Published in final edited form as:
BioDrugs. 2011 December 1; 25(6): 365–379. doi:10.2165/11595950-000000000-00000.

Targeting T Cells with Bispecific Antibodies for Cancer Therapy

Lawrence G. Lum[1,2,3] and **Archana Thakur**[1]

[1]Department of Oncology, Wayne State University and Barbara Ann Karmanos Cancer Center, Detroit, MI, USA

[2]Department of Medicine, Wayne State University and Barbara Ann Karmanos Cancer Center, Detroit, MI, USA

[3]Department of Immunology and Microbiology, Wayne State University and Barbara Ann Karmanos Cancer Center, Detroit, MI, USA

Abstract

Bispecific antibodies (BiAbs) offer a unique opportunity to redirect immune effector cells to kill cancer cells. BiAbs combine the benefits of different binding specificities of two monoclonal antibodies (mAbs) into a single construct. This unique feature of BiAbs enables approaches that are not possible with single mAbs. Advances in antibody engineering and antigen profiling of malignant cells have led to the development of a number of BiAb formats and their combinations for redirecting effector cells to tumor targets. There have been significant advances in the design and application of BiAbs for intravenous and local injection. The initial barrier of cytokine storm has been partially overcome by more recent constructs that have improved clinical effectiveness without dose-limiting toxicities. Since the recent revival of BiAbs, there has been multiple, ongoing, phase I/II and III trials, and some promising clinical outcomes have been reported in completed clinical studies. This review focuses on arming T cells with BiAbs to create the 'poor man's cytotoxic lymphocyte'.

1. Introduction

The advances in molecular antibody engineering and high-throughput methods for screening and identifying specific tumor antigens provide extraordinary tools for developing bispecific antibodies (BiAbs) to redirect immune cells to cancer cells. BiAbs combine the specificities of two antibodies into a single molecule, enabling the bridging of cytotoxicity-triggering receptors on an effector cell with selected surface molecules on a target cell. Targeting of two antigenic determinants was initially assessed in preclinical models, and phase I/II clinical trials were started nearly 20 years ago. However, initial clinical studies were disappointing, mainly due to low efficacy, severe adverse effects without a significant impact on the clinical outcome of disease, and the immunogenicity of the BiAbs. These shortcomings gave rise to the development of numerous formats of BiAb fragments and whole IgG molecules. The formats of BiAbs include chemical heteroconjugation of two whole monoclonal antibodies (mAbs) or fragments of mAbs, quadroma $F(ab')_2$, diabodies, tandem diabodies, single-chain variable fragment (scFv) antibodies, and dock-and-lock multivalent-multifunctional antibodies.[1-4] The designs are limited only by one's imagination, with the engineering process ranging from simple chemistry to complex recombinant technology to produce BiAbs that target effector cells, drugs, prodrugs, toxins,

Correspondence: Dr Lawrence G. Lum, Barbara Ann Karmanos Cancer Institute, Rm 740.1 HWCRC, 4100 John R., Detroit, MI 48201, USA. luml@karmanos.org.

DNA, enzymes, anti-vascular agents, vectors, or radionuclides to tumor-associated antigens (TAAs) on malignant cells. BiAb engineering and glycoengineering can achieve the desired effector function, pharmacokinetics, and clinical outcome. The engineering of BiAb constructs to optimize tissue penetration, in vivo stability, targeting specificity, and binding affinity for tumor cells has been reviewed elsewhere[5,6] and will not be discussed here. This review focuses on the use of BiAbs to redirect effector cells to target cancer cells. The preclinical approaches and their clinical translation and the pros and cons of *in vivo* infusions of BiAb versus *ex vivo* 'franking' or arming of effector cells with BiAb will be detailed in this review.

2. The Challenges of Immune Cell Therapy

2.1 Adoptive T-Cell Therapy

Adoptively transferred lymphokine-activated killer cells (LAK),[7,8] tumor-infiltrating lymphocytes (TIL),[9] anti-CD3-activated T cells (ATC),[10,11] and anti-CD3/anti-CD28 co-activated T cells (COACTs)[12-14] have been used to eliminate or reduce tumor burden in preclinical murine models. However, translating these approaches to patients has been challenging. Although results were initially encouraging in patients with malignant melanoma (MM) or renal cell carcinoma using TIL infusions,[9,15] subsequent studies have not clearly shown improved remission or overall survival rates with these approaches. Since 1986, clinical immunologists have sought to develop preclinical models to dissect the mechanisms responsible for the lack of anti-tumor responses and to demonstrate that effector cell therapy can induce sustained memory anti-tumor responses. Clinical studies in advanced MM showed some encouraging results.[16] Infusions of specific cytotoxic T lymphocytes (CTL) in combination with 720 000 IU of interleukin (IL)-2/kg given every 8 hours induced clinical responses 7 days after non-myeloablative chemotherapy with cyclophos-phamide (60 mg/kg×2 days) and fludarabine (25 mg/m^2 × 5 days).[16] A mean of 7.8×10^{10} ($2.3–13.7\times10^{10}$) anti-melanoma CTL were infused. Six of 13 patients had objective clinical responses and 4 of 13 (30%) patients had mixed responses. Although TIL, ATC, and COACTs can usually be expanded to large numbers, they failed to induce objective clinical responses in most clinical studies. This may be due to intrinsic T-cell defects caused by the malignancy,[17] inadequate numbers of specific CTL, chemotherapy, or a combination of factors.

The *sine qua non* of successful immunotherapy is the allogeneic graft-vs-leukemia (GVL) effect seen after allogeneic stem cell transplant (SCT). The original observation was that SCT patients who developed chronic graft-vs-host disease (GVHD) had lower relapse rates.[18] This GVL effect was also seen in patients who received donor lymphocyte infusions (DLIs) for relapsed chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), and other hematologic malignancies.[19,20] DLI can induce cytogenetic and molecular remissions in patients with CML.[20,21] A similar GVL effect was observed in patients who developed Epstein-Barr virus (EBV)-driven lymphoproliferative disorder (LPD) after SCT with a T-cell-depleted allograft.[22] Infusions of donor-derived EBV-specific CTL induced clinical remissions in patients who had developed LPD.[23,24] Unfortunately, DLI is less effective against AML and ALL.[18] The use of DLI for the treatment of solid tumors remains a challenge.

2.2 Tumor Escape

Tumors evade immune surveillance by expressing low levels of tumor or human leukocyte antigens (HLA).[25,26] Altered HLA expression has been reported in breast,[27] prostate,[28] colon,[29] lung,[30] and pancreatic[31] cancers and MM.[32] Furthermore, tumor-derived suppressive cytokines inhibit differentiation of myeloid cells and promote accumulation of

both myeloid and lymphoid (regulatory T [T_{reg}] cells) suppressive cells in the neoplastic bed and in the secondary lymphoid organs. T_{reg} cells, myeloid-derived suppressor cells, and tumor-associated macrophages can inhibit the cellular and humoral immune responses to cell-based therapies or vaccines. Cytokines (transforming growth factor-β, IL-10, and IL-6) secreted by tumors and suppressor cells downregulate the synthesis of T-helper type 1 (T_h1) cytokines IL-2 and interferon (IFN)-γ. The suppression of IL-2 and IFNγ inhibits T-cell proliferation and blocks the production of perforin granules and granzyme B, which are needed for non-major histocompatibility complex (MHC)-restricted killing.[33] The presence of suppressive cytokines is known to decrease responses to treatment with IL-2 or IFNα.[34,35] Immune escape mechanisms challenge the effectiveness of natural, adoptively transferred T cells and vaccines responses. Besides tumor escape and sabotage of immune responses, tumors provide a physical barrier with a well fortified perimeter consisting of pressure gradients that is difficult for immune effectors and antibodies to infiltrate/penetrate. Redirecting T cells with BiAbs may circumvent tumor escape mechanisms.

3. Clinical Infusions of Bispecific Antibodies (BiAbs)

Since 1997, when rituximab (Rituxan®) was approved, there have been nine additional US FDA-approved mAbs for cancer therapy as of June 2011. Currently, there are more than 22 mAbs approved for clinical use by the FDA, beginning with muromonab (anti-CD3) for transplant rejection in 1986. Most indications are for organ graft rejection, anti-platelet therapy, rheumatoid arthritis, respiratory syncytial virus infections, Crohn disease, breast cancer, colon cancer, asthma, and hematologic malignancies. Unconjugated, radioimmunoconjugated, and chemoimmunoconjugated mAbs have been approved for use based on their clinical efficacy and impact through specific targeting of CD20-positive lymphomas and epidermal growth factor receptor (EGFR)- and human epidermal growth factor receptor-2 (HER2)-positive solid tumors. The FDA-approved mAbs provide a unique source of material that can be paired with anti-CD3 through heteroconjugation to create BiAbs for targeting tumor cells with T cells. Table I summarizes the ongoing or completed clinical trials of BiAbs in various cancer types.

3.1 Whole IgG-based BiAbs

Most of the original targeting strategies were designed to infuse the BiAb into the patient, with the assumption that the infused BiAb would activate and redirect immune effector cells to tumor cells *in vivo*, leading to the lysis of the tumor target. In most of the clinical trials, BiAbs have been infused as ‘drugs’. Shortly after the first BiAbs were made in the early 1980s, clinical applications for targeting cancer were recognized.[56-58] The first phase I clinical trial was conducted in patients with CD19-expressing non-Hodgkin's lymphoma and chronic lymphocytic leukemia (CLL) using the anti-CD3 ×anti-CD19 BiAb SHR-1.[38] The clinical approach was to determine if SHR-1 infusions could redirect endogenous T cells to lymphomas. With the exception of thrombocytopenia, SHR-1 doses ranging from 5 to 10mg did not cause toxicity. However, no clear clinical effects were seen in chemotherapy-resistant CLL patients. Failure was thought to be related to rapid clearance of SHR-1.

The next BiAb, anti-CD3× anti-epidermal glycoprotein 2 (EGP-2) [BIS-1], was designed to prolong *in vivo* serum half-life and was clinically effective for tumor imaging.[59] BIS-1 was made to target carcinoma cells expressing the 38 kDa epithelial carcinoma-associated transmembrane glycoprotein EGP-2.[38] In a phase I trial in renal cell cancer patients, intravenous infusions of BIS-1 with IL-2 induced high levels of specific cytotoxicity associated with elevated serum tumor necrosis factor (TNF)-α and IFNγ levels.[38] The maximum tolerated dose was reached at 5 μg/kg, with dose-limiting toxicities (DLTs) of dyspnea, vasoconstriction, and fever. This study showed that preclinical toxicology did not predict clinical toxicities. On the other hand, injecting autologous, *ex vivo*, IL-2-activated

peripheral blood mononuclear cells (PBMCs) and BIS-1 into carcinomatous ascites or pleural effusions did not cause dose-limiting side effects.[39] Local administration most likely avoided cytokine storm effects caused by BiAb binding to Fc-receptor-bearing cells in circulation. FcγR-bearing cells may have aggregated with BiAb-armed T cells, triggering T cells to secrete cytokines (see figure 1).

