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Abstract

With 23 approvals in the US and other countries and 4 approvals outside US, antibodies are now widely recognized as therapeutic molecules. The therapeutic and commercial successes met by rituximab, trastuzumab, cetuximab and other mAbs have inspired antibody engineers to improve the efficacy of these molecules. Consequently, a new wave of antibodies with engineered Fc leading to much higher effector functions such as antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity is being evaluated in the clinic, and several approvals are expected soon. In addition, research on a different class of antibody therapeutics, bispecific antibodies, has recently led to outstanding clinical results, and the first approval of the bispecific antibody catumaxomab, a T cell retargeting agent that was approved in the European Union in April 2009. This review describes the most recent advances and clinical study results in the field of bispecific antibodies, a new class of molecules that might outshine conventional mAbs as cancer immunotherapeutics in a near future.

MESH Keywords Animals ; Antibodies, Bispecific ; immunology ; therapeutic use ; Antibody-Dependent Cell Cytotoxicity ; genetics ; immunology ; Drug Approval ; Europe ; Genetic Engineering ; Humans ; Immunoglobulin Fc Fragments ; genetics ; immunology ; Neoplasms ; immunology ; therapy ; United States

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Introduction

Monoclonal antibodies (mAbs) are endowed with exquisite specificities. Since 1975, when Kohler and Milstein published an efficient way of producing these molecules,1 they have raised many hopes for the development of novel therapies, particularly as cancer treatments. However, extensive optimization through antibody engineering was required before effective IgG molecules could be produced; the first anti-tumor mAb, rituximab, was finally approved in 1997. Since then, a total of nine mAbs have been approved for cancer therapy in the US and other countries.2 These molecules are generally very well-tolerated and lead to significant clinical results, especially in the case of hematologic malignancies, as seen with rituximab. Unfortunately, none of them are able to cure cancer as single agent. Several clinical outcomes and animal studies have highlighted major limitations in their modes of action, including redundancy of molecular pathways leading to cancer cell survival, effects of the microenvironment, suboptimal interaction with effector cells due to alternative Fc glycosylation or Fc receptor polymorphism, activation of inhibitory receptors, and competition with circulating IgG.2 However, as hypothesized very early,3 many mAb shortcomings could be overcome by creating bispecific antibodies (bsAbs) capable of simultaneous binding to two different targets. Such molecules would be capable of retargeting a large variety of payloads to cancer cells. The potential of this approach has been demonstrated by several studies over the years, but the difficulty of producing large amounts of homogenous bsAbs using the available techniques (e.g., hybrid hybridomas, chemical cross-linking) hindered wider adoption and development of this approach. However, using advanced antibody engineering, new recombinant formats have been designed and validated to a certain extent. These formats include tandem scFv, diabodies, tandem diabodies, dual variable domain antibodies, and heterodimerization using a motif such as CH1/Ck domain or the Dock and Lock motif (reviewed in Ref. 4). The development of single domain antibodies from Camelid antibodies or engineered VH domain should also facilitate design of improved antibody therapeutics.5 However, few candidates based on these formats have reached the clinic. This review focuses on novel antibody formats of particular interest, highlighting triomabs and BiTEs, which are two formats that have yielded outstanding results in recent clinical trials.

First generation bsAbs: chemically cross-linked bispecific antibodies

The potential of using bispecific antibodies to retarget effector cells toward tumor cells was demonstrated in the 1980s, 3 , 6 , 7 and, several Phase 1clinical studies were launched in the early nineties. These early bispecific molecules were mainly generated using either of two approaches, chemical cross-linking, or hybrid hybridomas or quadromas. Despite some obvious biological effects, none of these approaches led to a significant impact in the clinical course of the disease.8 The first studies of bsAbs highlighted two major limitations of the first generation molecules, including the difficulty of producing large, homogeneous batches, and the lack of efficacy of murine antibody fragments. Human anti-mouse antibody (HAMA) responses were seen in most treated patients, which severely decreased the efficacy of the murine molecules and excluded the possibility of multiple administrations.

A series of clinical trials were also performed with chemically linked bispecific (Fab′)2 molecules targeting the breast and ovarian cancer tumor antigens HER2 or EGFR, 9 –12 which are overexpressed in many epithelial tumors such as colorectal, head and neck, bladder, renal, non-small cell lung carcinoma. The second specificity of these bsAbs was directed against FcγRI (CD64), which is notably expressed on monocytes and macrophages and up-regulated upon activation on neutrophils. Since this last population represents 60–70 % of leukocytes, co-administration of granulocyte-colony stimulating factor (G-CSF) was thought to enhance the activity of the injected bsAb. Biological effects were seen in some clinical trials of bsAbs MDX-210 (targeting Her2 and CD64), MDX-H210 (humanized version of MDX-210) and MDX-447 (targeting EGFR and CD64), but none of these treatments led to consistent antitumor activity.9 –12 These results might be explained by preclinical data for MDX-210 indicating that measurable tumor-cell lysis required high bsAb concentrations (0.1–1 $mg.mL^{-1}$) and effector-to-target cell ratios of at least 40:1, even when human neutrophils that had been prestimulated with IFN-γ and G-CSF were used.13 More encouraging results were obtained in two clinical trials involving HRS-3/A9, a bispecific F(ab′)2 antibody targeting the CD30 antigen on Hodgkin and Reed-Sternberg cells in patients with Hodgkin Disease (HD), and receptor Fcγ RIII (CD16) expressed by natural killer and macrophages. Two Phase 1 clinical studies performed with this molecule led to one complete remission (CR) and one partial remission (PR) in a group of 15 treated patients;14 this was followed by one CR and three PR in a second clinical trial.15 The construct was the first instance of bsAb treatment leading to a complete remission. However, the low production yield (2.8 g of bsAb obtained from 44 g of IgG) and the high immunogenicity of this bsAb precluded further clinical studies.

