing, C. and Pluckthun, A. 1994, J. Mol. Biol., 242, 655.
13. Michaeli, Y., Denkberg, G., Sinik, K., Lantzy, L., Chih-Sheng, C., Beauverd, C., Ziv, T., Romero, P., and Reiter, Y. 2009, J. Immunol., 182, 6328.
14. Wittman, V. P., Woodburn, D., Nguyen, T., Neethling, F. A., Wright, S., and Weidanz, J. A. 2006, J. Immunol., 177, 4187.
15. Denkberg, G., Lev, A., Eisenbach, L., Benhar, I., and Reiter, Y. 2003, J. Immunol., 171, 2197.
16. Epel, M., Carmi, I., Soueid-Baumgarten, S., Oh, S. K., Bera, T., Pastan, I., Berzofsky, J., and Reiter, Y. 2008, Eur. J. Immunol., 38, 1706.

17. Klechevsky, E., Gallegos, M., Denkberg, G., Palucka, K., Banchereau, J., Cohen, C., and Reiter, Y. 2008, Cancer Res., 68, 6360.
18. Kohler, G. and Milstein, C. 1975, Nature, 256, 495.
19. Milstein, C. and Cuello, A. C. 1983, Nature, 305, 537.
20. Tiebout, R. F., van Boxtel-Oosterhof, F., Stricker, E. A., and Zeijlemaker, W. P. 1987, J. Immunol., 139, 3402.
21. Sebastian, M., Passlick, B., Friccius-Quecke, H., Jäger, M., Lindhofer, H., Kanniess, F., Wiewrodt, R., Thiel, E., Buhl, R., and Schmittel, A. 2007, Cancer Immunology, Immunotherapy, 56, 1637.
22. Glennie, M. J., McBride, H. M., Worth, A. T., and Stevenson, G. T. 1987, J. Immunol., 139, 2367.
23. Graziano, R. F. and Guptill, P. 2004, Methods Mol. Biol., 283, 71.
24. Stevenson, G. T., Pindar, A., and Slade, C. J. 1989, Anticancer Drug Des., 3, 219.
25. Brandão, J. G., Scheper, R. J., Lougheed, S. M., Curiel, D. T., Tillman, B. W., Gerritsen, W. R., van den Eertwegh, A. J. M., Pinedo, H. M., Haisma, H. J., and de Gruijl, T. D. 2003, Vaccine, 21, 2268.
26. Kipriyanov, S. M., Moldenhauer, G., Strauss, G., and Little, M. 1998, International Journal of Cancer, 77, 763.
27. Kranz, D. M., Gruber, M., and Wilson, E. R. 1995, J. Hematother., 4, 403.
28. Kufer, P., Mack, M., Gruber, R., Lutterbüse, R., Zettl, F., and Riethmüller, G. 1997, Cancer Immunology, Immunotherapy, 45, 193.
29. McDonagh, C. F., Huhalov, A., Harms, B. D., Adams, S., Paragas, V., Oyama, S., Zhang, B., Luus, L., Overland, R., Nguyen, S., Gu, J., Kohli, N., Wallace, M., Feldhaus, M. J., Kudla, A. J., Schoeberl, B., and Nielsen, U. B. 2012, Molecular cancer therapeutics, 11, 582.
30. Holliger, P. and Winter, G. 1997, Cancer Immunol Immunother, 45, 128.
31. Dreier, T., Baeuerle, P. A., Fichtner, I., Grun, M., Schlereth, B., Lorenczewski, G., Kufer, P., Lutterbuse, R., Riethmuller, G., Gjorstrup, P. and Bargou, R. C. 2003, J. Immunol., 170, 4397.
32. Dreier, T., Lorenczewski, G., Brandl, C., Hoffmann, P., Syring, U., Hanakam, F., Kufer, P., Riethmuller, G., Bargou, R., and Baeuerle, P. A. 2002, Int. J. Cancer, 100, 690.
33. Wolf, E., Hofmeister, R., Kufer, P., Schlereth, B., and Baeuerle, P. A. 2005, Drug Discovery Today, 10, 1237.
34. Bluemel, C., Hausmann, S., Fluhr, P., Sriskandarajah, M., Stallcup, W. B., Baeuerle, P. A., and Kufer, P. 2010, Cancer Immunol Immunother, 59, 1197.
35. Baeuerle, P. A. and Reinhardt, C. 2009, Cancer Res., 69, 4941.
36. Witlox, M. A., Van Beusechem, V. W., Grill, J., Haisma, H. J., Schaap, G., Bras, J., Van Diest, P., De Gast, A., Curiel, D. T., Pinedo, H. M., Gerritsen, W. R., and Wuisman, P. I. 2002, J. Gene. Med., 4, 510.
