

Bispecific T-Cell Engaging Antibodies for Cancer Therapy

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Abstract

There is increasing evidence that T cells are able to control tumor growth and survival in cancer patients, both in early and late stages of the disease. However, tumor-specific T-cell responses are difficult to mount and sustain in cancer patients, and are limited by numerous immune escape mechanisms of tumor cells selected during immunoediting. An alternative approach to engage T cells for cancer therapy are antibodies, which are bispecific for a surface target antigen on cancer cells, and for CD3 on T cells. These are capable of connecting any kind of cytotoxic T cell to a cancer cell, independently of T-cell receptor specificity, costimulation, or peptide antigen presentation. Here, we review the principle of a new class of bispecific antibodies called BiTE (for “bispecific T-cell engager”) antibodies. Recent results from clinical studies with a CD19/CD3-bispecific BiTE antibody suggest that this therapeutic paradigm is finally showing promise for treatment of both bulky and minimal residual disease. [Cancer Res 2009;69(12):4941–4]

Evolution of Bispecific Antibodies

More than 25 years ago, monoclonal antibody and recombinant DNA technologies enabled the construction of antibody-based molecules that normally do not occur in nature. The first bispecific antibodies looked like normal immunoglobulin G (IgG) molecules but had their two binding arms equipped with distinct binding specificities. The concept of using such bispecific antibodies to engage cytotoxic T cells for cancer cell lysis was shown by Staerz and colleagues (1). Since then, different kinds of constructs were made for this purpose, the properties and limitations of which have been reviewed previously (2, 3). Likewise, bispecific antibodies for engagement of other cytotoxic immune cells were constructed, for instance, targeting Fcγ RI/CD64 on macrophages and Her2/neu or EGFR on tumor cells. Apparently, their efficacy in clinical trials has been insufficient for further development (4).

Further progress in the development of bispecific antibodies came from reduction of antibodies to their minimal binding domains. Because these are bipartite with variable fragments (Fv) coming from two different genes, a linker sequence was introduced in order to align the two variable domains on a single polypeptide chain, resulting in a so-called single-chain antibody (scFv) (ref. 5). This enabled combination with an additional linker sequence of two single-chain antibodies of different binding specificity in tandem, in such a way that four gene products are ultimately aligned on a single polypeptide chain of approximately 55 kDa (Fig. 1). Pioneering work by Kufer and colleagues (6) showed that

CD3/target antigen-bispecific antibodies of this particular design had an exceptionally high potency, and could likewise engage CD8⁺ and CD4⁺ T cells for redirected lysis of cancer cells at very low effector to target (E:T) ratios. By using just one CD3-specific binding arm, such bispecific antibodies can monovalently bind to all T cells, but do so only with rather low affinity (7), which will not trigger T-cell signaling by CD3, unless the BiTE antibody is presented to the T cell in a multivalent fashion by a target cell (8). This mode of action is being referred to as BiTE for “bispecific T-cell engager” technology. The technology and improved, newly developed BiTE antibodies are described in recent review articles (9, 10). BiTE antibodies consistently show a high potency of redirected lysis *in vitro*, and high antitumor activity in various animal models. Animal models were based on either immunodeficient severe combined immunodeficiency disease (SCID) or immunocompetent mice, and used as xenografts cancer tissue of patients, human cancer cell lines, or syngeneic murine cancer cell lines producing lung metastases, orthotopic, or subcutaneous tumors. Tested BiTE antibodies were either human- or murine-specific, or had a dual species specificity, referred to as “hybrid BiTE antibody.”

Two BiTE antibodies are currently being tested in clinical trials. Blinatumomab (also known as MT103) is bispecific for CD3 and CD19. It is currently being tested in a phase 1 trial in patients with late stage, relapsed non-Hodgkin's lymphoma (NHL) (ref. 11), and in a phase 2 trial in patients with B-precursor acute lymphoblastic leukemia (B-ALL) having minimal residual disease in their bone marrow (12). CD19 is a surface antigen suitable to address a wide range of B-cell malignancies. The other BiTE antibody in clinical development is called MT110 and is bispecific for CD3 and epithelial cell adhesion molecule (EpCAM) (ref. 13). It is currently being tested in a phase 1 trial with lung and gastrointestinal cancer patients. EpCAM is very frequently expressed on human adeno- and some squamous cell carcinoma, and also on cancer stem cells (14).