A different approach, and indeed the most obvious application of bsAb, is T cell retargeting. Cytotoxic T cells are considered the most potent killer cells of the immune system. They are abundant, efficiently proliferate upon activation, capable of killing multiple times, 16 and efficiently infiltrate tumors, but do not express Fcγ receptors. The idea of using T cells to efficiently kill tumor cells using bsAb emerged in the 1980.3 Bispecific antibodies directed against a tumor marker and CD3 have the potential to redirect and activate any circulating T cells against tumors. However, T cells have a major drawback. Without the secondary signal given by the interaction between CD28 and one of its ligands (e.g., B7), T cells are not fully activated, and might even become anergic.17 The first anti-CD3 bsAbs were thus administered in combination with anti-CD28 antibodies, but the combination yielded mixed results.18

Alternatively, it should be possible to pre-stimulate the effector T cells. Surprisingly, treatment with bsAbs of the most basic format, i.e., chemical cross-linking of full size monoclonal antibodies, yielded some success in clinical studies. Such antibodies were primarily developed for use with polyclonally activated T cells (PACTs); bsAb-loaded PACTs were generated ex vivo and then administered to patients. . Specifically, the patient's T cells are purified, expanded and activated in vitro to very large amount (>300×10^9). Next, these cells are incubated with an anti-CD3 × tumor target bispecific antibody, before being administered to the patient. This approach has the advantage of avoiding a direct systemic injection of bsAb in the patient, thereby significantly minimizing toxicity associated with free murine Fc bearing molecules.19 The approach also provides a large amount of activated and armed T cells artificially redirected to tumor cells, and has been applied to arm patient PACTs for targeting breast and hormone-refractory prostate cancers, non-Hodgkin lymphoma (NHL), EGFR+ cancers, and CA-125+ ovarian cancers.

Interestingly, it was shown that bsAb-retargeted PACTs can divide and secrete cytokines upon restimulation with target cells, while retaining their specificity and cytotoxicity for more than two weeks, which might be an important feature for therapeutic settings.20 A Phase 1/2 clinical trial using Her2Bi (OKT3 × trastuzumab)-armed activated T cells for the treatment of breast cancer and prostate cancer has resulted in partial responses persisting for two months following completion. In addition, a new Phase 1 clinical study of autologous activated T cells retargeted by CD20bi (OKT3 × rituximab) in combination with chemotherapy for the treatment of multiple myeloma was recently designed. The treatment will be followed by autologous peripheral blood stem cell transplantation to replace the blood-forming cells that were destroyed by the chemotherapy (ClinicalTrials.gov Identifier NCT00938626).

TriomAbs

Triomabs probably represent one of the most impressive and unexpected success in the field of bispecific antibodies. In 1995, Lindhofer and collaborators published a paper describing a major improvement of the classical quadroma approach to produce bsAbs.21 By using an original subclass combination (mouse IgG2a and rat IgG2b), they demonstrated a preferential species-restricted heavy/light chain pairing, in contrast to the random pairing in conventional mouse/mouse or rat/rat quadromas, as well as use of sequential pH elution on protein A to easily separate the desired bsAb from the parental mAb. Surprisingly, the resulting hybrid rat/mouse Fc portion efficiently interacted with activating human Fc receptors (FcγRI and FcγRIII), but not inhibitory ones (FcγRIIB), thereby reaching the goal that other groups had hoped to achieve using human Fc engineering.22 , 23 The investigators used this approach to create an anti-CD3 × anti-EpCAM bsAb, and demonstrated that this antibody was capable of binding to target cells and human T cells, but was also capable of activating dendritic cells (DC), inducing NK-dependent ADCC and stimulating tumor cell phagocytosis by macrophages. 22 , 23 In short, this Fc adds two crucial functions to regular anti-CD3 × target bsAbs: additive tumor killing capabilities through the efficient recruitment of macrophages and NK cells, and, most importantly, efficient co-stimulation of T cells through direct contact with accessory cells such as macrophages and DC (B7/CD28, CD40/CD40L, LFA3/CD2) or cytokine secretion (IL-2, IL-6, IL-12).