37. Korn, T., Nettelbeck, D. M., Volkel, T., Muller, R., and Kontermann, R. E. 2004, J. Gene. Med., 6, 642.
38. Wimberger, P., Xiang, W., Mayr, D., Diebold, J., Dreier, T., Baeuerle, P. A., and Kimmig, R. 2003, Int. J. Cancer, 105, 241.
39. Haagen, I. A., Geerars, A. J., Bast, E. J., de Gast, G. C., van de Winkel, J. G., and de Lau, W. B. 1994, Ther. Immunol., 1, 279.
40. Maletz, K., Kufer, P., Mack, M., Raum, T., Pantel, K., Riethmuller, G., and Gruber, R. 2001, Int. J. Cancer, 93, 409.
41. Koristka, S., Cartellieri, M., Theil, A., Feldmann, A., Arndt, C., Stamova, S., Michalk, I., Töpfer, K., Temme, A., Kretschmer, K., Bornhäuser, M., Ehninger, G., Schmitz, M., and Bachmann, M. 2011, The Journal of Immunology, 188, 1551.
42. Herrmann, I., Baeuerle, P. A., Friedrich, M., Murr, A., Filusch, S., Ruttinger, D., Majdoub, M. W., Sharma, S., Kufer, P., Raum, T., and Munz, M. 2010, PLoS One, 5, e13474.
43. Cioffi, M., Dorado, J., Baeuerle, P. A., and Heeschen, C. 2011, Clinical Cancer Research, 18, 465.
44. Bargou, R., Leo, E., Zugmaier, G., Klinger, M., Goebeler, M., Knop, S., Noppeney, R., Viardot, A., Hess, G., Schuler, M., Einsele, H., Brandl, C., Wolf, A., Kirchinger, P., Klappers, P., Schmidt, M., Riethmuller, G., Reinhardt, C., Baeuerle, P. A., and Kufer, P. 2008, Science, 321, 974.

45. Nagorsen, D. and Baeuerle, P. A. 2011, Exp. Cell Res., 317, 1255.
46. Topp, M. S., Kufer, P., Gökbuget, N., Goebeler, M., Klinger, M., Neumann, S., Horst, H.-A., Raff, T., Viardot, A., Schmid, M., Stelljes, M., Schaich, M., Degenhard, E., Köhne-Volland, R., Brüggemann, M., Ottmann, O., Pfeifer, H., Burmeister, T., Nagorsen, D., Schmidt, M., Lutterbuese, R., Reinhardt, C., Baeuerle, P. A., Kneba, M., Einsele, H., Riethmüller, G., Hoelzer, D., Zugmaier, G., and Bargou, R. C. 2011, Journal of Clinical Oncology, 29, 2493.
47. Stanglmaier, M., Faltin, M., Ruf, P., Bodenhausen, A., Schröder, P., and Lindhofer, H. 2008, International Journal of Cancer, 123, 1181.
48. Boehrer, S., Schroeder, P., Mueller, T., Atz, J., and Chow, K. U. 2011, Anticancer Drugs, 22, 519.
49. Linke, R., Klein, A., and Seimetz, D. 2010, MAbs, 2, 129.
50. Seimetz, D. 2011, J. Cancer, 2, 309.
51. Baeuerle, P. A., Kufer, P., and Bargou, R. 2009, Curr. Opin. Mol. Ther., 11, 22.
52. Atkins, M. B., Lotze, M. T., Dutcher, J. P., Fisher, R. I., Weiss, G., Margolin, K., Abrams, J., Sznol, M., Parkinson, D., Hawkins, M., Paradise, C., Kunkel, L., and Rosenberg, S. A. 1999, J. Clin. Oncol., 17, 2105.
53. Keilholz, U., Stoter, G., Punt, C. J., Scheibenbogen, C., Lejeune, F., and Eggermont, A. M. 1997, Cancer J. Sci. Am., 3 Suppl. 1, S22.
54. Mule, J. J., Yang, J. C., Afreniere, R. L., Shu, S. Y., and Rosenberg, S. A. 1987, J. Immunol., 139, 285.
55. Malek, T. R. 2008, Annual Review of Immunology, 26, 453.
56. Gillies, S. D., Reilly, E. B., Lo, K. M., and Reisfeld, R. A. 1992, Proc. Natl. Acad. Sci. USA, 89, 1428.
57. Xiang, R., Lode, H. N., Dolman, C. S., Dreier, T., Varki, N. M., Qian, X., Lo, K. M., Lan, Y., Super, M., Gillies, S. D., and Reisfeld, R. A. 1997, Cancer Res., 57, 4948.