BiTE antibodies have so far been constructed to more than 10 different target antigens, including CD19, EpCAM, Her2/neu, EGFR, CD66e (or CEA, CEACAM5), CD33, EphA2, and MCSP (or HMW-MAA) (ref. 10). Key hallmarks of BiTE antibodies that, in their combination, distinguish them from other bispecific antibody constructs, include (1) a high potency of redirected lysis with EC50 values ranging from 0.1 to 50 pmol/L (2–1,000 pg/mL) (ref. 10), (2) strictly target cell-dependent activation of T cells (8), and (3) support of serial lysis by activated T cells, i.e., activity at low E:T ratios. BiTE antibodies are typically produced as recombinant, nonglycosylated proteins secreted by higher eukaryotic cell lines.

Why Engage T Cells by Bispecific Antibodies?

There is a choice of immunological effector cells for engagement by bispecific antibodies. With BiTE antibodies, we have selected T cells because of their high cytotoxic potential, abundance, search and destroy function, and evidence for their potential to initially control malignant diseases, which apparently is getting lost with

Note: BiTE is a registered trademark.

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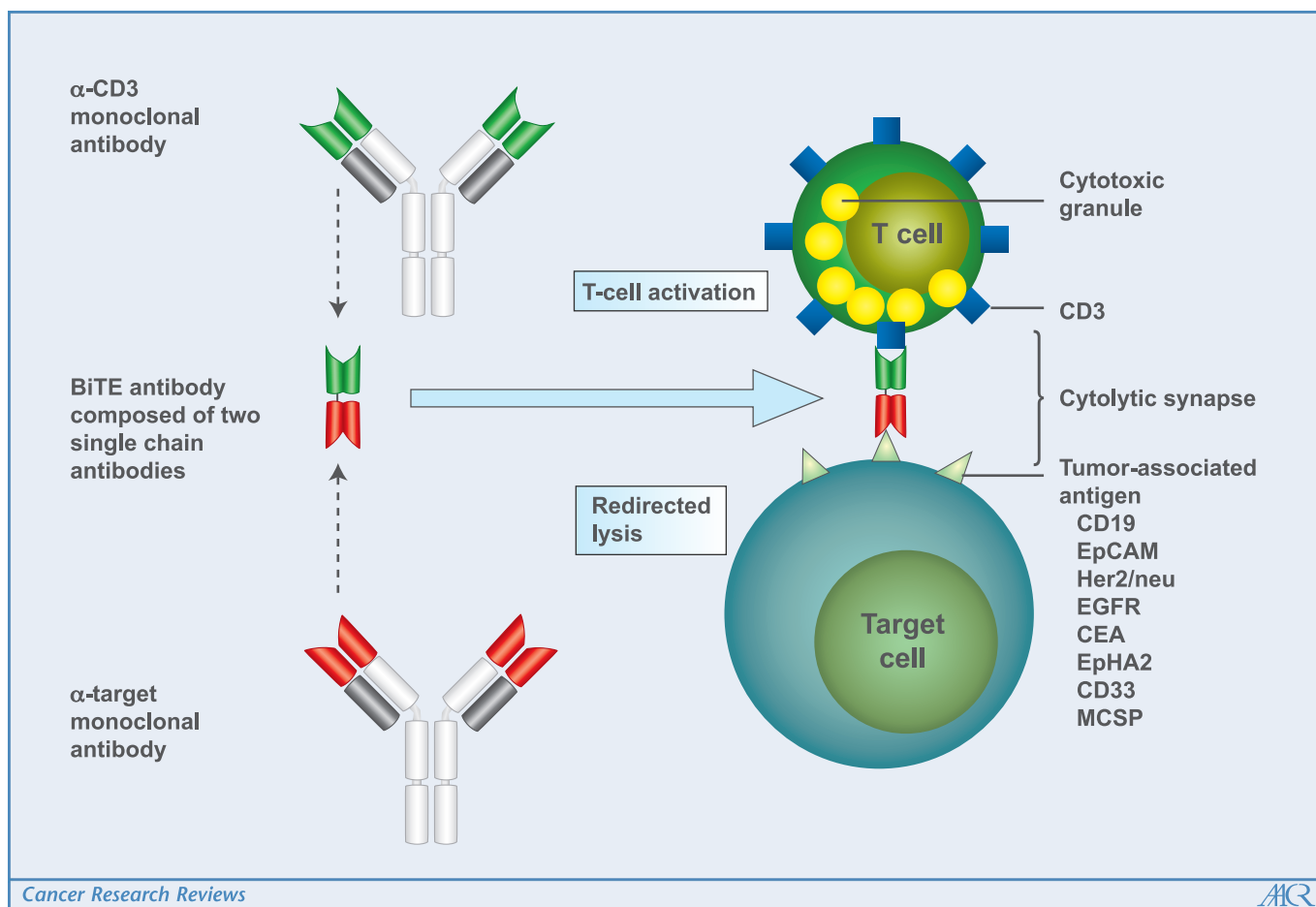