Catumaxomab (Removab®)

CatumaxomAb, which targets the tumor antigen EpCAM, was the first triomab produced. EpCAM (CD326) is expressed on essentially all human adenocarcinoma, certain squamous cell carcinoma, retinoblastoma, and hepatocellular carcinoma. EpCAM is also expressed in normal cells, but is predominantly located in intercellular spaces where epithelial cells form very tight junctions. It is thought that EpCAM is sequestered on epithelia, and thus much less accessible to antibodies compared to EpCAM in cancer tissue, where it is homogeneously distributed on the cancer cell surface. Moreover, EpCAM is very often on cancer stem cells, a very attractive feature for a tumor marker.24

In vitro and in vivo preclinical data demonstrated that a mouse surrogate of this triomab, targeting mouse CD3 and human EpCAM, was able to kill tumor cells very efficiently, at low concentration (10 pM range), without any additional costimulation of effector cells. In animal studies, 100% of mice administered an injection of 4 μg of triomabs survived after intraperitoneal (IP) injection of a lethal dose of EpCAM+ melanoma cells.25 Moreover, initial treatment of the tumor with this bsAb led not only to total tumor eradication, but also to the induction of immune protection. All mice rechallenged on day 144 after the primary challenge were still able to reject the tumor, indicating the high efficacy and long duration of the antitumor response. This effect was dependent on the presence of the hybrid Fc, and involved a humoral response directed against the non-transfected (EPCAM^{-}) tumor cells.

In a Phase 1/2 clinical trial, patients in ovarian cancer patients with malignant ascites were treated with IP administration of triomab (4 to 5 doses of 5 to 200 μg); 22/23 patients did not require paracentesis between the last infusion and the end of study at day 37. Tumor cell monitoring revealed a reduction of EpCAM-positive malignant cells in ascites by up to 5 logs.26 By early 2009, the results of a large international Phase 2/3 pivotal study involving 258 patients demonstrated a statistically significant improvement of the primary endpoint, puncture-free survival. Patients receiving catumaxomab had a four-fold increase in puncture-free survival compared to those receiving paracentesis therapy only. Below the maximum tolerated dose (200 μg), side effects, including fever, nausea and vomiting, were predictable, limited, manageable and mostly fully transient.27 Consequently, the European commission approved catumaxomab in April 2009 for the treatment of malignant ascites in patients with EpCAM positive carcinomas in cases where standard therapy is not available or no longer feasible. This first marketing approval clearly represents an important milestone in the field of bispecific antibodies. It is also interesting to note that the high immunogenicity of this rat/mouse hybrid molecule did not constitute a major issue. Moderate anti mouse and anti rat responses were seen in the majority of the patients but they do not seem to affect the efficiency of the treatment.26 This is probably due to the extremely small amounts administered to the patients (around 100 μg, compared to 3 g for rituximab, i.e., 30,000 fold less), the short duration of the treatment (10 days), and probably to the IP route of administration. However, Intravenous injections will be required for other indications. In a phase I study for the treatment of non-small cell lung cancer patients, it was established that the maximum tolerated dose for multiple catumaxomab i.v. administration was 5 μg, together with a pre-medication of 40 mg dexamethasone and antihistamines.28 This low amount probably reflects the mode of action of triomabs, potentially leading to tumor-cell independent cross-linking of T-cells with accessory cells followed by cytokine release-related symptoms. At this dose, no patient developed HAMA or HARA within 28 days after the single dose treatment. Thus, repeated administration of the antibody can be considered in the future and must be investigated in further clinical trials. Although survival analysis was not the primary endpoint, the survival observed in this study was very favorable with several advanced patients still alive 28 months after catumaxomab treatment. Future randomized trials are needed to determine if these low amounts of bsAb will be enough to achieve a therapeutic effect in this setting.

Ertumaxomab (rexomun®)

Ertumaxomab is triomab targeting HER2, a well-characterized breast tumor marker that is also targeted trastuzumab (Herceptin). This triomab possesses the same hybrid Fc portion as catumaxomab, and displays the same efficiency in vitro.29 Ertumaxomab was also compared to trastuzumab for its ability to kill tumor cells expressing various level of HER2. Exposure to ertumaxomab led to the efficient lysis of cells expressing very low amount of tumor antigen, whereas trastuzumab was completely ineffective even at high concentration.30 This difference can probably be explained by the mode of action of these antibodies. Trastuzumab triggers NK-mediated ADCC while ertumaxomab relies on T cell mediated killing and the interaction between T cells and accessory cells, as demonstrated by the release of proinflammatory cytokines such as IL-6, IFN-γ and TNF-α.

In an early clinical trial of patients with malignant ascites due to peritoneal carcinomatosis, administration of total doses of ertumaxomab as low as 40–140 μg lead to a complete elimination of tumor cells in ascites, and disappearance of ascites accumulation in all patients.29 This antibody is currently in Phase 2 trials for the treatment of metastatic breast cancer.