58. King, D. M., Albertini, M. R., Schalch, H., Hank, J. A., Gan, J., Surfus, J., Mahvi, D., Schiller, J. H., Warner, T., Kim, K., Eickhoff, J., Kendra, K., Reisfeld, R., Gillies, S. D., and Sondel, P. 2004, Journal of Clinical Oncology, 22, 4463.
59. Shusterman, S., London, W. B., Gillies, S. D., Hank, J. A., Voss, S. D., Seeger, R. C., Reynolds, C. P., Kimball, J., Albertini, M. R., Wagner, B., Gan, J., Eickhoff, J., DeSantes, K. B., Cohn, S. L., Hecht, T., Gadbaw, B., Reisfeld, R. A., Maris, J. M., and Sondel, P. M. 2010, Journal of Clinical Oncology, 28, 4969.
60. Gillies, S. D., Lan, Y., Hettmann, T., Brunkhorst, B., Sun, Y., Mueller, S. O., and Lo, K.-M. 2011, Clinical Cancer Research, 17, 3673.
61. Brack, S. S., Silacci, M., Birchler, M., and Neri, D. 2006, Clin. Cancer Res., 12, 3200.
62. Pini, A., Viti, F., Santucci, A., Carnemolla, B., Zardi, L., Neri, P., and Neri, D. 1998, J. Biol. Chem., 273, 21769.
63. Villa, A., Trachsel, E., Kaspar, M., Schliemann, C., Sommavilla, R., Rybak, J. N., Rosli, C., Borsi, L., and Neri, D. 2008, Int. J. Cancer, 122, 2405.
64. Johannsen, M., Spitaleri, G., Curigliano, G., Roigas, J., Weikert, S., Kempkensteffen, C., Roemer, A., Kloeters, C., Rogalla, P., Pecher, G., Miller, K., Berndt, A., Kosmehl, H., Trachsel, E., Kaspar, M., Lovato, V., Gonzalez-Iglesias, R., Giovannoni, L., Menssen, H. D., Neri, D., and de Braud, F. 2010, Eur. J. Cancer, 46, 2926.
65. Begent, R., Sharma, S. and Chester, K. (2010). Antibody-Dependent Enzyme Prodrug Therapy (ADEPT) Antibody Engineering. R. Kontermann and S. Dübel, Springer Berlin Heidelberg: 431.
66. Blakey, D. C., Davies, D. H., Dowell, R. I., East, S. J., Burke, P. J., Sharma, S. K., Springer, C. J., Mauger, A. B., and Melton, R. G. 1995, Br. J. Cancer, 72, 1083.
67. Francis, R. J., Sharma, S. K., Springer, C., Green, A. J., Hope-Stone, L. D., Sena, L., Martin, J., Adamson, K. L., Robbins, A., Gumbrell, L., O'Malley, D., Tsiompanou, E., Shahbakhti, H., Webley, S., Hochhauser, D., Hilson, A. J., Blakey, D., and Begent, R. H. J. 2002, Br. J. Cancer, 87, 600.
68. Mahato, R., Tai, W., and Cheng, K. 2011, Adv. Drug Deliv. Rev., 63, 659.
69. Chen, K.-C., Wu, S.-Y., Leu, Y.-L., Prijovich, Z. M., Chen, B.-M., Wang, H.-E., Cheng, T.-L., and Roffler, S. R. 2011, Bioconjugate Chemistry, 22, 938.

70. Mayer, A., Sharma, S. K., Tolner, B., Minton, N. P., Purdy, D., Amlot, P., Tharakan, G., Begent, R. H., and Chester, K. A. 2004, Br. J. Cancer, 90, 2402.
71. Cassady, J. M., Chan, K. K., Floss, H. G., and Leistner, E. 2004, Chem. Pharm. Bull (Tokyo), 52, 1.
72. Doronina, S. O., Toki, B. E., Torgov, M. Y., Mendelsohn, B. A., Cerveny, C. G., Chace, D. F., DeBlanc, R. L., Gearing, R. P., Bovee, T. D., Siegall, C. B., Francisco, J. A., Wahl, A. F., Meyer, D. L., and Senter, P. D. 2003, Nat. Biotechnol., 21, 778.
73. Hinman, L. M., Hamann, P. R., Wallace, R., Menendez, A. T., Durr, F. E., and Upeslacis, J. 1993, Cancer Res., 53, 3336.