Cytokine storm has been a major limitation for strategies that use anti-CD16 (anti-FcγRIII) ×anti-TAA BiAbs, which bind to and redirect natural killer (NK) cells and neutrophils to target tumor antigens. The anti-CD16 ×anti-HER2 BiAb, 2B1, was used to target NK cells to HER2-positive tumors in a phase I clinical trial involving 15 patients. There was one complete response (CR), one partial response (PR), three minor responses, and one mixed response. Treatment induced a 100-fold increase in circulating levels of TNFα, IL-2, and IL-8 and slight increases in the levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IFNγ.[60] Fourteen of 15 patients developed human anti-mouse antibody (HAMA) responses. In a phase I/II trial using the BiAb HRS-3/A9 (anti-CD16×anti-CD30) to treat patients with refractory Hodgkin's disease, there were encouraging clinical results.[61] In a follow-up study involving 16 patients, there was one CR, three PRs, and three patients with stable disease (SD). Even though clinical responses have been seen with whole IgG-based BiAbs, cytokine storm has been a major limitation. Since whole-cell IgG-based BiAbs showed nonspecific activation of Fc receptor-expressing innate immune cells, the next modification step was to remove the Fc portions of the BiAb constructs.

3.2 The Heterogeneous F(ab′)$_2$ Molecule-Based BiAbs

Using the same platform as 2B1 and targeting the same epitope on HER2, a humanized anti-CD64 (FcγRI) Fab′ was chemically linked with a murine anti-HER2 Fab′ (anti-CD64×anti-HER2) to produce the BiAb MDX-H210. Multiple phase I studies using MDX-H210 with various dose ranges and responses have been carried out[2-4,62-65] and are summarized in table II.

The BiAb MDX-447 (anti-CD64×anti-EGFR) was tested in a phase I study in 64 patients to target renal cell carcinoma or head and neck cancer. Patients received MDX-447 at dose levels ranging from 1 to 40mg/m^2/week alone or in combination with G-CSF. Hypotension was dose limiting with grade III toxicity. Other toxicities included fever, hypertension, arrhythmia, allergic reaction, dyspnea, and tumor pain.[48]

Conjugated Fab′ fragments of anti-CD64×anti-CD30 (H22×Ki-4) at doses ranging from 1 to 20mg/m^2/day on days 1, 5, and 7 were given to ten patients with refractory Hodgkin's disease in a phase I dose study. Side effects included hypotension (4 of 10 patients), tachycardia (6 of 10 patients), fatigue (10 of 10 patients), and fever (2 of 10 patients). There was one CR, three PRs, and four patients with SD.[66]

These formats of BiAbs showed some clinical responses but their efficacy in clinical trials has either been insufficient or associated with DLTs. Moreover, the serum stability, poor yield, and immunogenicity of the above-mentioned BiAb formats have restricted their use. These limitations prompted the development of a new generation of BiAbs.

4. New Generation BiAbs

4.1 Trifunctional BiAbs

The development of trifunctional antibodies (TriFAbs), a new generation of whole IgG-based BiAbs, is an impressive and significant improvement on the classical quadroma approach.[67,68] The major improvement was a preferential species-restricted heavy-/light-

chain pairing, in contrast to the random pairing in conventional mouse/mouse or rat/rat quadromas using an original subclass combination (mouse IgG2a and rat IgG2b).

The TriFAb ertumaxomab (anti-CD3 × anti-HER2 BiAb, Fresenius Biotech GmbH; Munich, Germany) was used to treat women with metastatic breast cancer in a phase I clinical trial.[69] Ertumaxomab, which was designed with a modified Fcγ type I/III receptor, creates a tri-cell complex consisting of T cells, Fc-receptor-positive cells, and tumor cells.[69] Patients who received 100 μg of ertumaxomab showed mild and transient side effects and there was one CR, two PRs, and two patients had SD. However, a higher intravenous dose (150–200 μg) of ertumaxomab was not well tolerated; 7 of 17 patients experienced serious adverse events and in three patients (18%), the serious adverse events were classified as drug related.[69]

Another TriFAb, catumaxomab (anti-CD3 × anti-epithelial cell adhesion molecule [EpCAM]; Removab®; Trion Pharma, Munich, Germany), when administered intraperitoneally to 23 patients with malignant ascites in a phase I/II study of refractory ovarian cancer, resulted in a 5-log reduction in EpCAM-positive tumor cells in the ascitic fluid and had an acceptable safety profile.[44] Catumaxomab received EU approval in 2009 for the treatment of EpCAM-positive ovarian cancer ascites, making it the first BiAb to be approved for clinical use. A phase I study using catumaxomab was done in non-small-cell lung cancer to evaluate the safety and tolerability of intravenous treatment. Grade 3 and 4 DLTs were observed at dose level IV (dexamethasone 10 mg premedication, catumaxomab 5μg) and V (dexamethasone 40 mg followed by catumaxomab 7.5 μg). The maximum tolerated dose was defined as dose level III (dexamethasone 40 mg followed by catumaxomab 5(μg).[45] Combining the use of catumaxomab with dexamethasone reduced the cytokine side effects successfully.[45]

A novel future application of Removab® would be to target T cells to solid tumors in patients undergoing allogeneic SCT while simultaneously preventing the development of acute GVHD (US patent 2000601154810 entitled *Treating tumor growth and metastasis by using TriFAb antibodies to reduce the risk for GVHD in allogeneic anti-tumor cell therapy*). If successful, this would be an extraordinary clinical advance for the use of a trifunctional BiAb construct in treating solid tumors in combination with an allogeneic SCT.

4.2 BiAb Format Based on Single-Chain Variable Fragment

ScFv-based bispecific T-cell engager (BiTE) represents a highly innovative advance towards the development of a new generation of BiAbs. BiTEs combine the minimal binding domains (Fv fragments) of two different mAbs on one polypeptide chain of ~55 kDa. Studies using the BiTE format have shown some promising clinical results. BiTE antibodies induced lysis of target antigen-expressing cells at pico- to femtomolar concentrations without the need to pre-stimulate or co-stimulate the T cells. BiTE may induce cytolytic immunological synapses between cytotoxic T cells and target cells that are similar to normal T-cell synapses.[70,71] Phase I/II clinical trials are being performed with the BiTE antibody MT103 (anti-CD19 × anti-CD3; blintumomab).[72-74] Ongoing or completed phase I/II studies with MT103 suggest that T cells engage and lyse tumors.[75] In 38 patients with follicular lymphoma, mantle cell lymphoma, or CLL who received doses ranging from 0.0005 to 0.06mg/m^2/day, 11 patients had CRs. The four CRs and seven PRs occurred at doses of 0.015 mg/m^2/day and higher. Seven patients who received 0.06mg/m^2/day had objective responses. Doses of 0.015mg/m^2/day and higher cleared tumor cells from the blood, lymph nodes, spleen, and bone marrow. In 9 of 11 cases with bone marrow involvement, immunohistochemical staining and flow cytometry had partial (3/11) or complete (6/11) clearance of tumor cells.[75] A phase II trial using MT103 to treat patients with precursor B-cell acute lymphoblastic leukemia is ongoing, wherein tumor cells are

detected only by PCR.[76] These results show that MT103 can effectively recruit and redirect T cells against both bulky and minimal residual disease in hematological malignancies;[77] however, clinical efficacy of the BiTE BiAb format in the treatment of solid tumors has not been reported.

Several BiTE antibodies directed at CD19, EpCAM, HER2, EGFR, CD66e (or carcinoembryonic antigen [CEA] or carcinoembryonic antigen-related cell adhesion molecule 5 [CEACAM5]), CD33, EphA2, and melanoma-associated chondroitin sulfate proteoglycan (MCSP) [or high molecular weight melanoma-associated antigen] are in the developmental pipeline.[78] The cetuximab-based BiTE antibody showed promising results in preclinical models. It prevented tumor growth of *KRAS*- and *BRAF*-mutated human colorectal cancer (CRC) xenografts at very low doses, where cetuximab showed no effect.[79] During treatment for 3 weeks in non-human primates, complete lysis of EGFR-overexpressing cancer cells was observed. These data suggest that EGFR-specific BiTE antibodies may be an effective treatment for CRC that is not responsive to conventional antibodies.[79,80]

The critical points in design to improve clinical effectiveness without inducing DLTs are: (i) the design of the anti-effector activating or engaging construct directed at CD3, CD16, or CD64; (ii) the modification of the Fc-portions to attenuate cytokine storm; and (iii) the use of drugs or chemotherapy that can modulate the cytokine storm and thus improve the tolerability of BiAb infusions.

5. Clinical Infusions of BiAb with Effector Cells or BiAb-Armed (Coated) Effector Cells

The critical element of a BiAb is that it takes advantage of the binding specificities of two antibodies and combines them with the powerful effector functions of cytotoxic immune cells.[56,57,81,82] Arming ATC with anti-CD3× anti-TAA BiAb transforms every polyclonal ATC into a CTL directed at a TAA.[83] In the early 1990s, initial studies were done using *ex vivo* expanded LAK or ATC armed with BiAbs. The general strategy took advantage of T-cell proliferation over 10–14 days so that large numbers of effector cells could be armed.

5.1 Redirecting Immune Effectors

Co-injection of autologous LAK and chemically heteroconjugated anti-CD3 ×anti-glioma BiAb was first reported in 1990.[36] BiAb-armed and -unarmed LAK were injected into the brain tumors of ten patients. Four of ten patients who received armed LAK had tumor regression and four patients had improved overall survival (OS); OS and progression-free survival (PFS) appeared to be better than in patients who received unarmed LAK cells. In a subsequent phase I trial, ATC were co-injected with anti-CD3 × anti-EGFR BiAb and anti-CD28 × anti-EGFR BiAb into an Ommaya reservoir that was connected to the surgical cavity of glioma patients. Two often patients had CRs who received 70 and 250 × 10^6 ATC. It was remarkable that such a small number of ATC led to clinical responses; however, the infusions were associated with transient fever, nausea, headache, and the aggravation of preexisting neurologic deficits.[84]

In the early 1990s, several phase I and II studies were conducted in which ovarian carcinoma patients were treated with intraperitoneal injections of phytohemagglutinin- or anti-CD3-ATC armed with anti-CD3 × anti-Mov28 (ovarian carcinoma-associated antigen) or anti-CD3 × anti-folate receptor (OC/TR), with encouraging clinical responses.[85-87] The product contained both CD4+ and CD8+ T cells. In a series of advanced ovarian carcinoma patients, intraperitoneal injections of ATC armed with OC/TR induced regression in patients who had debulking laparatomies for advanced disease.[87] The patients received two cycles

of five daily intraperitoneal doses of ATC armed with OC/TR BiAb and 0.6×10^6 IU of IL-2. Ten patients received between 4 and 9×10^9 armed ATC. Despite poor prognostic features, 7 of 26 (27%) patients had a CR (4) or PR (3). Metastatic lesions that were >2 cm disappeared after therapy. The side effects were mild to moderate fever, nausea, and emesis. However, HAMA responses developed in 84% of the patients.