Bi20 (Lymphomun™ or fBTA05)

Bi20 triomab utilizes the same hybrid Fc portion as catumaxomab and ertumaxomab, and targets CD20. Expressed on all stages of B cell development from pre-B cells through memory cells, but not on either pro-B cells or plasma cells, CD20 represents an attractive target for the treatment of B cell malignancies. Notably, CD20 is targeted by rituximab. In vitro, Bi20 was shown to mediate an efficient and specific lysis of Bcell lines and B cells with low CD20 expression levels that were derived from chronic lymphocytic leukemia (CLL) patients.31 Remarkably, T cell activation and tumor cell killing occurred in an entirely autologous setting, i.e., without additional effector cells, in 5 of 8 samples. In comparison, rituximab demonstrated a significantly lower B cell eradication rate.31 In a pilot study, Bi20 was administered to six patients with recurrent B cell malignancies, CLL or highly malignant lymphoma after allo-stem cell transplantation. All alemtuzumab- and rituximab-refractory patients showed a prompt but transient clinical and hematological response, highlighting the high therapeutic potential of this triomab. Bi20 is currently in Phase 1 clinical study.32

Of note, two other triomabs, targeting melanoma-associated proteoglycans or melanomaassociated gangliosides (GD2 and GD3),33 are in preclinicalstudies as potential treatments for malignant melanoma.

The new generation: recombinant bsAbs

In the nineties, advances in antibody engineering provided innovative solutions antibody design problems, allowing production of small antibody fragments recapitulating the entire binding activity of the parent molecule [Fig. 1]. One fragment, the single chain variable fragment (scFv), made by association of the heavy and light variable domain through a peptidic linker, has especially inspired antibody engineers. Two formats have been intensively studied. Tandem scFvs (TaFv) are two scFv fragments linked via an extra peptide linker that is expected to confer good flexibility to each fragment. Although difficult to produce in E. coli , this 60 kDa molecule is well-expressed by mammalian cells, and. By reducing the length of the peptide linker between variable domains so that these cannot assemble, it is possible to force the pairing of domains between two different polypeptides, leading to a compact bsAbs called diabody (Db).34 These molecules can be expressed at high yields in bacteria, and have been shown by crystallography experiments to adopt several conformations.35 This format has been improved by adding an extra peptide linker between the two polypeptides in order to further decrease the amount of homodimers, yielding fragments called single chain diabodies (scDb). Numerous studies have demonstrated the potency of these formats in preclinical studies.36 Examples of bsAb fragments with potential as therapeutic candidates include bispecific anti-CD19 × CD3 and anti-CD19 × CD16 diabodies that demonstrated a synergistic antitumor effect in a preclinical model of NHL,37 a promising anti-EGFR × CD3 diabody able to cure xenografted mice in combination with lymphokine activated killer cells,38 and the efficient treatment of xenografted mice bearing prostate cancer cell induced tumors using an anti-PSMA × CD3 diabody and peripheral blood lymphocytes.39 Surprisingly, no bispecific diabodies have been tested so far in clinical trials.

Although no bispecific diabodies have been administered to humans, tandem scFvs have been studied in clinical trials. One atypical molecule was rM28, a bispecific tandem molecule targeting the co-stimulatory molecule CD28 expressed on T cells and NG2, a melanoma-associated proteoglycan. This molecule spontaneously forms stable dimers, and was shown to be capable of inducing T-cell activation and effective tumor-cell killing in vitro and in vivo without a signal through the TCR/CD3 complex, a so called "targeted supra-agonistic stimulation".40 This effective mode of action was recently extended to the cytolysis of lymphoma cells using an anti-CD28 × CD20 bispecific tandem scFv endowed with the same supra-agonistic properties41 . In 2005, a Phase 1/2 clinical trial was conducted to analyze the safety and efficiency of intralesional administration of rM28 and autologous peripheral blood mononuclear cells in patients with metastatic stage III/IV melanoma and unresectable metastasis. However, in March 2006, TGN1412, a monospecific 'superagonistic' CD28 antibody induced systemic T-cell activation and severe cytokine release syndrome when injected into six healthy volunteers,42 and since then concerns have been raised about the use of immunomodulatory molecules. However, it should be noted that a fundamental difference exists between TGN1412 and bispecific molecules such as rM28. Indeed, unlike the activity of TGN1412, supra-agonistic CD28 stimulation by rM28 was shown to be strictly target-cell restricted over a wide concentration range, i.e., not induced if NG2-expressing target cells are absent.43 This, in principle, should avoid side effects such as those observed after TGN1412 administration.

