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Chimeric antigen receptors and bispecific antibodies to retarget T cells in pediatric oncology

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Abstract

Cancer immunotherapy using antigen-specific T cells has broad therapeutic potential. Chimeric antigen receptors and bispecific antibodies can redirect T cells to kill tumors without human leukocyte antigens (HLA) restriction. Key determinants of clinical potential include the choice of target antigen, antibody specificity, antibody affinity, tumor accessibility, T cell persistence, and tumor immune evasion. For pediatric cancers, additional constraints include their propensity for bulky metastatic disease and the concern for late toxicities from treatment. Nonetheless, the recent preclinical and clinical developments of these T cell based therapies are highly encouraging.

Keywords

bispecific antibodies; chimeric antigen receptors; immunotherapy; pediatric oncology; T cells

Introduction

The late nineteenth century witnessed the birth of cancer immunotherapy when Dr. William Coley treated cancer patients with mixtures of heat-killed streptococcal organisms and *Serratia marcescens*, called “Coley’s toxin”, based on his observation of tumor regression following erysipelas in patients with inoperable sarcomas [1]. Supplanted by radiotherapy throughout the early twentieth century, immunotherapy did not gain momentum until the 1950s when the concept of cancer immunosurveillance was put forward by Drs. Burnet and Thomas, and allogeneic hematopoietic stem cell transplant for leukemia was first performed by Dr. E. Thomas[2-4]. Cancer therapeutics continued to be dominated by intensive radiotherapy and chemotherapy, designed to match the unrelenting recurrences and aggressiveness of metastatic solid tumors. Cancer immunotherapy was not an accepted modality until the 1990s, upon the Food and Drug Administration (FDA) approval of monoclonal antibodies. Since then, the concepts of “cancer immunosurveillance” and

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Potential Conflict of Interest Statement

NK. V. Cheung has ownership interest (including patents) in scfv constructs of anti-GD2 antibodies, therapy-enhancing glucan, use of mAb 8H9, methods for preparing and using scFv, GD2 peptide mimics, methods for detecting MRD, anti-GD2 antibodies, generation and use of HLA-A2–restricted peptide-specific mAbs and CARs, high-affinity anti-GD2 antibodies, and multimerization technologies. No potential conflicts of interest were disclosed by the other authors.

"cancer immunoediting" have shaped the development of cancer immunotherapy. Over the past two decades, a variety of clinical strategies including adoptive T cell therapies, cancer vaccines, and monoclonal antibodies have emerged and continually optimized following their initial clinical successes. However, these clinical strategies have only been sporadically applied in pediatric oncology. Recent successes in treating refractory cancers by using T cells redirected by chimeric antigen receptors (CARs) or by bispecific antibodies (BsAbs) have energized the field.

Immunosurveillance and Immunoediting

To better understand how host immunity can target malignancy, one must evaluate how immune cells and tumor cells interact. The endogenous immune system can recognize malignant transformation because of its accompanying neo-antigens. However, cancer cells quickly evolve evasive or immune-suppressive mechanisms to avoid detection and/or eradication. This process of cancer "immunosurvelliance" and "immunoediting" has been summarized into three sequential phases; elimination, equilibrium, and escape [5]. During the "elimination phase", both innate and adaptive immune effectors combine to control the cancer growth. The innate immune cells such as macrophages, natural killer (NK), NK-T, and dendritic cells, cooperate to recognize and eliminate the transformed cells. Through their Fc receptors, they lyse or phagocytose tumor cells in the presence of anti-tumor antibodies. The professional antigen-presenting cells prime the CD4(+) and CD8(+) T cells in the adaptive immune system. When CD4(+) cells engage the HLA-class II-peptide complex, they secrete cytokines such as interferon (INF)-γ and interleukins (e.g. IL-2) to orchestrate other effectors (including B lymphocytes) for an optimal anti-tumor response. CD8(+) T cells recognize tumor cells through tumor peptides presented on the human HLA-class I antigen, injecting their granzymes and perforins to kill. Rare cancer cell mutants with inherent or acquired capacities to evade the immune system can survive, and the tumor enters the "equilibrium phase", where the rate of tumor growth is equal to the rate of tumor elimination. Finally, in the "escape phase", additional tumor cell variants can completely escape recognition by the adaptive immune system. Many mechanisms can facilitate this escape, including the loss of HLA or the tumor antigen from the tumor cell surface, defects in tumor antigen processing, altered tumor microenvironment that is T-cell suppressive by recruiting regulatory T cells (Tregs) [6], myeloid-derived suppressor cells [7], or tumor associated M2 macrophages [8]. To combat this tumor "escape", cancer biologists have recently focused on releasing the brake at immune checkpoints (e.g. CTLA4, PD1, PDL1) [9, 10]. The clinical potential of such manipulations assumes a preexisting tumor-specific T cell immunity. Unfortunately, if the tumor downregulates their HLA or target, or if the clonal frequency of these T cells are low (especially after immunosuppressive chemotherapy or radiation therapy), removing the brakes may not be adequate. If the preexisting immunity is not tumor-specific, autoimmune complications are expected. To overcome these limitations, CARs and BsAbs can provide powerful platforms to engage T cells for robust anti-tumor responses. The characteristics of these two platforms are the focus of this review.