74. Kim, K. M., McDonagh, C. F., Westendorf, L., Brown, L. L., Sussman, D., Feist, T., Lyon, R., Alley, S. C., Okeley, N. M., Zhang, X., Thompson, M. C., Stone, I., Gerber, H. P., and Carter, P. J. 2008, Molecular cancer therapeutics, 7, 2486.
75. Schmidt, M. M. and Wittrup, K. D. 2009, Molecular cancer therapeutics, 8, 2861.
76. Bross, P. F., Beitz, J., Chen, G., Chen, X. H., Duffy, E., Kieffer, L., Roy, S., Sridhara, R., Rahman, A., Williams, G. and Pazdur, R. 2001, Clin Cancer Res, 7, 1490.
77. Petersdorf, S., Kopecky, K., Stuart, R. K., Larson, R. A., Nevill, T. J., Stenke, L., Slovak, M. L., Tallman, M. S., Willman, C. L., Erba, H., and Appelbaum, F. R. 2009, ASH Annual Meeting Abstracts, 114, 790.
78. Younes, A., Bartlett, N. L., Leonard, J. P., Kennedy, D. A., Lynch, C. M., Sievers, E. L., and Forero-Torres, A. 2010, N. Engl. J. Med., 363, 1812.
79. Burris, H. A., 3rd, Rugo, H. S., Vukelja, S. J., Vogel, C. L., Borson, R. A., Limentani, S., Tan-Chiu, E., Krop, I. E., Michaelson, R. A., Girish, S., Amler, L., Zheng, M., Chu, Y. W., Klencke, B., and O'Shaughnessy, J. A. 2011, J. Clin. Oncol., 29, 398.
80. Takeshita, A., Shinjo, K., Yamakage, N., Ono, T., Hirano, I., Matsui, H., Shigeno, K., Nakamura, S., Tobita, T., Maekawa, M., Ohnishi, K., Sugimoto, Y., Kiyoi, H., Naoe, T., and Ohno, R. 2009, Br. J. Haematol., 146, 34.
81. Endo, Y., Mitsui, K., Motizuki, M., and Tsurugi, K. 1987, J. Biol. Chem., 262, 5908.
82. Choudhary, S., Mathew, M., and Verma, R. S. 2011, Drug Discovery Today, 16, 495.
83. Kreitman, R. J., Squires, D. R., Stetler-Stevenson, M., Noel, P., FitzGerald, D. J., Wilson, W. H., and Pastan, I. 2005, J. Clin. Oncol., 23, 6719.
84. Kreitman, R. J., Stetler-Stevenson, M., Margulies, I., Noel, P., Fitzgerald, D. J., Wilson, W. H., and Pastan, I. 2009, J. Clin. Oncol., 27, 2983.
85. Kreitman, R. J. and Pastan, I. 2011, Clin. Cancer Res., 17, 6398.
86. Salvatore, G., Beers, R., Margulies, I., Kreitman, R. J., and Pastan, I. 2002, Clin. Cancer Res., 8, 995.
87. Schnell, R., Borchmann, P., Staak, J. O., Schindler, J., Ghetie, V., Vitetta, E. S., and Engert, A. 2003, Ann. Oncol., 14, 729.
88. Chaudhary, V. K., Queen, C., Junghans, R. P., Waldmann, T. A., FitzGerald, D. J., and Pastan, I. 1989, Nature, 339, 394.
89. Baluna, R., Rizo, J., Gordon, B. E., Ghetie, V., and Vitetta, E. S. 1999, Proc. Natl. Acad. Sci. USA, 96, 3957.
90. Govindan, S. V. and Goldenberg, D. M. 2010, ScientificWorld Journal, 10, 2070.
91. Pouget, J. P., Navarro-Teulon, I., Bardies, M., Chouin, N., Cartron, G., Pelegrin, A., and Azria, D. 2011, Nat. Rev. Clin. Oncol., 8, 720.
92. Goldsmith, S. J. 2010, Semin. Nucl. Med., 40, 122.
93. Palanca-Wessels, M. C. and Press, O. W. 2010, Cancer, 116, 1126.
94. Pagel, J. M., Gooley, T. A., Rajendran, J., Fisher, D. R., Wilson, W. A., Sandmaier, B. M., Matthews, D. C., Deeg, H. J., Gopal, A. K., Martin, P. J., Storb, R. F., Press, O. W., and Appelbaum, F. R. 2009, Blood, 114, 5444.
95. Liersch, T., Meller, J., Kulle, B., Behr, T. M., Markus, P., Langer, C., Ghadimi, B. M., Wegener, W. A., Kovacs, J., Horak, I. D., Becker, H. and Goldenberg, D. M. 2005, Journal of Clinical Oncology, 23, 6763.