BiTEs: bispecific T cell Engagers

Another type of tandem scFvs aimed at activating T cells has met with more success in clinical trials. BiTEs, or bispecific T cell Engagers, are made by fusing an anti-CD3 scFv to an anti-TAA scFv via a short 5 residue peptide linker (GGGGS). In 1995, Kufer and collaborators produced such a tandem scFv targeting EpCAM (epithelial 17-1A antigen) and human CD3 in CHO cells. This new kind of bsAb proved to be highly cytotoxic at nanomolar concentrations against various cell lines, using unstimulated human PBMCs in the absence of co-signaling.44 Later, Löffler et al. published similar data obtained using a fusion between a murine anti-CD19 scFv and a murine anti-CD3 scFv.45 This molecule demonstrated outstanding in vitro properties, including efficient cytotoxicity induced by extremely low concentrations of bsAb (10–50 $pg.mL^{-1}$, i.e. subpicomolar), at low effector:target ratio (2:1), and in only four hours, without the need for pre-stimulation of T cells or, most surprisingly, the need of co-signaling (e.g., through CD28). These results were in marked contrast with the majority of published studies based on anti-CD3 bispecific constructs.

Since then, Baeuerle and collaborators have accumulated data demonstrating the properties of BiTEs.46 The most impressive property of this class of molecules, beside the absence of requirement for any kind of pre or co stimulation of effector T cells, is the low concentration required to achieve anti-tumor activity. Indeed, most tumor cell lines can be lysed in the presence of 0.2 to 2 pM of BiTEs for half maximal target cell lysis. In some cases, up to 18 fM was shown to be enough to induce in vitro cell lysis, suggesting that a low two digit number of BiTEs bound between T and target cells is sufficient to induce lysis.47 Moreover, BiTEs are capable of inducing efficient lysis at effector to target ratios (E:T) as low as 1:10. This suggests that BiTEs mediate serial killing of many target cells, and this was actually demonstrated using video assisted microscopy.16 Despite this extreme efficiency, the killing remains strictly target-cell dependent, and T cells cannot be activated in the absence of target cells, even at concentrations exceeding EC50 values by several thousand fold.48

The biological properties of BiTEs are not yet clearly understood. In the case of molecules targeting CD19 on B cells, one cannot exclude a possible co-signal triggered by the interaction between CD28 and B7, known to be expressed on normal and malignant B cells. However, BiTE molecules targeting EpCAM expressed on a variety of solid tumor of epithelial origin that do not express B7 show similar efficacy.

BiTEs have been demonstrated to induce immunological cytolytic synapses identical to synapses induced by regular T cell stimuli,49 even in the absence of MHC class I molecules, as shown by the lysis of Ep-CAM-expressing K562 cells or transfected rodent cells expressing the target antigen by human effector cells. The small size (60 kDa) of BiTEs, which ensures close proximity of T cells and target cell membranes, might therefore be responsible for their high efficiency by leading to the active displacement of negative regulatory proteins from the forming synapse, as demonstrated in the case of CD45.47 It should be stressed that the possibility to efficiently form cytolytic synapses by a mere interaction between CD3 and a target antigen via a BiTE molecule, in the absence of MHC class I/TCR interaction, has the potential to overcome many tumor cell escape mechanisms such as MHC class I, proteasome and intracellular peptide transporter (TAP1 and 2) down-regulation.

The absence of a need for co-signaling through CD28 for these BiTEs might also be explained by the observation that among all T cell subtypes, $CD8^+$ effector memory cells $CD45RO^+$ (TEM) and $CD8^+$ effector memory $CD45RA^+$ (TEMRA) contribute the most to BiTE activity, whereas naïve T cells do not contribute at all to the killing efficiency.50 It is believed that memory T cells do not require CD28 costimulation for expansion during secondary responses, which could explain the efficiency of BiTEs. However, this dogma has recently been challenged.51

Blinatumomab (MT103)

Blinatumomab, a murine anti-human CD3 × anti-human CD19 was the first BiTE developed and is the most advanced BiTE in clinical trials. The candidate is being studied as a treatment of lymphoma and leukemia. In preclinical trials, Dreier et al. demonstrated that submicrogram amounts of this molecule were sufficient to prevent tumor growth in a mouse model.52 Later, studies performed in chimpanzees showed that repeated 2 h treatments with doses as low as 0.1 $\mu g.kg^{-1}$ were well-tolerated, and led to cumulative depletion of peripheral B cells due to fully reversible T cell activation.53 In 2008, the first result of a Phase 1 clinical study indicated that doses as low as 5 μg per square meter per day in relapsed NHL patients led to an elimination of target cells in blood. All seven patients treated at a dose level of 60 μg experienced a tumor regression.54 Because of its small size (60 kDa), blinatumomab is characterized by a short serum half life of several hours, and so continuous intravenous infusion by portable mini-pumps is required. It should be highlighted that cumulative doses of several milligrams were sufficient to lead to the notable response in patients, whereas conventional antibody treatments, such as rituximab, consume gram amounts per treatment cycle.