In preclinical studies, we showed that ATC armed with chemically heteroconjugated anti-CD3 × anti-HER2 BiAbs (anti-CD3 × trastuzumab) [HER2Bi] could (i) kill HER2 breast,[88] prostate,[89] ovarian,[90] and pancreatic[91] cancer cell lines; (ii) secrete cytokines (IFNγ, TNFα, GM-CSF) and chemokines (RANTES [Regulated on Activation, Normal T cell Expressed and Secreted], and MIP-1α [macrophage inflammatory protein 1 alpha]);[92] (iii) prevent the development of tumors in severe combined immune deficiency (SCID)/Beige mice when co-injected in Winn assays or induce remission when directly injected into established prostate PC-3 xenografts;[93] (iv) traffic to tumors in SCID/Beige mice;[93] (v) inhibit and eliminate ovarian cancer in SCID mice;[90] and (vi) proliferate and kill tumors multiple times without undergoing apoptosis. If *in vivo* targeting of a tumor leads to tumor lysis and release of cytokines/chemokines by T cells at the tumor site, endogenous monocytes and T cells could be recruited and activated to induce immune responses. TAAs could then be processed and presented by dendritic cells to T cells (figure 2). Table III summarizes the preclinical studies using BiAbs that redirected T cells and NK-T cells. Table I summarizes the clinical trials for cancer involving infusions of redirected T cells or *in vivo* arming of effector cells by infusions of BiAb.

Our initial phase I clinical trials involved eight infusions of HER2Bi (anti-CD3 × trastuzumab)-armed ATC given over 4 weeks in combination with low-dose IL-2 (300 000 IU/m^2 daily) and GM-CSF (250 (μg/m^2 twice weekly) starting 3 days prior to the first ATC infusion and ending 1 week after the last infusion for the treatment of patients with high-risk HER2 0-3+ stage II–IV breast cancer, hormone-refractory prostate cancer, and advanced pancreatic cancer. ATC activated with muromonab and expanded in the presence of IL-2 for 14 days were harvested, armed with 50 ng HER2Bi/10^6 ATC, washed, aliquoted, and cryopreserved. There were two DLTs that occurred in the dose range between 80 and 160×10^9 armed ATC per patient. One patient died of heart failure most likely due to digoxin toxicity and one patient developed a subdural hematoma associated with high blood pressure due to GM-CSF administration and armed ATC infusions. The subdural hematoma was evacuated without any neurologic sequelae. The non-dose-limiting toxicity profile of the remaining patients included chills, fever, hypotension, and fatigue. The side effects were easily managed with prophylactic antihistamines, antipyretics, and vigorous hydration. A total of 22 women with metastatic breast cancer, nine women with high-risk breast cancer, six men with hormone-refractory prostate cancer, and one patient with pancreatic cancer were treated with HER2Bi-armed ATC. Women with breast cancer and men with prostate cancer reported decreased bone pain.[126] Circulating ATC bearing HER2Bi could be detected for up to several weeks after the infusions.[127] T_h1 cytokines (GM-CSF, IFN-γ, TNFα, IL-2), IL-12, RANTES, and MIP-1α and low levels of T_h2 cytokines (IL-10, IL-4) were detected in the serum during infusions.[128]

In a phase I clinical trial in patients with refractory, resistant, or high-risk CD20-positive non-Hodgkin's lymphoma, up to a total of 80×10^9 anti-CD3 × Rituxan® (CD20Bi)-armed ATC were given in four divided doses over 4 weeks after high-dose chemotherapy and peripheral blood SCT, without DLTs.[129]

5.2 *In Situ* Vaccination by Redirected T Cells

ATC release TNFα, IFNγ, GM-CSF, IL-4, IL-6, and IL-10 during BiAb-mediated tumor lysis in an IL-2 independent manner.[100] Analysis of cytokine levels in patients treated with

BiAb-armed T cells showed increases in TNFα and IFNγ but not IL-4.[100] TNFα and IFNγ secretion during BiAb targeting shifts the *in vivo* milieu towards a T_h1 anti-tumor environment. Repeated stimulation of T cells armed with anti-CD3×anti-CD19 or anti-CD3×anti-HER2 may improve survival and enhance in vivo cytotoxicity.[100,130] Our phase I trial using HER2Bi (anti-CD3 ×anti-HER2)-armed ATC showed not only a shift towards a T_h1 cytokine pattern but also the secretion of RANTES, and MIP-1α,[130] which would attract endogenous antigen-presenting cells and naïve T cells to the tumor site. This process would immunize the patient's endogenous immune system against the TAAs (figure 2).

5.3 Activated T Cells Armed with BiAb Survive to Kill Again

CTL that express Fas ligand (FasL) are known to kill tumor cells and the antigen-dependent binding of T cells to tumors releases IFNγ, which is known to upregulate Fas expression on tumor targets.[131] The FasL-Fas interaction between the T cells and tumor cells induces apoptosis of tumor cells; however, the tumor cells counterattack by inducing apoptosis in the effector T cells.[132-134] This counterattack may eliminate effector T cells before the immune response begins,[135] but T-cell receptor (TCR) stimulation can protect CD8+ T cells from CD95-mediated apoptosis.[136] BiAb-armed ATC, upon TCR restimulation, may be resistant to activation-induced cell death (AICD) since binding to the TCR and other receptors via the BiAb-complex may mimic secondary signals.[92] We showed that armed ATC can repeatedly kill tumor targets and not undergo AICD.[92] The data suggest that BiAb arming of ATC may not only re-stimulate the ATC to proliferate and secrete cytokines, but also enhance *in vivo* T-cell survival.

6. Strategies to Enhance Tumor Targeting

6.1 Co-Activation and Redirecting of T Cells

Co-stimulatory signals for T-cell activation have emerged as a promising strategy for tumor immunotherapy. A recombinant single-chain BiAb, rM28, directed to a melanoma-associated proteoglycan (NG2) and the co-stimulatory CD28 molecule on T cells, induced T-cell activation, which resulted in tumor-cell killing without additional TCR/CD3 stimulation. Presentation of a CD28 antibody within a suitable recombinant, bispecific format may result in ‘targeted supra-agonistic stimulation’ of the CD28 molecule, which leads to effective tumor-cell killing.[137,138] On a cautionary note, when TGN1412, a mono-specific ‘superagonistic’ CD28 antibody, was injected into six healthy volunteers it induced life-threatening systemic T-cell activation and severe cytokine storm[139] that raised concerns about the use of immunomodulatory molecules. However, as rM28 is a bispecific molecule, it will not be activated in the absence of target cells, in contrast to the systemic T-cell activation seen withTGN1412 in the absence of target cells.

In addition, co-stimulation with anti-CD3/anti-CD28 and targeting with BiAb has been reported and may be critical to obtaining long-term memory responses. Co-stimulation with anti-CD28 alone or anti-CD28×anti-TAA induces enhanced signaling,[140,141] cytokine synthesis,[142] enhanced killing in leukemia/lymphoma models,[95,98,105] and cytotoxicity in colon cell lines.[143] It has also been reported that BiAb and CD28 co-stimulation induces T_h1 differentiation.[143] However, it remains unclear whether co-stimulation correlates with more effective long-term clinically relevant immune responses.

6.2 T Cells Expressing Chimeric Antibody Receptors

T cells have been transduced with genes that express chimeric scFv domain receptors, creating T cells with chimeric antibody receptors (CARs) [i.e. ‘T-bodies’] to deliver lethal hits to tumors.[144-149] Earlier constructs using retroviral vectors have been replaced with lentiviral vectors that have better transduction efficiency. Most CARs include heavy and

light chain-derived variable regions connected by peptide linkers and activating signaling chains that consist of a gamma chain and CD28 signaling chains.[150] Although early phase I clinical trials using T-bodies were not encouraging,[151] recent studies involving the addition of a CD28 signaling chain to the gamma or zeta chain indicate that this approach may increase the function and survival of CARs in the body. Constructs have been made to target CD19,[152] CEA,[153] and prostate-specific membrane antigen [154] and have shown encouraging clinical responses.

6.3 Immunologic Space

Both preclinical and clinical studies strongly suggest that depletion of T_{reg} cells or creating immunologic space using cyclophosphamide can lead to improved anti-tumor activity.[155-158] An early study showed that infusions of purified CD4+ cells induced remissions in patients with solid tumors after lymphodepleting cyclophosphamide doses ranging from 500 to 1000 mg/m^2.[159]

6.4 Affinity of BiAbs

The affinity, isotype, targeted CD3 epitope, and arming dose of the BiAb may affect signaling, proliferation, cytokine synthesis, and cytotoxicity. Changing the valency may alter the ability of the anti-CD3-based BiAb to induce specific T cell functions. In a series of scFv anti-HER2 constructs with affinities ranging from 10^{-11} to 10^{-7}, increasing binding affinity led to increasing cytotoxicity.[160]

6.5 Ex Vivo BiAb-Armed ATC versus BiAb Infusions

Activated and fresh unactivated T cells can be armed with BiAbs to target tumors in a non-MHC-restricted manner.[161] Fresh T cells in PBMCs can be armed with BiAb, inducing proliferation and cytotoxicity.[161] Most investigators choose to infuse BiAb instead of performing *ex vivo* expansion of T cells followed by arming with BiAb to induce anti-tumor cytotoxicity. We adopted the *ex vivo* expansion and arming approach to avoid *in vivo* activation of a very large number of endogenous T cells.[88] Infusing BiAb alone results in substantially more BiAb (micrograms to milligrams per kg) being infused into patients compared with arming ATC with 25–50ng BiAb/million cells. Infusing free BiAb would result in binding to all circulating T cells, tumor targets, and Fc-receptor-bearing cells immediately after the infusion, potentially leading to the development of a cytokine storm. The BiTE format with significantly reduced doses of 0.015 mg/m^2/day given as a continuous infusion may have overcome the cytokine storm limitation of infusing BiAb alone.[75] Obviously, the binding to effector cells and tumor cells will be dependent upon the affinity of each arm of the BiAb construct and the Fc-binding ability of the Fc-portion of the BiAb.

In addition, immobilized BiAb on the surface of T cells, NK cells, monocytes, or neutrophils may resist clearance from the circulation, whereas circulating single-chain antibodies, diabodies, minibodies, leucine-zippered antibodies, or ‘knobs-into-holes’ constructs are cleared more rapidly than effector cells.[5] The arming concentration of the BiAb to re-activate T cells upon tumor engagement may be critical. Overloading ATC with BiAb may trigger or induce activation-induced cell death.

7. Conclusions

The use of BiAbs for redirecting immune effector cells shows promise. As the understanding of the interactions between cancer stem cells and the cells involved in the inflammatory response to tumors improves, cell- and BiAb-based engineering will enable construction of customized BiAb molecules for optimal targeting of specific tumors. The

key considerations for successful manipulation of immunologic responses are (i) the mode and state of activation of the effector cells; (ii) the binding affinity of the BiAb to the effector cells; (iii) the presence and functional capacity of regulatory or suppressor cells; (iv) the type of BiAb construct (chemically conjugated whole or fragments of antibodies, Fab2, scFv, trifunctional, BiTE etc.); (v) the presence of competing decoy antigens; (vi) tumor antigen modulation after BiAb engagement; (vii) the rate and route of delivering the BiAb alone or as armed effector cells; and (viii) the type of tumor and the overall immunologic state of the patient. All in all, antibody engineering, immunologic approaches and concepts on a single platform offer excitement and promise in targeting cancer.