96. Sauer, S., Erba, P. A., Petrini, M., Menrad, A., Giovannoni, L., Grana, C., Hirsch, B., Zardi, L., Paganelli, G., Mariani, G., Neri, D., Durkop, H., and Menssen, H. D. 2009, Blood, 113, 2265.
97. Knox, S. J., Goris, M. L., Tempero, M., Weiden, P. L., Gentner, L., Breitz, H., Adams, G. P., Axworthy, D., Gaffigan, S., Bryan, K., Fisher, D. R., Colcher, D., Horak, I. D., and Weiner, L. M. 2000, Clin. Cancer Res., 6, 406.
98. Li, L., Quang, T. S., Gracely, E. J., Kim, J. H., Emrich, J. G., Yaeger, T. E., Jenrette, J. M., Cohen, S. C., Black, P., and Brady, L. W. 2010, J. Neurosurg., 113, 192.
99. DeNardo, S. J., Richman, C. M., Albrecht, H., Burke, P. A., Natarajan, A., Yuan, A., Gregg, J. P., O'Donnell, R. T., and DeNardo, G. L. 2005, Clin. Cancer Res., 11, 7187s.
100. Shen, S., Forero, A., Meredith, R. F., and LoBuglio, A. F. 2011, Cancer Biother Radiopharm, 26, 127.
101. Meredith, R. F., Khazaeli, M. B., Macey, D. J., Grizzle, W. E., Mayo, M., Schlom, J., Russell, C. D., and LoBuglio, A. F. 1999, Clin. Cancer Res., 5, 3254s.
102. Zalutsky, M. R., Reardon, D. A., Akabani, G., Coleman, R. E., Friedman, A. H., Friedman, H. S., McLendon, R. E., Wong, T. Z., and Bigner, D. D. 2008, J. Nucl. Med., 49, 30.
103. Dudley, M. E., Wunderlich, J. R., Robbins, P. F., Yang, J. C., Hwu, P., Schwartzentruber, D. J., Topalian, S. L., Sherry, R., Restifo, N. P., Hubicki, A. M., Robinson, M. R., Raffeld, M., Duray, P., Seipp, C. A., Rogers-Freezer, L., Morton, K. E., Mavroukakis, S. A., White, D. E., and Rosenberg, S. A. 2002, Science, 298, 850.
104. Besser, M. J., Shapira-Frommer, R., Treves, A. J., Zippel, D., Itzhaki, O., Hershkovitz, L., Levy, D., Kubi, A., Hovav, E., Chermoshniuk, N., Shalmon, B., Hardan, I., Catane, R., Markel, G., Apter, S., Ben-Nun, A., Kuchuk, I., Shimoni, A., Nagler, A., and Schachter, J. 2010, Clin. Cancer Res., 16, 2646.
105. Dudley, M. E., Wunderlich, J. R., Yang, J. C., Sherry, R. M., Topalian, S. L., Restifo, N. P., Royal, R. E., Kammula, U., White, D. E., Mavroukakis, S. A., Rogers, L. J., Gracia, G. J., Jones, S. A., Mangiameli, D. P., Pelletier, M. M., Gea-Banacloche, J., Robinson, M. R., Berman, D. M., Filie, A. C., Abati, A., and Rosenberg, S. A. 2005, J. Clin. Oncol., 23, 2346.
106. Morgan, R. A., Dudley, M. E., Wunderlich, J. R., Hughes, M. S., Yang, J. C., Sherry, R. M., Royal, R. E., Topalian, S. L., Kammula, U. S., Restifo, N. P., Zheng, Z., Nahvi, A., de Vries, C. R., Rogers-Freezer, L. J., Mavroukakis, S. A., and Rosenberg, S. A. 2006, Science, 314, 126.
107. Eshhar, Z., Waks, T., Bendavid, A., and Schindler, D. G. 2001, J. Immunol. Methods, 248, 67.
108. Stauss, H. J., Cesco-Gaspere, M., Thomas, S., Hart, D. P., Xue, S. A., Holler, A., Wright, G., Perro, M., Little, A. M., Pospori, C., King, J., and Morris, E. C. 2007, Mol. Ther., 15, 1744.
109. Porter, D. L., Levine, B. L., Kalos, M., Bagg, A., and June, C. H. 2011, New England Journal of Medicine, 365, 725.
110. Berger, C., Flowers, M. E., Warren, E. H., and Riddell, S. R. 2006, Blood, 107, 2294.