MT103 is also currently being tested in a Phase 2 trial in patients with B-precursor acute lymphoblastic leukemia (B-ALL) having minimal residual disease in their bone marrow. The first results from an ongoing Phase 2 trial in patients with B-ALL indicate that T cells engaged by blinatumomab are able to locate and eradicate rare disseminated tumor cells in the bone marrow that can only be detected by quantitative PCR assays detecting tumor cell-specific genomic aberrations. Thirteen out of 16 evaluable patients (81%) became minimum residual disease (MRD) negative, with ongoing responses lasting for up to 47 weeks on May the 25^{th} 2009 (data presented in Recombinant Antibodies meeting, June 2009, Cologne). This result is remarkable since MRD positive ALL patients have very limited treatment options, and around 60% of patients die within 2 years, with a median time to hematological relapse of only 4.1 months. Interestingly, this trial was associated with significantly reduced toxicity compared to NHL patients at the same dose level, suggesting that toxicity might be correlated to the number of target cells present in patients, rather than to the BiTE molecule itself.

MT110

MT110, an anti-human EpCAM × anti-human CD3 TaFv, was the second BiTE tested in clinical trial, and the first directed to a wide spectrum of solid tumors. In vitro characterizations of MT110 have recapitulated the results obtained with MT103 on tumor cell lines, thereby demonstrating the generality of the BiTE format.55 A study in NOD/SCID mice demonstrated that as little as 100 ng of MT110 can prevent tumor outgrowth after an injection of a 1:1 ratio of human colon carcinoma cells mixed with unstimulated human peripheral mononuclear cells. MT110 was also active against human ovarian metastatic tissues grafted in immunodeficient mice in the absence of human peripheral mononuclear cells. Since MT110 does not activate murine T cells, this result suggests that tumor resident human T cells, being tolerized or anergized, could be reactivated, and were present in sufficient number to eliminate the xenograft.56 Preclinical data from studies with muS110, a BiTE surrogate targeting murine EpCAM and murine CD3 demonstrated that EpCAM-specific BiTE antibodies can effectively discriminate between target antigen expressed on tumor and normal epithelial tissue.57 MT110 is currently being tested in a Phase 1 study with lung, colorectal and gastrointestinal cancer patients; initial results are expected by the end of 2009.

Other BiTEs in the pipeline

MT111/MEDI-565 targets the carcinoembryonic antigen (CEA, also called CEACAM5), a widely expressed tumor antigen highly expressed in colorectal cancer, and also in a substantial proportion of carcinomas of the lung, pancreas, stomach, ovary, uterus, breast, and a subset of melanomas.58 However, CEA can be shed by phospholipases from the cell surface through cleavage of its glycosylphosphatidylinositol-linkage, which causes the protein to be released in the circulation. This soluble CEA (sCEA) might therefore interfere with antibody based therapies. To test this hypothesis, Lutterbuese et al. compared the activity of several anti-CEA × anti-CD3 BiTE in the absence or presence of sCEA, and found that the cytotoxic activity of some of these molecules was not competitively inhibited by sCEA at concentrations that exceeded levels found in the serum of most cancer patients.59 MT111 is therefore a promising molecule soon to be tested in clinical trials.

Two other approaches to rapidly generate new relevant BiTEs are also being pursued by Micromet. The company has elaborated a platform allowing the selection of human scFvs against new tumor antigens and against CD3. Interestingly, these new scFvs are directly selected to be cross-reactive with orthologous antigens of non-human primates, allowing straightforward assessment of the molecule safety and pharmacology. Examples of fully human BiTEs in discovery that were obtained this way include BiTEs that target CD33, a protein expressed in > 90% of acute myeloid leukemia, including cancer stem cells, and melanoma associated chondroitin sulfate proteoglycan, a 450 kDa antigen expressed in >90% of all melanomas. Both BiTEs have already been investigated in studies with macaque monkeys that have validating these new molecules, both in terms of efficacy and safety.60 A second approach involves the reformatting of approved therapeutic antibodies as BiTE molecules. Examples include trastuzumab, panitumumab (Vectibix), cetuximab (Erbitux) and omalizumab (Xolair), leading to efficient BiTEs against HER2, EGFR and IgE, that have EC50 values between 90 fM and 3.6 pM. These molecules should rapidly enter preclinical phases.

DNL antibodies for radioimmunotherapy

The Dock and Lock (DNL) method was originally published in 2006,61 and represents a convenient and efficient way to create bispecific antibodies. It relies on the spontaneous association of a dimer of the 45 amino acids peptide DDD2, derived from the regulatory subunit of human cAMP-dependent protein kinase (PKA) with the 21 residues peptide AD2, derived from the anchoring domains (AD) of human A kinase anchor proteins (AKAPs). Upon association, two disulfide bonds are created, resulting in a covalent complex that is stable for more than a week at 37°C in human serum. This approach can be used to efficiently create any kind of bsAb but was so far used to create bispecific molecules dedicated to radioimmunotherapy.