Acknowledgments

We appreciate the efforts of the physicians, immunotherapy technical staff, clinical coordinators, nursing staff, administrative staff, and the leadership of the Barbara Ann Karmanos Cancer Institute and Roger Williams Medical Center. Special thanks to Wendy Young, Annette Olson, Lori Hall, Patricia Steele, and Karen Myers for their dedicated efforts to serve the immunotherapy patients. The studies were supported by NIH grants R01 CA 092344 (LGL), R01 CA 140314 (LGL); Cancer Center Support Grant P30 CA022453-25; Translational Grants #6092-09 (LGL) and #6066-06 (LGL) from the Leukemia and Lymphoma Society; Susan G. Komen Foundation grant BCTR0707125 (LGL); startup funds from the Karmanos Cancer Institute (LGL); and a gift from the Bill Young Foundation for breast cancer immunotherapy (LGL). LGL is a founder of Transtarget, Inc. AT has no conflict of interest.

References

1. Dreier T, Lorenczewski G, Brandl C, et al. Extremely potent, rapid and costimulation-independent cytotoxic T-cell response against lymphoma cells catalyzed by a single-chain bispecific antibody. Int J Cancer. 2002 Aug 20; 100(6):690–7. [PubMed: 12209608]
2. Asano R, Ikoma K, Kawaguchi H, et al. Application of the Fc fusion format to generate tag-free bi-specific diabodies. FEBS J. 2010; 277(2):477–87. [PubMed: 20015073]
3. Asano R, Watanabe Y, Kawaguchi H, et al. Highly effective recombinant format of a humanized IgG-like bispecific antibody for cancer immunotherapy with retargeting of lymphocytes to tumor cells. J Biol Chem. 2007 Sep 21; 282(38):27659–65. [PubMed: 17644522]
4. Rossi EA, Goldenberg DM, Cardillo TM, et al. Stably tethered multifunctional structures of defined composition made by the dock and lock method for use in cancer targeting. Proc Natl Acad Sci U S A. 2006; 103(18):6841–6. [PubMed: 16636283]
5. Cao Y, Lam L. Bispecific antibody conjugates in therapeutics. Adv Drug Deliv Rev. 2003 Feb 10; 55(2):171–97. [PubMed: 12564976]
6. Segal DM, Weiner GJ, Weiner LM. Introduction: bispecific antibodies. J Immunol Methods. 2001 Feb 1; 248(1-2):1–6. [PubMed: 11223064]
7. Grimm EA, Mazumder A, Zhang HZ, et al. Lymphokine-activated killer cell phenomenon: lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med. 1982; 155:1823–41. [PubMed: 6176669]
8. Anderson PM, Bach FH, Ochoa AC. Augmentation of cell number and LAK activity in peripheral blood mononuclear cells activated with anti-CD3 and interleukin-2: preliminary results in children with acute lymphocytic leukemia and neuroblastoma. Cancer Immunol Immunother. 1988; 27:82–8. [PubMed: 3260824]
9. Rosenberg SA, Spiess P, Lafreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science. 1986; 233:1318–21. [PubMed: 3489291]
10. Uberti JP, Joshi I, Ueda M, et al. Preclinical studies using immobilized OKT3 to activate human T cells for adoptive immunotherapy: optimal conditions for the proliferation and induction of non-MHC restricted cytotoxicity. Clin Immunol Immunopathol. 1994; 70:234–40. [PubMed: 8313660]
11. Ueda M, Joshi ID, Dan M, et al. Preclinical studies for adoptive immunotherapy in bone marrow transplantation, II: generation of anti-CD3 activated cytotoxic T cells from normal donors and autologous bone marrow transplant candidates. Transplantation. 1993; 56:351–6. [PubMed: 8356589]

12. Lum LG, LeFever AV, Treisman JS, et al. Immune modulation in cancer patients after adoptive transfer of anti-CD3/anti-CD28-costimulated T cells: phase I clinical trial. J Immunother. 2001 Sep; 24(5):408–19.
13. Fowler DH, Odom J, Steinberg SM, et al. Phase I clinical trial of costimulated, IL-4 polarized donor CD4+ T cells as augmentation of allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2006 Nov; 12(11):1150–60. [PubMed: 17085308]
14. Garlie NK, LeFever AV, Siebenlist RE, et al. T cells co-activated with immobilized anti-CD3 and anti-CD28 as potential immunotherapy for cancer. J Immunother. 1999; 4:335–45.
15. Rosenberg SA, Packard BS, Aebersold PM, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma: a preliminary report. N Engl J Med. 1988; 319:1676–80. [PubMed: 3264384]
16. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002 Oct 25; 298(5594):850–4. [PubMed: 12242449]
17. Whiteside TL. Signaling defects in T lymphocytes of patients with malignancy. Cancer Immunol Immunother. 1999; 48:346–52. [PubMed: 10501846]
18. Weiden PL, Sullivan KM, Flournoy N, et al. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med. 1981 Jun 18; 304(25):1529–33. [PubMed: 7015133]
19. Deol A, Lum LG. Role of donor lymphocyte infusions in relapsed hematological malignancies after stem cell transplantation revisited. Cancer Treat Rev. 2010 Nov; 36(7):528–38. [PubMed: 20381970]
20. Kolb HJ, Mittermuller J, Clemm C, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood. 1990; 76:2462–5. [PubMed: 2265242]
21. Kolb HJ, Holler E. Adoptive immunotherapy with donor lymphocyte transfusions. Curr Opin Oncol. 1997; 9:139–45. [PubMed: 9161791]
22. Liu Z, Savoldo B, Huls H, et al. Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for the prevention and treatment of EBV-associated posttransplant lymphomas. Recent Results Cancer Res. 2002; 159:123–33. [PubMed: 11785836]
23. Rooney CM, Smith CA, Ng CY, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998 Sep 1; 92(5):1549–55. [PubMed: 9716582]
24. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010 Feb 4; 115(5):925–35. [PubMed: 19880495]
25. Rivoltini L, Barracchini KC, Viggiano V, et al. Quantitative correlation between HLA class I allele expression and recognition of melanoma cells by antigen-specific cytotoxic T lymphocytes. Cancer Res. 1995 Jul 15; 55(14):3149–57. [PubMed: 7541714]
26. Cohen SB, Katsikis PD, Feldmann M, et al. IL-10 enhances expression of the IL-2 receptor alpha chain on T cells. Immunology. 1994 Nov; 83(3):329–32. [PubMed: 7835955]
27. Concha A, Cabrera T, Ruiz-Cabello F, et al. Can the HLA phenotype be used as a prognostic factor in breast carcinomas? Int J Cancer Suppl. 1991; 6:146–54. [PubMed: 2066180]
28. Blades RA, Keating PJ, McWilliam LJ, et al. Loss of HLA class I expression in prostate cancer: implications for immunotherapy. Urology. 1995 Nov; 46(5):681–6. [PubMed: 7495121]
29. Browning M, Petronzelli F, Bicknell D, et al. Mechanisms of loss of HLA class I expression on colorectal tumor cells. Tissue Antigens. 1996 May; 47(5):364–71. [PubMed: 8795136]
30. Redondo M, Concha A, Oldiviela R, et al. Expression of HLA class I and II antigens in bronchogenic carcinomas: its relationship to cellular DNA content and clinical-pathological parameters. Cancer Res. 1991 Sep 15; 51(18):4948–54. [PubMed: 1654207]
31. Torres MJ, Ruiz-Cabello F, Skoudy A, et al. Loss of an HLA haplotype in pancreas cancer tissue and its corresponding tumor derived cell line. Tissue Antigens. 1996 May; 47(5):372–81. [PubMed: 8795137]

32. Ferrone S, Marincola FM. Loss of HLA class I antigens by melanoma cells: molecular mechanisms, functional significance and clinical relevance. Immunol Today. 1995 Oct; 16(10): 487–94. [PubMed: 7576053]
33. Smyth MJ, Strobl SL, Young HA, et al. Regulation of lymphokine-activated killer activity and pore-forming protein gene expression in human peripheral blood CD8+ T lymphocytes: inhibition by transforming growth factor-β. J Immunol. 1991; 146:3289–97. [PubMed: 1827481]
34. Blay JY, Negrier S, Combaret V, et al. Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma. Cancer Res. 1992 Jun 15; 52(12):3317–22. [PubMed: 1596890]
35. Tartour E, Blay JY, Dorval T, et al. Predictors of clinical response to interleukin-2-based immunotherapy in melanoma patients: a French multi-institutional study. J Clin Oncol. 1996 May; 14(5):1697–703. [PubMed: 8622090]
36. Nitta T, Sato K, Yagita H, et al. Preliminary trial of specific targeting therapy against malignant glioma. Lancet. 1990; 335:368–71. [PubMed: 1968115]
37. Bolhuis RL, Lamers CH, Goey SH, et al. Adoptive immunotherapy of ovarian carcinoma with bs-mAb-targeted lymphocytes: a multicenter study. Int J Cancer Suppl. 1992; 7:78–81. [PubMed: 1428412]
38. de Gast GC, van Houten AA, Haagen IA, et al. Clinical experience with CD3 × CD19 bispecific antibodies in patients with B cell malignancies. J Hematother. 1995 Oct; 4(5):433–7. [PubMed: 8581381]
39. Kroesen BJ, Nieken J, Sleijfer DT, et al. Approaches to lung cancer treatment using the CD3 × EGP-2-directed bispecific monoclonal antibody BIS-1. Cancer Immunol Immunother. 1997 Nov; 45(3-4):203–6. [PubMed: 9435874]
40. Hartmann F, Renner C, Jung W, et al. Treatment of refractory Hodgkin's disease with an anti-CD16/CD30 bispecific antibody. Blood. 1997; 89(6):2042–7. [PubMed: 9058726]
41. Chen J, Bashey A, Holman P, et al. A phase I dose escalating study of infusion of a bispecific antibody (BsAb) for relapsed/refractory acute myeloid leukemia (AML). Blood. 1999 Nov 15.94(10):227B. abstract.
42. Jmes ND, Atherton PJ, Jones J, et al. A phase II study of the bispecific antibody MDX-H210 (anti-HER2 × CD64) with GM-CSF in HER2+ advanced prostate cancer. Br J Cancer. 2001; 85(2):152–6. [PubMed: 11461069]
43. Valone FH, Kaufman PA, Guyre PM, et al. Phase Ia/Ib trial of bispecific antibody MDX-210 in patients with advanced breast or ovarian cancer that overexpresses the proto-oncogene HER-2/neu. J Clin Oncol. 1995; 13(9):2281–92. [PubMed: 7545221]
44. Burges A, Wimberger P, Kumper C, et al. Effective relief of malignant ascites in patients with advanced ovarian cancer by a trifunctional anti-EpCAM × anti-CD3 antibody: a phase I/II study. Clin Cancer Res. 2007; 13(13):3899–905. [PubMed: 17606723]
45. Sebastian M, Passlick B, Friccius-Quecke H, et al. Treatment of non-small cell lung cancer patients with the trifunctional monoclonal antibody catumaxomab (anti-EpCAM × anti-CD3): a phase I study. Cancer Immunol Immunother. 2007; 56(10):1637–44. [PubMed: 17410361]
46. Borghaei H, Alpaugh RK, Bernardo P, et al. Induction of adaptive Anti-ER2/neu immune responses in a Phase 1B/2 trial of 2B1 bispecific murine monoclonal antibody in metastatic breast cancer (E3194): a trial coordinated by the Eastern Cooperative Oncology Group. J Immunother. 2007 May; 30(4):455–67. [PubMed: 17457220]
47. Weiner LM, Clark JI, Davey M, et al. Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and Fc gamma RIII. Cancer Res. 1995; 55(20):4586–93. [PubMed: 7553634]
48. Fury MG, Lipton A, Smith KM, et al. A phase-I trial of the epidermal growth factor receptor directed bispecific antibody MDX-447 without and with recombinant human granulocyte-colony stimulating factor in patients with advanced solid tumors. Cancer Immunol Immunother. 2008 Feb; 57(2):155–63. [PubMed: 17602224]
49. Barbara Ann Karmanos Cancer Institute. Donor T cells, low-dose aldesleukin, and low-dose GM-CSF after donor stem cell transplant in treating patients with relapsed or refractory non-Hodgkin's lymphoma. US National Institutes of Health; ClinicalTrials.gov identifier: NCT00521261ClinicalTrials.gov [online]. Available from URL: http://clinicaltrials [Accessed 2011 Sep 8]