Figure 1. The BiTE antibody principle. Generation of a BiTE antibody from the variable domains of two distinct monoclonal antibodies is depicted on the left. The anti-CD3 specific single-chain antibody (green) is shared by all BiTE antibodies. The target antigen-specific single-chain antibody (red) is different for each BiTE antibody, and can recognize for instance CD19 (blinatumomab; MT103) or EpCAM (MT110). As shown on the right, a BiTE antibody can transiently connect a T cell and a cancer cell by simultaneously binding CD3 and a target antigen. This will trigger T-cell activation involving cytotoxic granule fusion, transient cytokine release, and proliferation. Redirected lysis of the attached cancer cell involves membrane perforation by perforin, and subsequent programmed cell death as induced by granzymes. Of note, BiTE-activated T cells recognize the same surface antigens as regular monoclonal antibodies.

disease progression. Apart from numerous reports on tumor rejection by T cells in animal models, the mere presence of T cells inside tumors can very significantly correlate with overall survival, as recently reported for patients with colon cancer (15) and NHL (16). A process called immunoeediting is gradually leading to a loss of control by T cells and other immune cells over cancer cells, most likely because cancer cells undergo Darwinian selection for immune escape mechanisms that predominantly interfere with antigen presentation, or T-cell activation or differentiation (17). The potential of T cells in treating even late-stage solid tumors is evident from clinical results treating melanoma by vaccines, adoptive T-cell transfer and cytokines (18), or using antibodies blocking CTLA-4, a negative regulator of T-cell activation (19). Likewise, the graft-versus-leukemia effect observed after allogeneic stem cell transplantation relies on the elimination of tumor cells by T cells originating from the grafted marrow cells. Other approaches in cancer patients to bolster specific T-cell responses such as administration of interleukin-2 (IL-2), granulocyte macrophage stimulating factor (GM-CSF), vaccines, or Toll receptor agonists have occasionally shown tumor regressions in clinical trials albeit with rather low response rates.

A limitation of all these approaches is that generation and sustained performance of tumor-specific T-cell clones is a very com-

plicated and tightly controlled process, which is frequently compromised in cancer patients. Moreover, even if active T-cell clones happen to appear with high frequency in cancer patients, they may no longer recognize tumor cells because, for instance, tumor cells with defective antigen presentation have been selected (20). The promise of BiTE antibodies is that their activity no longer relies on generation of specific T-cell clones or a regular antigen presentation by dendritic and tumor cells, which is why major immune escape mechanisms are unlikely to impact the approach. It has been shown that any existing T-cell clone can be engaged by a bispecific antibody to recognize a frequent surface antigen on the tumor cell, and that expression of MHC class I is not required (10). Moreover, not only cytotoxic $CD8^+$ T cells can be redirected for lysis, but also $CD4^+$ T cells. The latter show a dramatic upregulation of granzyme B when stimulated by BiTE-decorated tumor cells (8, 21). The impact of regulatory T cells on BiTE activity is under investigation.