TF2

The first described bsAb built using the DNL method was a molecule of 157 kDa, devoid of Fc fragment, comprising two Fab fragments derived from humanized anti-human CEA mAb hMN-14 and one Fab fragment from humanized mAb h679 that is able to strongly bind the hapten histamine-succinyl-glycine (HSG). This bispecific Tri-Fab molecule named TF2 was used for tumor imaging purposes in xenografted nude mice, based on a previously validated pretargeted approach where the bsAb is first injected, then the ^{99m} Tc-labeled hapten is injected after the bsAb has cleared from the circulation. Exceptionally high tumor blood ratios were observed, ranging from 13 at 0.5 h to 395 at 24 h, with high tumor uptake of 30% ID/g, whereas the levels in blood and normal tissues, except kidney, were <1% ID/g at1 h after injection. These outstanding results obtained in imaging approaches prompted the use of this bsAb for therapy. In order to take advantage of possible dimerization of the bsAb on the surface of target cells (a process named affinity enhancement system) to increase tumor retention, a new peptide; called IMP-288, was designed to contain two HSG epitopes.62 The divalent peptide was labeled with Lutetium 177 for use in a Phase 1 clinical study involving patients with CEA expressing advanced colorectal tumors that was initiated in July 2009. ^{177} Lu is a very promising radionuclide for targeted radiotherapy because its relatively long half-life of 6.71 days allows employment of sophisticated procedures to synthesize and purify the radiopharmaceutical, and allows transport to more distant customers. Furthermore, the emitted β radiation with a maximum energy of 498 keV is effective in destroying small tumors and metastasis while sparing normal tissue, and its γ-rays are of low energy (113 and 208 keV) and abundance (7% and 11%), which simplifies radiation protection and patient-handling issues. Thus, the combination of bsAb-enabled pretargeting approaches and this radionuclide might have a high therapeutic potential.

Other DNL based bsAbs in the pipeline

TF4 is a second bsAb built using the DNL method, directed against CD20 for the treatment of NHL. Studies performed in xenografted nude mice that compared TF4 as a pretargeting agent of an ^{111} In-HSG-peptide with a one step approach using the parental anti-CD20 mAb (hA20) directly labeled with ^{90} Y demonstrated an impressive 1600 fold improvement of the tumor-to-blood ratio, as well as a 1.6 fold improvement of tumor uptake.63 This molecule should soon enter Phase 1 clinical study.

TF10 is another tri-Fab that is directed against MUC-1 and the HSG hapten. PAM-4, the parental anti-MUC-1 antibody has been shown to bind a MUC1 epitope that is not detected in normal pancreas but is expressed in 87% of invasive pancreatic adenocarcinomas, including early stage 1 disease.64 When compared with its parental mAb in studies conducted in nude mice bearing CaPan1 human pancreatic cancer xenografts, TF10 led to much greater tumor/blood ratios of ^{111} In-IMP-288 (1,000:1 at 3 hours) compared to ^{111} In-PAM4-IgG (5:1 at 24 hours). The high therapeutic potential of this approach should thus soon be tested in the clinic.

Conclusion

After years of disappointment and frustrations, clinical trials of bsAbs are finally providing exciting results, with the most impressive ones being delivered by triomab and BiTE molecules. These results do not represent a mere improvement compared to those obtained by approved therapeutic antibodies, but are a real leap in terms of therapeutic efficiency. For the first time, the possibility of actually curing patients using antibodies seems within reach. Further randomized studies are eagerly awaited to demonstrate whether these encouraging results from Phase 1 and 2 trials will result in prolonged overall survival. Interestingly, after a race toward fully human antibody molecules experienced during the last decade, these impressive biological activities were obtained with fully murine molecules which, because of their high efficiency, can be injected in doses four to five log lower than those usually used by therapeutic mAbs. In the case of BiTEs, the short half life traditionally perceived as a limitation was actually exploited to achieve an exquisite control of drug levels in patients using mini-pump devices developed for insulin delivery.

By choosing to develop treatments for malignant ascites and acute lymphoblastic leukemia, Trion pharma, which produces triomabs, and Micromet, which produces BiTE molecules, have decided to pursue approval for niche indications. This approach is likely to be the fastest and safest route to approval. There is no doubt that both companies have promising candidates with very large potential markets, and that candidates against several malignancies will soon be tested in clinical trials. With these developments, it might well be that we are currently experiencing a turning point in the field of bispecific antibody, and more generally of cancer immunotherapy.

Abbreviation

mAb : monoclonal antibodies
bsAbs : bispecific antibodies
ADCC : antibody dependent cell mediated cytotoxicity
scFv : single chain Fv fragment
BiTE : bispecific T cell engager
MHC : major histocompatibility
DC : dendritic cells
NK : natural killer cells
TEM : T effector memory
MRD : minimum residual disease

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Figure 1

A conventional antibody is depicted in green (light for light chain, dark for heavy chain, blue triangle indicate the glycosylation site) and the derived fragments (shaded areas represent the binding sites). The orange color symbolizes a different specificity. The blue and red shapes represent the DDD2 and AD2 peptides of the dock and lock (DNL) method. All flexible linkers are in grey. bsAb: bispecific antibody. bsFab: bispecific Fab fragment. scFv: single chain Fv fragment. dAb: domain antibody.