50. Barbara Ann Karmanos Cancer Institute. Laboratory-treated T cells after second-line chemotherapy in treating women with HER2/neu-negative metastatic breast cancer. US National Institutes of Health; ClinicalTrials.gov identifier: NCT01022138ClinicalTrials.gov [online]. Available from URL: http://clinicaltrials.gov [Accessed 2011 Sep 8]
51. AGO Study Group. open-label, phase IIa study with the intraperitoneally infused trifunctional bispecific antibody Removab™ (anti-EpCAM × anti-CD3) to select the better dose level in platinum refractory epithelial ovarian cancer patients. US National Institutes of Health; Randomized, multicenter, 2-dose level. ClinicalTrials.gov identifier: NCT00189345ClinicalTrials.gov [online]. Available from URL: http://clinicaltrials.gov [Accessed 2011 Sep 8]
52. Fresenius Biotech GmbH Phase II study with the trifunctional antibody ertumaxomab to treat metastatic breast cancer progressing after endocrine treatment. US National Institutes of Health; ClinicalTrials.gov identifier: NCT00452140ClinicalTrials.gov [online] Available from URL: http://clinicaltrials.gov [Accessed 2011 Sep 8]
53. University of Tuebingen (Germany). Phase I/II study with local treatment of metastatic melanoma with autologous lymphocytes and the bispecific antibody rM28. US National Institutes of Health; ClinicalTrials.gov identifier: NCT00204594ClinicalTrials.gov [online]. Available from URL: http://clinicaltrials.gov [Accessed 2011 Sep 8]
54. Micromet, AG. Safety study of the bispecific t-cell engager blinatumomab (MT103) in patients with relapsed NHL. US National Institutes of Health; ClinicalTrials.gov identifier: NCT00274742ClinicalTrials.gov [online]. Available from URL: http://clinicaltrials.gov [Accessed 2011 Sep 8]
55. Micromet, AG. Phase II study of the BiTE® Blinatumomab (MT103) in patients with minimal residual disease of B-precursor acute ALL. US National Institutes of Health; ClinicalTrials.gov identifier: NCT00560794ClinicalTrials.gov [online]. Available from URL: http://clinicaltrials.gov [Accessed 2011 Sep 8]
56. Raso V, Griffin T. Hybrid antibody with dual specificity for the delivery of ricin to immunoglobin bearing target cells. Cancer Res. 1981; 41:2073–8. [PubMed: 7237414]
57. Titus JA, Perez P, Kaubisch A, et al. Human K/natural killer cells targeted with hetero-cross-linked antibodies specifically lyse tumor cells in vitro and prevent tumor growth in vivo. J Immunol. 1987; 139:3153–8. [PubMed: 2959724]
58. Perez P, Hoffman RW, Shaw S. Specific targeting of cytotoxic T cells by antiT3 linked to anti-target cell antibody. Nature. 1985; 316:354–6. [PubMed: 3160953]
59. Kosterink JG, de Jonge MW, Smit EF, et al. Pharmacokinetics and scintigraphy of indium-111-DTPA-MOC-31 in small-cell lung carcinoma. J Nucl Med. 1995 Dec; 36(12):2356–62. [PubMed: 8523132]
60. Weiner LM, Clark JI, Davey M, et al. Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and Fc gamma RIII. Cancer Res. 1995 Oct 15; 55(20):4586–93. [PubMed: 7553634]
61. Hartmann F, Renner C, Jung W, et al. Treatment of refractory Hodgkin's disease with an anti-CD16/Cd30 bispecific antibody. Blood. 1997; 89:2042–7. published erratum appears in Blood 1998, 91: 1832. [PubMed: 9058726]
62. Valone FH, Kaufman PA, Guyre PM, et al. Phase Ia/Ib trial of bispecific antibody MDX-210 in patients with advanced breast or ovarian cancer that overexpresses the proto-oncogene HER-2/neu. J Clin Oncol. 1995; 13:2281–92. [PubMed: 7545221]
63. Schwaab T, Lewis LD, Cole BF, et al. Phase I pilot trial of the bispecific antibody MDXH210 (anti-Fc gamma RI × anti-HER-2/neu) in patients whose prostate cancer overexpresses HER-2/neu. J Immunother. 2001 Jan; 24(1):79–87. [PubMed: 11211151]
64. James ND, Atherton PJ, Jones J, et al. A phase II study of the bispecific antibody MDX-H210 (anti-HER2 × CD64) with GM-CSF in HER2+ advanced prostate cancer. Br J Cancer. 2001 Jul 20; 85(2):152–6. [PubMed: 11461069]
65. Posey JA, Raspet R, Verma U, et al. Apilot trial of GM-CSF and MDX-H210 in patients with erbB-2-positive advanced malignancies. J Immunother. 1999 Jul; 22(4):371–9. [PubMed: 10404439]

66. Borchmann P, Schnell R, Fuss I, et al. Phase 1 trial of the novel bispecific molecule H22xKi-4 in patients with refractory Hodgkin lymphoma. Blood. 2002 Nov 1; 100(9):3101–7. [PubMed: 12384405]

67. Zeidler R, Reisbach G, Wollenberg B, et al. Simultaneous activation of T cells and accessory cells by a new class of intact bispecific antibody results in efficient tumor cell killing. J Immunol. 1999; 163(3):1246–52. [PubMed: 10415020]

68. Zeidler R, Mysliwietz J, Csanady M, et al. The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumour cells. Br J Cancer. 2000 Jul; 83(2):261–6. [PubMed: 10901380]

69. Kiewe P, Hasmuller S, Kahlert S, et al. Phase I trial of the trifunctional anti-ER2 × anti-CD3 antibody ertumaxomab in metastatic breast cancer. Clin Cancer Res. 2006; 12(10):3085–91. [PubMed: 16707606]

70. Baeuerle PA, Kufer P, Lutterbuse R. Bispecific antibodies for polyclonal T-cell engagement. Curr Opin Mol Ther. 2003; 5(4):413–9. [PubMed: 14513685]

71. Brischwein K, Parr L, Pflanz S, et al. Strictly target cell-dependent activation of T cells by bispecific single-chain antibody constructs of the BiTE class. J Immunother. 2007; 30(8):798–807. [PubMed: 18049331]

72. Dreier T, Lorenczewski G, Brandl C, et al. Extremely potent, rapid and costimulation-independent cytotoxic T-cell response against lymphoma cells catalyzed by a single-chain bispecific antibody. Int J Cancer. 2002; 100(6):690–7. [PubMed: 12209608]

73. Dreier T, Baeuerle PA, Fichtner I, et al. T cell costimulus-independent and very efficacious inhibition of tumor growth in mice bearing subcutaneous or leukemic human B cell lymphoma xenografts by a CD19-/CD3- bispecific single-chain antibody construct. J Immunol. 2003; 170(8): 4397–402. [PubMed: 12682277]

74. Loffler A, Kufer P, Lutterbuse R, et al. A recombinant bispecific single-chain antibody, CD19 × CD3, induces rapid and high lymphoma-directed cytotoxicity by unstimulated T lymphocytes. Blood. 2000; 95(6):2098–103. [PubMed: 10706880]

75. Bargou R, Leo E, Zugmaier G, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science. 2008; 321(5891):974–7. [PubMed: 18703743]

76. Topp MS. Treatment with anti-CD19 BiTE antibody blinatumomab (MT103/MEDI-538) is able to eliminate minimal residual disease (MRD) in patients with B-precursor acute lymphoblastic leukemia (ALL): first results of an ongoing phase II study. Blood. 2008; 112(Suppl.):1926. abstract.

77. Baeuerle PA, Reinhardt C. Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res. 2009; 69(12):4941–4. [PubMed: 19509221]

78. Baeuerle PA, Kufer P, Bargou R. BiTE: teaching antibodies to engage T-cells for cancer therapy. Curr Opin Molecular Ther. 2009 Feb; 11(1):22–30.