Activity of Antibody-Engaged T Cells against Large Tumors and Disseminated Disease

The first clinical results with CD3/CD19-bispecific BiTE antibody blinatumomab from ongoing phase 1 and 2 studies have confirmed

that T cells can be potently engaged within cancer patients for redirected tumor cell lysis (11, 12). The BiTE antibody showed activity as a single-agent therapy in treating bulky disease in follicular and mantle cell lymphoma patients who had received multiple previous therapies, including repeated cycles of anti-CD20 antibodies in combination with standard chemotherapy. Objective complete and partial regressions of lymph node tumors were observed in relapsed NHL patients at doses as low as 0.015 mg blinatumomab per square meter per day (11). By continuous infusion over 4 to 8 weeks, steady state serum concentrations in patients at this dose level were below 1 ng/ml, indicating a dramatically higher potency of the BiTE antibody than regular IgG1 antibodies used in this indication, which typically require serum concentrations above 10 µg/mL. Complete target cell elimination in peripheral blood was seen at a dose level as low as 0.005 mg/m² per day. The activity observed with blinatumomab appeared to be dose-dependent, because only the dose level of 0.06 mg/m² per day made all seven patients respond with a tumor regression. In nine patients with bone marrow infiltration treated at 0.015 mg/m² per day and higher, biopsies revealed complete or partial clearance from tumor cells in all but one patient. In biopsies taken from one patient before and after treatment, tumor cell elimination was also observed in infiltrated liver tissue.

The majority of adverse events (AEs) occurred during the first few days of treatment. Leukopenia and/or lymphopenia, chills, pyrexia, and C-reactive protein increase were the most frequent AEs (11). In most cases, AEs normalized under continued treatment. Out of 40 patients, treatment was discontinued in nine patients, seven of which because of fully reversible symptoms of the central nervous system (CNS), such as confusion, disorientation, and speech disorder. It is likely that CNS events were related to a collateral effect of target cell elimination in the brain. In peripheral blood, blinatumomab caused a selective expansion of CD8⁺ and CD4⁺ effector memory T cells, which in *in vitro* studies is the T-cell population showing the highest cytotoxic potential with blinatumomab and other BiTE antibodies (9).

The first results from an ongoing phase 2 trial in patients with B-precursor acute lymphoblastic leukemia (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 (12). Residual tumor cells in the bone marrow of B-ALL patients (referred to as minimal residual disease, or MRD), as can be found after extensive chemotherapy, pose a very high risk of relapse and reduced survival when compared with MRD-negative patients. In four patients reported so far (12), three showed clearance of their bone marrow during the first 4-week cycle at a dose level of 0.015 mg blinatumomab/m² per day. Overall, the side effect profile of blinatumomab in B-ALL patients appeared to be very favorable. The most frequent AEs, such as fever and chills, were of only moderate intensity. CNS events have not occurred so far. This reduced toxicity compared with NHL patients at the same dose level may be due to a much lower amount of target cells present in this population of B-ALL patients.

The observation of antitumor activity of BiTE-activated T cells against both bulky and minimal residual disease is in line with a highly effective search-and-destroy and cytotoxic activity of T cells. Although elimination of just a few disseminated tumor cells in bone marrow or blood may be achieved with low numbers of existing T cells, elimination of larger tumor masses may require invasion and local proliferation of T cells. Completed and future

clinical trials will depict the full potential of cancer therapy by BiTE antibody-engaged T cells in various cancer indications.

Challenges for BiTE Antibodies

What could potentially stop a cytotoxic effector memory T cell from killing a tumor cell when it is transiently connected by a BiTE antibody? A significant down-modulation of CD3 on T cells by a BiTE antibody, in contrast to bivalent anti-CD3 antibodies, has not been observed in an animal model.¹ Exhaustion of cytotoxic granules of T cells during serial lysis may occur, but appears well balanced by upregulation of granzyme B and perforin expression as a consequence of BiTE-mediated activation (8, 21). On the tumor cell side, a major escape could result from selection of target antigen-negative cells, a problem that should likewise arise for conventional antibodies acting by ADCC, complement fixation, or receptor blockade. Targeting antigens that have been clinically validated by antibody-based therapies, are expressed at high level and frequency and occur on cancer stem cells, and those that cancer cells depend upon for survival and growth may minimize the risk of antigen loss.

BiTE-activated T cells were shown to kill cancer cells by membrane perforation using perforin, and by subsequent induction of apoptosis using granzyme B and, possibly, the activities of four more granzymes, each one with different protein substrate specificity (21). It is difficult to envision that anti-apoptotic proteins (e.g., Bcl-2) or protease inhibitors (e.g., serpins) within tumor cells can efficiently counteract the destructive activity of the highly toxic protein cocktail delivered by cytotoxic granules of T cells. Future studies will be designed to look for BiTE-resistant tumor cells and, should they become selected under therapy, study their phenotype. Of note, apart from surface antigen loss, the same resistance mechanism would limit the activity of tumor-specific T-cell clones as can be generated by other immunotherapeutic approaches.