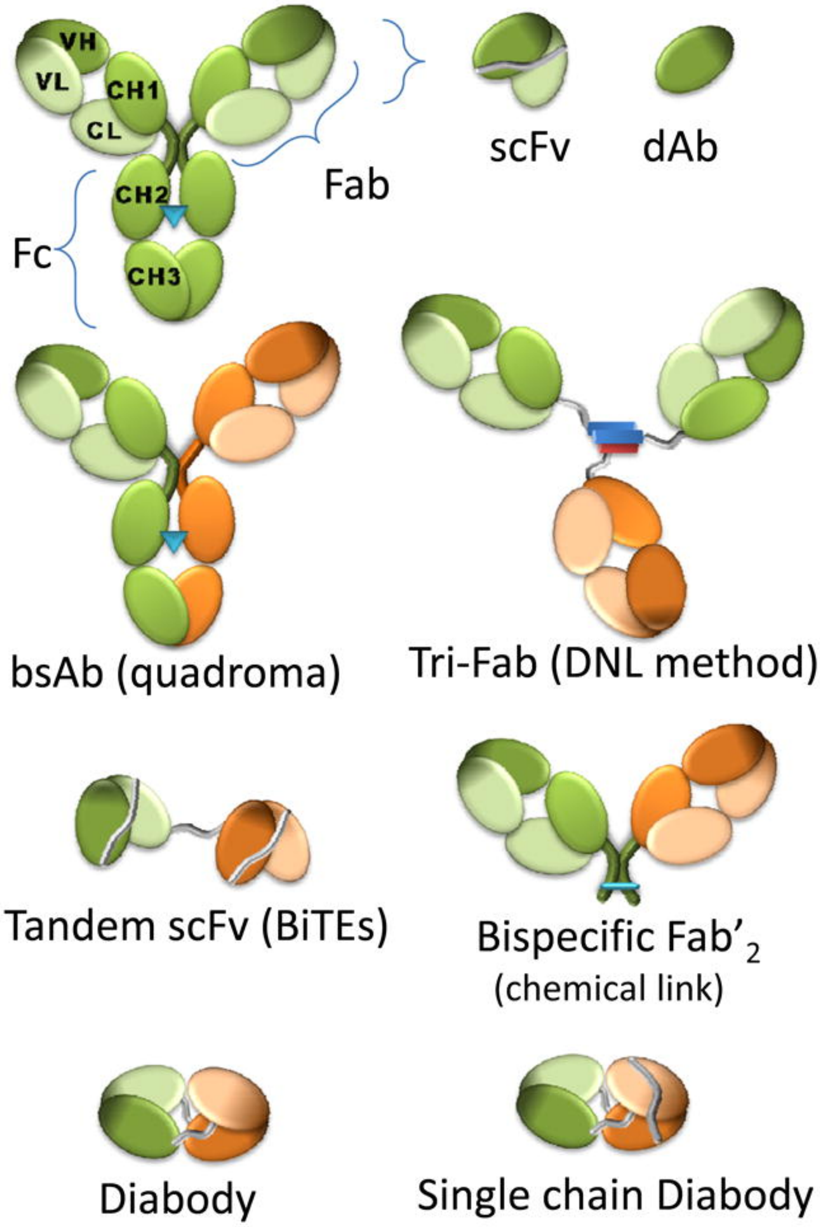

Table 1

Most recent clinical trials involving the use of bispecific antibodies.

Name	Format	Target	Cancer type	Stage	Ref.
MDX-210	(Fab′)2	HER2 × CD64	Breast/ovarian	Phase 1	13
MDX-H210	(Fab′)2	HER2 × CD64	Breast/ovarian	Phase 1	9
MDX-447	(Fab′)2	EGFR × CD64	Lung, colorectal…	Phase 1	12
HRS-3/A9	(Fab′)2	CD30 × CD16	Hodgkin Disease	Phase 1	14, 15
Her2Bi	Cross-linked IgGs	HER2 × CD3	Breast/prostate	Phase 1/2	19
CD20Bi	Cross-linked IgGs	CD20 × CD3	Multiple myeloma	Phase 1	*
Catumaxomab	Triomab	EpCAM × CD3	Malignant ascites	EMEA appr.	27
Ertumaxomab	Triomab	HER2 × CD3	Metastatic breast	Phase 2	29
Bi20	Triomab	CD20 × CD3	B-cell malignancies	Phase 1	32
rM28	Dimeric TaFv	NG2 × CD28	Melanoma	Phase 1/2	40
Blinatumomab	BiTE	D19 × CD3	HL and B-ALL	Phase 1 and 2	54
MT110	BiTE	EpCAM × CD3	Lung, colorectal…	Phase 1	55
TF2	DNL triFab	CEA × HSG	Colorectal	Phase 1	61

* ClinicalTrials.gov Identifier: NCT00938626