79. Lutterbuese R, Raum T, Kischel R, et al. T cell-engaging BiTE antibodies specific for EGFR potently eliminate KRAS- and BRAF-mutated colorectal cancer cells. Proc Natl Acad Sci USA. 2010 Jul 13; 107(28):12605–10. [PubMed: 20616015]

80. Schlereth B, Fichtner I, Lorenczewski G, et al. Eradication of tumors from a human colon cancer cell line and from ovarian cancer metastases in immunodeficient mice by a single-chain Ep-CAM-/CD3-bispecific antibody construct. Cancer Res. 2005 Apr 1; 65(7):2882–9. [PubMed: 15805290]

81. Perez P, Titus JA, Lotze MT, et al. Specific lysis of human tumor cells by T cells coated with anti-T3 cross-linked to anti-tumor antibody. J Immunol. 1986; 137(7):2069–72. [PubMed: 2944946]

82. Segal DM, Garrido MA, Perez P, et al. Targeted cytotoxic cells as a novel form of cancer immunotherapy. Mol Immunol. 1988; 25:1099–103. [PubMed: 3265476]

83. Renner C, Held G, Ohnesorge S, et al. Role of perforin, granzymes and the proliferative state of the target cells in apoptosis and necrosis mediated by bispecific-antibody-activated cytotoxic T cells. Cancer Immunol Immunother. 1997; 44:70–6. [PubMed: 9177467]

84. Jung G, Brandl M, Eisner W, et al. Local immunotherapy of glioma patients with a combination of 2 bispecific antibody fragments and resting autologous lymphocytes: evidence for in situ t-cell activation and therapeutic efficacy. Int J Cancer. 2001 Jan 15; 91(2):225–30. [PubMed: 11146449]

85. Lamers CHJ, van de Griend RJ, Braakman E, et al. Optimization of culture conditions for activation and large-scale expansion of human T lymphocytes for bispecific antibody-directed cellular immunotherapy. Int J Cancer. 1992; 51:973–9. [PubMed: 1386349]
86. Lamers CH, Bolhuis RL, Warnaar SO, et al. Local but no systemic immunomodulation by intraperitoneal treatment of advanced ovarian cancer with autologous T lymphocytes re-targeted by a bi-specific monoclonal antibody. Int J Cancer. 1997 Oct 9; 73(2):211–9. [PubMed: 9335445]
87. Canevari S, Stoter G, Arienti F, et al. Regression of advanced ovarian carcinoma by intraperitoneal treatment with autologous T lymphocytes retargeted by a bispecific monoclonal antibody. J Natl Cancer Inst. 1995; 87:1463–9. [PubMed: 7674333]
88. Sen M, Wankowski DM, Garlie NK, et al. Use of anti-CD3 × anti-HER2/neu bispecific antibody for redirecting cytotoxicity of activated T cells toward HER2/neu tumors. J Hematother Stem Cell Res. 2001 Apr.10:247–60. [PubMed: 11359672]
89. Lum HE, Miller M, Davol PA, et al. Preclinical studies comparing different bispecific antibodies for redirecting T cell cytotoxicity to extracellular antigens on prostate carcinomas. Anticancer Res. 2005 Jan; 25(1A):43–52. [PubMed: 15816517]
90. Chan JK, Hamilton CA, Cheung MK, et al. Enhanced killing of primary ovarian cancer by retargeting autologous cytokine-induced killer cells with bispecific antibodies: a preclinical study. Clin Cancer Res. 2006 Mar 15; 12(6):1859–67. [PubMed: 16551871]
91. Lum LG, Davol P, Grabert R, et al. Targeting pancreatic cancer with armed activated T cells directed at Her2/neu receptors. Exp Hematol. 2002; 30:56. abstract.
92. Grabert RC, Smith J, Tiggs J, et al. Anti-CD3 activated T cells armed with OKT3 × herceptin bispecific antibody, survive and divide, and secrete cytokines and chemokines after multiple cycles of killing directed at Her2/neu+ tumor targets. Am Assoc Cancer Res. 2003; 44:656a. abstract.
93. Davol PA, Smith JA, Kouttab N, et al. Anti-CD3 × Anti-HER2 bispecific antibody effectively redirects armed T cells to inhibit tumor development and growth in hormone-refractory prostate cancer-bearing SCID-Beige mice. Clin Prostate Cancer. 2004 Sep.3:112–21. [PubMed: 15479495]
94. Kroesen BJ, ter Haar A, Willemse P, et al. Local antitumor treatment in arcinoma patients with bispecific-monoclonal-antibody-redirected T cells. Cancer Immunol Immunother. 1993; 37(6): 401–7.
95. Demanet C, Brissinck J, De Jong J, et al. Bispecific antibody-mediated immunotherapy of the BCL_1 lymphoma: increased efficacy with multiple injections and CD28-induced costimulation. Blood. 1996; 87:4390–8. [PubMed: 8639800]
96. Hombach A, Tillmann T, Jensen M, et al. Specific activation of resting T cells against CA19-9+ tumor cells by an anti-CD3/CA19-9 bispecific antibody in combination with a costimulatory anti-CD28 antibody. J Immunother. 1997 Sep; 20(5):325–33. [PubMed: 9336739]
97. Kaneko T, Fusauchi Y, Kakui Y, et al. A bispecific antibody enhances cytokine-induced killer-mediated cytolysis of autologous acute myeloid leukemia cells. Blood. 1993; 81(5):1333–41. [PubMed: 8095165]
98. Bohlen H, Hopff T, Manzke O, et al. Lysis of malignant B cells from patients with B-chronic lymphocytic leukemia by autologous T cells activated with CD3 × CD19 bispecific antibodies in combination with bivalent CD28 antibodies. Blood. 1993; 82:1803–12. [PubMed: 7691238]
99. Bohlen H, Manzke O, Patel B, et al. Cytolysis of leukemic B-cells by T-cells activated via two bispecific antibodies. Cancer Res. 1993; 43:4310–4. [PubMed: 7689932]
100. Klein SC, Boer LH, de Weger RA, et al. Release of cytokines and soluble cell surface molecules by PBMC after activation with the bispecific antibody CD3 × CD19. Scand J Immunol. 1997; 46:452–8. [PubMed: 9393627]
101. Anderson PM, Crist W, Hasz D, et al. G19.4(aCD3) × B43(aCD19) mono-clonal antibody heteroconjugate triggers CD19 antigen-specific lysis of t(4;11) acute lymphoblastic leukemia cells by activated CD3 antigen-positive cytotoxic T cells. Blood. 1992; 80(11):2826–34. [PubMed: 1280479]
102. Bejeck BE, Wang D, Berven E, et al. Development and characterization of three recombinant single chain antibody fragments (scFvs) directed against the CD19 antigen. Cancer Res. 1995; 55:2346–51. [PubMed: 7538901]

103. de Gast GC, Haagen IA, van Houten AA, et al. CD8 T cell activation after intravenous administration of CD3 × CD19 bispecific antibody in patients with non-Hodgkin lymphoma. Cancer Immunol Immunother. 1995; 40:390–6. [PubMed: 7543021]
104. Gall JM, Davol PA, Grabert RC, et al. T cells armed with anti-CD3 × anti-D20 bispecific antibody enhance killing of CD20+ malignant B cells and bypass complement-mediated rituximab resistance in vitro. Exp Hematol. 2005 Apr; 33(4):452–9. [PubMed: 15781336]
105. Renner C, Jung W, Sahin U, et al. Cure of xenografted human tumors by bispecific monoclonal antibodies and human T cells. Science. 1994; 264:833–5. [PubMed: 8171337]
106. Renner C, Bauer S, Sahin U, et al. Cure of disseminated xenografted human Hodgkin's tumors by bispecifc monoclonal antibodies and human T cells: the role of human T-cell subsets in a preclinical model. Blood. 1996; 87(7):2930–7. [PubMed: 8639913]
107. Pohl C, Denfeld R, Renner C, et al. CD30-antigen-specific targeting and activation of T cells via murine bispecific monoclonal antibodies against CD3 and CD28: potential use for the treatment of Hodgkin's lymphoma. Int J Cancer. 1993; 54:820–7. [PubMed: 7686889]
108. Kuwahara M, Kuroki M, Arakawa F, et al. A mouse/human-chimeric bispecific antibody reactive with human carcinoembryonic antigen-expressing cells and human T-lymphocytes. Anticancer Res. 1997; 16:2661–8. [PubMed: 8917366]
109. Negri DR, Tosi E, Valota O, et al. In vitro and in vivo stability and anti-tumour efficacy of an anti-EGFR/anti-CD3 F(ab′)2 bispecific monoclonal antibody. Br J Cancer. 1995 Oct; 72(4):928–33. [PubMed: 7547242]
110. Reusch U, Sundaram M, Davol PA, et al. Anti-CD3 × anti-EGFR bispecific antibody redirects T cell cytolytic activity to EGFR-positive cancers in vitro and in an animal model. Clin Cancer Res. 2006; 12:183–90. [PubMed: 16397041]
111. Riesenberg R, Buchner A, Pohla H, et al. Lysis of prostate carcinoma cells by trifunctional bispecific antibodies (alpha EpCAM × alpha CD3). J Histochem Cytochem. 2001 Jul; 49(7):911–7. [PubMed: 11410615]
112. Luiten RM, Coney LR, Fleuren GJ, et al. Generation of chimeric bispecific G250/anti-CD3 monoclonal antibody, a tool to combat renal cell carcinoma. Br J Cancer. 1996 Sep; 74(5):735–44. [PubMed: 8795576]
113. Nitta T, Sato K, Okumura K, et al. Induction of cytotoxicity in human T cells coated with anti-glioma × anti-CD3 bispecific antibody against human glioma cells. J Neurosurg. 1990; 72:476–81. [PubMed: 2137533]
114. Shalaby MR, Shepard HM, Presta L, et al. Development of humanized bi- specific antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the *HER2* protooncogene. J Exp Med. 1992; 175:217–25. [PubMed: 1346155]
115. Shalaby MR, Carter P, Maneval D, et al. Bispecific Her2 × CD3 antibodies enhance T-cell cytotoxicity in vitro and localize to Her2-overexpressing xenograpfts in nude mice. Clin Immunol Immunopathol. 1995; 74:185–92. [PubMed: 7828373]
116. Brossart P, Stuhler G, Flad T, et al. Her-2/neu-derived peptides are tumorassociated antigens expressed by human renal cell and colon carcinoma lines and are recognized by in vitro induced specific cytotoxic T lymphocytes. Cancer Res. 1998; 58:732–6. [PubMed: 9485028]
117. Davol PA, Smith JA, Kouttab N, et al. Anti-CD3 × anti-HER2 bispecific antibody effectively redirects armed T cells to inhibit tumor development and growth in hormone-refractory prostate cancer-bearing severe combined immunodeficient beige mice. Clin Prostate Cancer. 2004 Sep; 3(2):112–21. [PubMed: 15479495]
118. Zhu Z, Lewis GD, Carter P. Engineering high affinity humanized antip185^{HER2}/anti-CD3 bispecific F(ab′)$_2$ for efficient lysis of p185^{HER2} over-expressing tumor cells. Int J Cancer. 1995; 62:319–24. [PubMed: 7628874]
119. Kostelny SA, Link BK, Tso JY, et al. Humanization and characterization of the anti-HLA-DR antibody 1D10. Int J Cancer. 2001 Aug 15; 93(4):556–65. [PubMed: 11477560]
120. Zhu Z, Ghose T, Lee SH, et al. Tumor localization and therapeutic potential of an antitumor-anti-CD3-heteroconjugate antibody in human renal cell carcinoma xenograft models. Cancer Lett. 1994 Oct 28; 86(1):127–34. [PubMed: 7954349]