Similar to every other cancer therapy, solid tumors with their mechanical barriers, disorganized vascularization, and highly immunosuppressive microenvironment are expected to also pose a challenge for BiTE-engaged T cells. Although the smaller BiTE molecules may penetrate better into tumors than IgG antibodies, T cells must also be present for activity. Adoptive T-cell transfer (18) as well as animal studies with green fluorescent protein-labeled T cells (22) suggested that T cells do not need to be present for tumor eradication inside the tumor but can effectively attack from the outside and subsequently invade tumors. The presence of so-called tumor-infiltrating lymphocytes (TILs) is expected to be an advantage because T cells no longer need to first penetrate into target tissue. Tumor-specific T-cell clones in tumors or elsewhere in the body may not be needed for BiTE activity in the first place, but, in case of their potential reactivation by BiTE antibodies, they may help to improve the durability of therapeutic effects induced by a preceding BiTE treatment. SCID mouse models using metastatic tissue from ovarian cancer patients as xenotransplants showed that low numbers of TILs were sufficient to eradicate the cancer tissue by intravenously administered BiTE antibodies (10, 12). This suggests that BiTE antibodies can reactivate TILs in the immunosuppressive microenvironment of the metastatic human tissue.

¹ M. Amann, P. Baeuerle, unpublished observation.

Side effects of BiTE antibodies may come from the onset of polyclonal T-cell activation, bystander effects of tumor cell lysis, damage to target-expressing normal tissues, or a combination thereof. Therefore, side effects are expected to be specific for the selected target antigen, correlate with tumor cell load, and may differ with the disease treated. The latter is suggested by the initially observed better tolerability at the same dose level of blinatumomab in four B-ALL patients with minimal residual disease compared with advanced, heavily pretreated NHL patients. In primate studies, we observed that a BiTE antibody specific for the macrophage-monocyte antigen CD33 was less well tolerated at high doses than a BiTE antibody specific for the melanoma antigen MCSP (10). Again, this difference may relate to target cell load and accessibility. Broad stimulation of T cells by BiTE antibodies may activate autoreactive T-cell clones. However, no autoimmune phenomena have thus far been observed in ongoing clinical trials with blinatumomab. In fact, the apparent lack of T-cell costimulation of BiTE antibodies via CD28 may avoid differentiation of potentially autoreactive naïve T-cell clones, and explains the predominant engagement for redirected lysis of effector memory T cells, which are no longer in need of CD28 costimulation. To date, no antibodies neutralizing blinatumomab have been detected in patients. With the development of a new BiTE antibody platform that is crossreactive between human and primates, BiTE candidates can now be tested early for potential side effects in primate studies.

The short serum half-life of BiTE antibodies of several hours may be perceived as a limitation, but allows for an exquisite control of

drug levels in patients. A straightforward solution is continuous intravenous infusion of BiTE antibodies by portable minipumps for 4 to 8 weeks, as is currently done with blinatumomab and MT110. This route has been shown to be practical and convenient for patients with late-stage NHL, adenocarcinoma, and B-ALL. Studies in rodents and primates have shown that various BiTE antibodies are also bioavailable after subcutaneous administration (10). Subcutaneous delivery by repeated bolus injection was feasible, as was a continuous subcutaneous delivery by an insulin minipump device. These approaches promise a further improvement of convenience.

In conclusion, BiTE antibodies may contribute in the future to immunotherapy of cancer by redirecting the vast number of existing T-cell clones in patients while ignoring many of the immune escape mechanisms that otherwise limit specific antitumor responses of T-cell clones. Further investigations are needed to study the impact of regulatory T cells, the contribution of genuine tumor-specific T-cell responses to clinical activity, possible mechanisms to evade BiTE-engaged T cells, and the broader utility of new BiTE antibodies for treatment of malignant diseases.

Disclosure of Potential Conflicts of Interest

PAB and CR are officers of Micromet Corporation, where blinatumomab and BiTE technology are being developed.

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