121. Katayose Y, Kudo T, Suzuki M, et al. MUC1-specific targeting immunotherapy with bispecific antibodies: inhibition of xenografted human bile duct carcinoma growth. Cancer Res. 1996; 56:4205–12. [PubMed: 8797593]
122. Katzenwadel A, Schleer H, Gierschner D, et al. Construction and in vivo evaluation of an anti-PSA × anti-CD3 bispecific antibody for the immunotherapy of prostate cancer. Anticancer Res. 2000 May; 20(3A):1551–5. [PubMed: 10928069]
123. Davico BL, De Monte LB, Spagnoli GC, et al. Bispecific monoclonal antibody anti-CD3 × anti-tenascin: an immunotherapeutic agent for human glioma. Int J Cancer. 1995 May 16; 61(4):509–15. [PubMed: 7538978]
124. Jost CR, Titus JA, Kurucz I, et al. A single-chain bispecific Fv2 molecule produced in mammalian cells redirects lysis by activated CTL. Mol Immunol. 1996 Feb; 33(2):211–9. [PubMed: 8649442]
125. Chapoval AI, Nelson H, Thibault C. Anti-CD3 × anti-tumor $F(ab')_2$ bifunctional antibody activates and retargets tumor-infiltrating lymphocytes. J Immunol. 1995; 155:1296–303. [PubMed: 7636196]
126. Davol PA, Gall JM, Grabert RC, et al. Infusions of T cells armed with antiCD3 × anti-her2/neu bispecific antibody modulate in vivo patient immune responses in phase I clinical trials for breast and hormone refractory prostate cancers. Blood. 2004 Nov 16.104(11):379a. abstract.
127. Lum LG, Rathore R, Colvin GA, et al. Targeting HER2/neu tumor cells with anti-CD3 activated T cells: clinical trials and trafficking studies. ASCO Meet Proc. 2003; 22:179. abstract.
128. Lum, LG.; Thakur, A.; Rathore, R., et al. ASCO Breast Cancer Symposium. Washington, DC: 2010 Oct 1-3. Phase I clinical trial involving infusions of activated T cells armed with anti-CD3 × anti-Her2neu bispecific antibody in women with metastatic breast cancer: clinical, immune, and trafficking results.
129. Lum, LG.; Thakur, A.; Al-Khadimi, Z., et al. Phase I dose escalation of activated T cells (ATC) armed with anti-CD3 × anti-CD20 bispecific antibody (CD20Bi) after stem cell transplant (SCT) in non-Hodgkin's lymphoma (NHL); The American Society of Hematology (ASH) Annual Meeting 2010; 2010 Dec 4-7; Orlando (FL). p. 488abstract
130. Grabert RC, Cousens LP, Smith JA, et al. Human T cells armed with Her2/neu bispecific antibodies divide, are cytotoxic, and secrete cytokines with repeated stimulation. Clin Cancer Res. 2006; 12(2):569–76. [PubMed: 16428502]
131. Mullbacher A, Lobigs M, Tha Hla R, et al. Antigen-dependent release of IFN-gamma by cytotoxic T cells up-regulates Fas on target cells and facilitates exocytosis-independent specific target cells lysis. J Immunol. 2002; 169:145–50. [PubMed: 12077239]
132. Zeytun A, Hassuneh M, Nagarkatti M, et al. Fas-Fas ligand-based interactions between tumor cells and tumor-specific cytotoxic T lymphocytes: a lethal two-way street. Blood. 1997; 90:1952–9. [PubMed: 9292529]
133. Zaks TZ, Chappell DB, Rosenberg SA, et al. Fas-mediated suicide of tumor-reactive T cells following activation by specific tumor: selective rescue by caspase inhibition. J Immunol. 1999; 162:3273–9. [PubMed: 10092779]
134. Walker PR, Saas P, Dietrich PY. Role of Fas ligand (CD95L) in immune escape: the tumor cell strikes back. J Immunol. 1997 May 15; 158(10):4521–4. [PubMed: 9144461]
135. O'Connell J, O'Sullivan GC, Collins JK, et al. The Fas counterattack: Fasmediated T cell killing by colon cancer cells expressing Fas ligand. J Exp Med. 1996; 184:1075–82. [PubMed: 9064324]
136. Karas M, Zaks TZ, Yakar S, et al. TCR Stimulation protects CD8+ T cells from CD95 mediated apoptosis. Hum Immunol. 2001; 62:32–8. [PubMed: 11165713]
137. Grosse-Hovest L, Hartlapp I, Marwan W, et al. A recombinant bispecific single-chain antibody induces targeted, supra-agonistic CD28-stimulation and tumor cell killing. Eur J Immunol. 2003 May; 33(5):1334–40. [PubMed: 12731059]
138. Otz T, Grosse-Hovest L, Hofmann M, et al. A bispecific single-chain antibody that mediates target cell-restricted, supra-agonistic CD28 stimulation and killing of lymphoma cells. Leukemia. 2009 Jan; 23(1):71–7. [PubMed: 18830257]

139. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibodyTGN1412. N Engl JMed. 2006 Sep 7; 355(10):1018–28. [PubMed: 16908486]

140. Alvarez-Vallina L, Hawkins RE. Antigen-specific targeting of CD28-mediated T cell co-stimulation using chimeric single-chain antibody variable fragment-CD28 receptors. Eur J Immunol. 1996; 26:2304–9. [PubMed: 8898938]

141. Renner C, Jung W, Sahin U, et al. The role of lymphocyte subsets and adhesion molecules in T cell-dependent cytotoxicity mediated by CD3 and CD28 bispecific monoclonal antibodies. Eur J Immunol. 1995; 25:2027–33. [PubMed: 7621876]

142. Mazzoni A, Mezzanzanica D, Jung G, et al. CD3-CD28 costimulation as a means of avoiding T cell preactivation in bispecific monoclonal antibody-based treatment of ovarian carcinoma. Cancer Res. 1996; 56:5443–9. [PubMed: 8968099]

143. Hombach A, Tillmann T, Jensen M, et al. Specific activation of resting T cells against tumour cells by bispecific antibodies and CD28-mediated co-stimulation is accompanied by Th1 differentiation and recruitment of MH-C independent cytotoxicity. Clin Exp Immunol. 1997; 108:352–7. [PubMed: 9158110]

144. Fitzer-Attas CJ, Eshhar Z. Tyrosine kinase chimeras for antigen-selective T-body therapy. Adv Drug Deliv Rev. 1998 Apr 6; 31(1-2):171–82. [PubMed: 10837624]

145. Eshhar Z, Waks T, Gross G, et al. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibodybinding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci USA. 1993; 90:720–4. [PubMed: 8421711]

146. Hwu P, Shafer GE, Treisman JS, et al. Lysis of ovarian cancer cells by human lymphocytes redirected with a chimeric gene composed of an antibody variable region and the Fc receptor gamma chain. J Exp Med. 1993; 178:361–6. [PubMed: 8315392]

147. Hwu P, Yang JC, Cowherd R, et al. In vivo antitumor activity of T cells redirected with chimeric antibody/T-cell receptor genes. Cancer Res. 1995; 55:3369–73. [PubMed: 7614473]

148. Fitzer-Attas CJ, Eshhar Z. Tyrosine kinase chimeras for antigen-selective T-body therapy. Adv Drug Delivery Rev. 1998; 31:171–82.

149. Altenschmidt U, Moritz D, Groner B. Specific cytotoxic T lymphocytes in gene therapy. J Mol Med. 1997; 75:259–66. [PubMed: 9151212]

150. Sadelain M, Riviere I, Brentjens R. Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer. 2003 Jan; 3(1):35–45. [PubMed: 12509765]

151. Kershaw MH, Westwood JA, Parker LL, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006 Oct 15; 12(20 Pt 1):6106–15. [PubMed: 17062687]

152. Cooper LJ, Al-Kadhimi Z, Serrano LM, et al. Enhanced antilymphoma efficacy of CD19-redirected influenza MP1-specific CTLs by cotransfer of T cells modified to present influenza MP1. Blood. 2005 Feb 15; 105(4):1622–31. [PubMed: 15507526]

153. Emtage PC, Lo AS, Gomes EM, et al. Second-generation anti-carcinoembryonic antigen designer T cells resist activation-induced cell death, proliferate on tumor contact, secrete cytokines, and exhibit superior antitumor activity in vivo: a preclinical evaluation. Clin Cancer Res. 2008 Dec 15; 14(24):8112–22. [PubMed: 19088026]

154. Ma Q, Safar M, Holmes E, et al. Anti-prostate specific membrane antigen designer T cells for prostate cancer therapy. Prostate. 2004 Sep 15; 61(1):12–25. [PubMed: 15287090]

155. Wang LX, Li R, Yang G, et al. Interleukin-7-dependent expansion and persistence of melanoma-specific T cells in lymphodepleted mice lead to tumor regression and editing. Cancer Res. 2005 Nov 15; 65(22):10569–77. [PubMed: 16288050]

156. Gattinoni L, Finkelstein SE, Klebanoff CA, et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med. 2005 Oct 3; 202(7):907–12. [PubMed: 16203864]

157. Grinshtein N, Ventresca M, Margl R, et al. High-dose chemotherapy augments the efficacy of recombinant adenovirus vaccines and improves the therapeutic outcome. Cancer Gene Ther. 2009 Apr; 16(4):338–50. [PubMed: 18989352]

158. Salem ML, az-Montero CM, Al-Khami AA, et al. Recovery from cyclophosphamide-induced lymphopenia results in expansion of immature dendritic cells which can mediate enhanced prime-boost vaccination antitumor responses in vivo when stimulated with the TLR3 agonist poly(I:C). J Immunol. 2009 Feb 15; 182(4):2030–40. [PubMed: 19201856]

159. Curti BD, Longo DL, Ochoa AC, et al. Treatment of cancer patients with *ex vivo* anti-CD3-activated killer cells and interleukin-2. J Clin Oncol. 1993; 11:652–60. [PubMed: 8257476]

160. McCall AM, Shahied L, Amoroso AR, et al. Increasing the affinity for tumor antigen enhances bispecific antibody cytotoxicity. J Immunol. 2001 May 15; 166(10):6112–7. [PubMed: 11342630]

161. Haagen IA, de Lau WB, Bast BJ, et al. Unprimed CD4+ and CD8+ T cells can be rapidly activated by a CD3 × CD19 bispecific antibody to proliferate and become cytotoxic. Cancer Immunol Immunother. 1994 Dec; 39(6):391–6. [PubMed: 7528094]

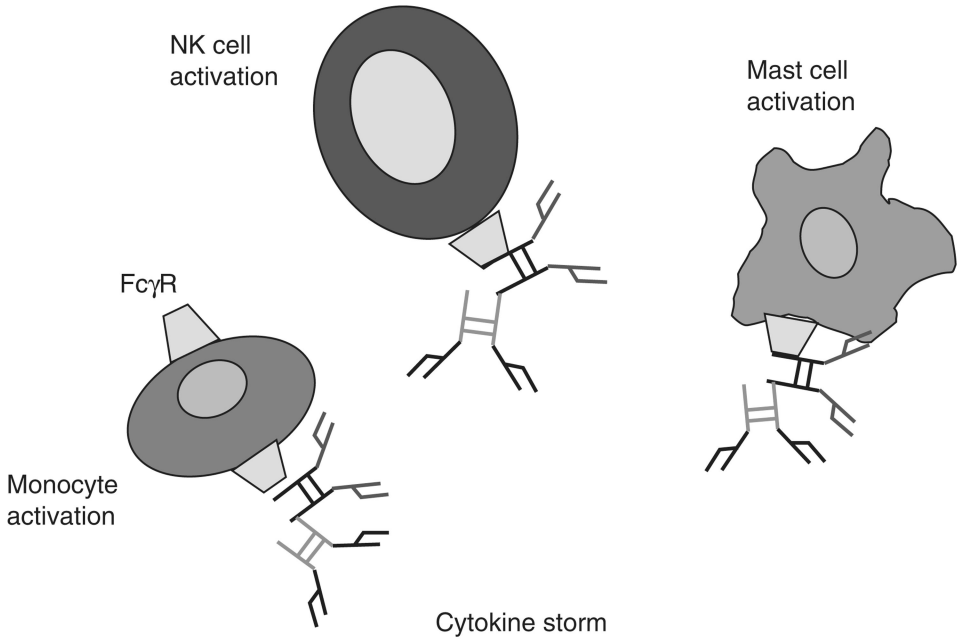

Fig. 1.

Fc-receptor binding to immune cells, leading to cytokine storm. Bispecific antibodies (BiAbs) alone can bind to FcγR on natural killer (NK) cells, mast cells, and monocytes and induce the release of cytokines/chemokines leading to the cytokine storm. The figure shows how the interaction between the Fc-portions on a BiAb can occur via available Fc-receptors on NK cells, mast cells, and monocytes.

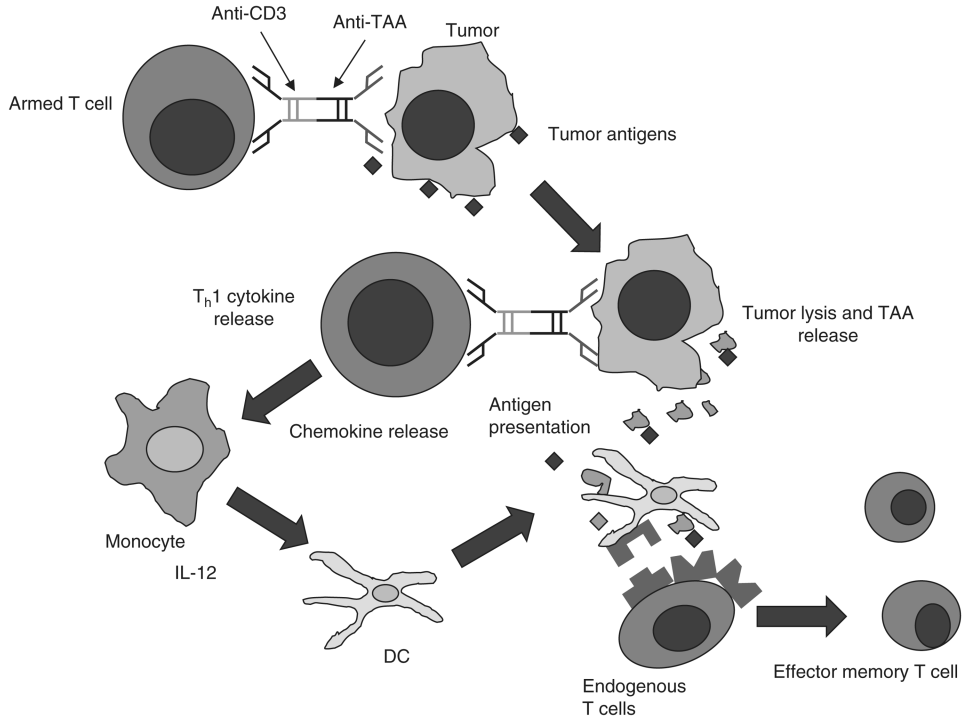

Fig. 2.

In situ immunization of the endogenous immune system. Armed, activated T cells engage the tumor by targeting tumor-associated antigens (TAAs) on the tumor. The targeting process induces T helper-1 (T_h1) cytokine secretion and the release of TAAs from the tumor. Differentiated dendritic cells (DCs), which are induced by interleukin (IL)-12 produced by monocytes, process the released TAAs and present them to endogenous naïve T cells recruited by chemokine release, leading to local *in situ* immunization that becomes systemic immunization.

Table I

Clinical trials using bispecific antibodies (BiAbs)

Targets	BiAb	INNs/trade names (company)	Effector cells	Clinical outcome	References
Gliomas	Anti-CD3×anti-glioma		LAK	DLTs	36
Ovarian carcinoma	Anti-OCAA×anti-CD3	OC/TR	T cells	DLTs	37
NHL	Anti-CD3×anti-CD19	SHR-1	T cells	No clear clinical responses	38
Lung cancer, renal cell cancer	Anti-CD3×anti-EGP-2	BIS-1	T cells	DLTs	39
Hodgkin's disease	Anti-CD16×anti-CD30	HRS-3/A9	NK cells	No clear-cut dose side effect or dose response	40
AML	CD64×CD33	251-22	Monocyte/macrophage	DLTs	41
Breast and prostate cancer	Anti-CD64×anti-HER2	MDX-210; MDX-H210	Monocyte/macrophage	DLTs	42,43
Malignant ascites in ovarian, gastric, colon, breast	Anti-CD3×anti-EpCAM	Removab® (Trion Pharma)	T cells	Objective clinical responses (market approved 2009)	44
Gastric, lung, colorectal carcinomas	Anti-CD3×anti-EpCAM	Removab® (Trion Pharma)	T cells	Objective clinical responses	45
HER2+ tumors	Anti-CD16×anti-HER2	2B1	NK cells	DLTs/cytokine storm	46,47
Solid tumors	Anti-CD64×anti-EGFR	MDX-447	Monocyte/macrophage	DLTs/cytokine storm	48
NHL	Anti-CD3×anti-CD20	CD20BiAb	T cells	Phase I	49
Metastatic breast cancer	Anti-CD3×anti-HER2	HER2BiAb	T cells	Phase II	50
Ovarian carcinoma	Anti-CD3×anti-EpCAM	Removab (Trion Pharma)	T cells	Phase II	51
Metastatic breast cancer	Anti-CD3×anti-HER2	Ertumaxomab (Trion Pharma)	T cells	Phase I/II	52
Metastatic melanoma	Anti-CD28×MAPG	rM28	T cells	Phase I/II	53
NHL	Anti-CD3×anti-CD19	Blinatumomab (Micromet)	T cells	Phase I/II	54
B-ALL	Anti-CD3×anti-CD19	Blinatumomab (Micromet)	T cells	Phase I/II	55
Relapsed/refractory B-ALL	Anti-CD3×anti-CD19	Blinatumomab (Micromet)	T cells	Phase II	55

ALL=acute lymphocytic leukemia; **AML**=acute myelogenous leukemia; **B-ALL**= precursor B-cell ALL; **DLTs** =dose-limiting toxicities; **EGFR**=epidermal growth factor receptor; **EGP2**= epithelial glycoprotein 2; **EpCAM**= epithelial cell adhesion molecule; **HER2**=human EGFR-2; **INN** = international non-proprietary name; **LAK** =lymphokine-activated killer cells; **MAPG**= melanoma-associated proteoglycan; **NHL**= non-Hodgkin's lymphoma; **NK cells**=natural killer cells; **OCAA**= ovarian cancer-associated antigen.

Table II

Phase I clinical trials using the bispecific antibody MDX-H210 (anti-CD64 ×anti-HER2)

Indication (no. of pts)	Dosage	Clinical outcome	Adverse effects	References
Refractory breast and ovarian cancer (10)	0.35 to 18mg/m^2	1 PR and 1 mixed response	'Flu-like' symptoms, chest pain, dyspnea, fever, chills, myalgias, fatigue, hypotension	2,62
Prostate cancer (6)	1 to 8mg/m^2	5 of 6 pts had stable PSA levels for over 40 days and decreases in HER2 levels	'Flu-like' symptoms, chest pain, dyspnea, fever, chills, myalgias, fatigue, hypotension	3,63
Advanced prostate cancer (25)	5 μg/kg/day 4 days/ week for6weeks	20 of 25 pts had >50% decrease in PSA levels (median duration 128 days)	Therapy stopped in 2 pts who developed heart failure, dyspnea, and an allergic reaction	4,64
Prostate cancer (13)	1 to 20mg/m^2, in combination with GM-CSF	Of 11 evaluable pts,1 near-PR, 6 SD, 3 PD	5 of 11 pts developed HAMAs	65

EGFR=epidermal growth factor receptor; **GM-CSF**=granulocyte-macrophage colony-stimulating factor; **HAMAs**=human anti-mouse antibodies; **HER2**= human EGFR-2; **PD** =progressive disease; **PR** = partial response; **PSA** =prostate-specific antigen; **pts**= patients; **SD**= stable disease.

Table III

Preclinical studies of anti-CD3× anti-tumor-associated antigen bispecific antibodies (BiAbs)

Tumor antigen	Year	BiAb	Tumor	References
AMOC-31	1993	Anti-CD3×anti-AMOC-31	Carcinomas expressing the 40kDa membrane-bound glycoprotein AMOC-31	94
B-cell idiotype	1996	Anti-CD3 ×anti-idiotype	Idiotype on BCL1 lymphoma in Balb/c mice	95
CA125	2006	Anti-CD3×anti-CA125	Ovarian carcinomas	90
CA19-9	1997	Anti-CD3 ×anti-CA19-9	CA19-9	96
CD13	1993	Anti-CD3×anti-CD13	$CD13^+$ acute myeloid leukemia	97
CD19	1993	Anti-CD3×anti-CD19	Leukemic B cells	98,99
CD19	1992	Anti-CD3×anti-CD19	Malignant B cells	98,100-103
CD20	2005	Anti-CD3×anti-CD20	NHL	104
CD20	2005	Anti-CD3×anti-CD20	Multiple myeloma	104
CD30	1993	Anti-CD3×anti-CD30	Hodgkin's lymphoma	105-107
CEA	1997	Anti-CD3×anti-CEA	Human CEA-expressing cells	108
EGFR	1995	Anti-CD3×anti-EGFR	Glioma, neoplastic keratinocytes	109
EGFR	2006	Anti-CD3×anti-EGFR	Colon, head and neck, and lung	110
EpCAM	2001	Anti-CD3×anti-EpCAM	Adenocarcinomas expressing EpCAM	111
G250	1996	Anti-CD3×anti-G250	Renal cell carcinoma	112
Glioma	1990	Anti-CD3 ×anti-glioma	Human glioma	113
HER-2/neu (HER2)	1992	Anti-CD3×anti-HER2	HER2 receptor-expressing renal cell, colon, breast, and prostate carcinomas	88,89,114-118
HLA-DR beta chains	2001	Anti-CD3×anti-HLA-DR beta chains	Malignant B cells	119
kDal K29	1994	Anti-CD3 ×anti-kDalK29	Renal cell carcinoma	120
MUC1	1939	Anti-CD3×anti-MUC1	Bile duct carcinoma	121
PSA	2000	Anti-CD3×anti-PSA	PSA-expressing prostate carcinomas	122
Tenascin	1995	Anti-CD3 ×anti-tenascin	Human glioma	123
Transferrin receptors	1996	Anti-CD3 ×anti-transferrin receptor	Tumors expressing transferrin receptors	124
Tumor ($F[ab']_2$)	1995	Anti-CD3 ×anti-tumor $F(ab')_2$	For retargeting TIL	125

CEA=carcinoembryonic antigen; **EGFR**=epidermal growth factor receptor; **EpCAM**=epithelial cell adhesion molecule; **HER2**=human EGFR-2; **HLA**= human leukocyte antigen; **NHL**=non-Hodgkin's lymphoma; **PSA**=prostate-specific antigen; **TIL**=tumor-infiltrating lymphocytes.