Chimeric antigen receptor (CAR)-modified T cells

CARs are genetically engineered receptors that redirect T cells to a chosen tumor antigen. CARs usually consist of three domains: an extracellular antigen-binding domain, a transmembrane domain, and at least one intracellular signal transduction domain. They are genetically inserted into T cells using viral vectors, DNA transposons, or RNA transfection. When CARs bind to tumor antigen, the intracellular signaling domain is activated and the tumoricidal process by T cells is initiated. First generation CAR-modified T cells (CAR T cells) carrying a single activation domain consisting of either CD3-ζchain or FcεRIγ did not show significant efficacy in clinical trials because many tumor cells lack costimulatory ligands [11], although a phase 1 study of anti-GD2 CAR T cells in neuroblastoma showed objective clinical effects including complete remission in 3 patients.[12, 13]. The second generation CAR T cells contain an additional costimulatory sequence such as CD28 or CD137 (4-1BB), with improvement in T cell proliferation and survival in both preclinical and clinical settings [14-16]. Several third generation CAR T cells which carry two costimulatory sequences in tandem (e.g. CD28 and CD137) have also been tested [17-19] (Fig.1). While the current paradigm of CAR technology is focused on driving T cells to the tumor, recent pre-clinical studies of cellular cargos (e.g. potent immunostimulatory agents such as IL-12) suggest additional strategies in arming T cells to overcome the immuno-suppressive tumor microenvironment are feasible [20].

Bispecific antibody (BsAb)

BsAbs are antibodies with dual specificities; the first specificity against a tumor antigen, and the second specificity for an activator on immune effectors (e.g. CD3 on T cells). When activated through CD3, cytotoxic T cells inject perforin and granzyme B into target cells to kill. First-generation BsAbs were IgG-shaped molecules produced by fusing two different hybridomas, each directed at a different target [21, 22]. With the advances in protein engineering and recombinant DNA technologies, such as the packaging of the binding domains of the variable domains (VH and VL) of an IgG into a smaller single chain viable fragment (scFv), second-generation BsAbs are now possible, exploiting a variety of formats with increasing numbers of variable domains, specialized Fc forms, and novel functionalities [23, 24]. Bispecific T-cell engager (BiTE®) antibodies have two scFvs joined together as a single polypeptide chain in tandem, one scFv engaging tumor cells and the other scFv engaging CD3 on T cells (Fig.1). Univalent binding to CD3 without crosslinking to avoid nonspecific T cell activation is the driving principle behind the BiTE® and other engineered antibody platforms [25, 26], although a IgG-scFv platform was recently constructed to have low nonspecific T cell activation despite its bivalency [27]. It is of importance, though it remains to be generalized, that BsAbs can even redirect regulatory T cells (Treg) to kill tumor cells [28]. Unlike CAR T cells, BiTE®-activated T cells may be less susceptible to the inhibition by Treg [29, 30].

Selection of tumor targets and antibody affinity

One of the critical decisions in CAR T cell or BsAb strategy is the selection of the target antigen. Ideally it should have high expression on tumor cells, including tumor stem lines,

and limited expression on normal cells. CAR T cells was shown to be more potent than BiTE®-redirected T cells when the target antigen density was low [31], likely due to BiTE® being monovalent. For CAR T cells, tumor engagement is likely multivalent, and tumor lysis did not correlate with the antigen-binding affinity [32-34]. For BiTE® platforms, increasing affinity enhanced anti-tumor effects in preclinical studies [35]. In contrast to the monovalent BiTE®, bivalent BsAbs can bind more avidly to the tumor cells to exert higher lytic potency even when target antigen is low [27, 36]. However, enhanced potency at low antigen density can also be a clinical risk. In a Phase 1 study using the third generation HER2 CAR T cells for metastatic HER2(+) cancer, a patient died from respiratory distress, a complication ascribed to T cells recognizing the low levels of HER2 in the lung [37]. Unless the cross-reactivity is restricted to non-essential organs, such sequelae could be serious. An example of non-essential tissue is the CD19(+) B cell population - when destroyed by CD19-directed CAR T cells, agammaglobulinemia develops in patients. Nevertheless, this condition is correctable by gamma globulin replacements. In order to reduce lethal cross-reactivities with critical organs, decreasing the T cell dose and/or the number of costimulatory sequences have been tried. Furthermore, a suicide gene system exploiting thymidine kinase (HSV-TK) or drug inducible caspase-9, can also be used to eliminate unwanted CAR T cells (NCT00182650, NCT01822652, NCT01953900).

Access of CAR T cells and BsAbs to tumor

Quantitative in vivo delivery of therapeutic agents to the target is another critical determinant of clinical efficacy. Solid cancers, especially bulky tumors, have mechanical barriers to intravenous therapies because of their disorganized vascularization and stiff tissue structure [38]. Most CAR T cells are infused intravenously with blood, bone marrow and lymph nodes being most accessible after intravenous injection. Hence, the efficacy is seen with hematogenous cancers, whereas the clinical potential for eradicating solid tumors is still uncertain [11, 13, 33]. To enhance T cell homing into the tumor, additional receptors such as chemokine/cytokine receptors (e.g. CXCR2 (CXCL1 receptor), CCR4 (CCL17 receptor)), or lymphotactin have been introduced into CAR T cells with some preclinical success [39-42].

BiTE® can penetrate into solid tumors because of their small size (about 60 kDa) and they redirect tumor-infiltrating lymphocytes (TILs) and even Tregs to kill tumor cells [28]. Larger BsAbs (160 kDa to 210 kDa) have also been successful in penetrating solid tumors in preclinical models [27, 36, 43, 44]. Compartmental administration can also overcome the difficulties in drug delivery to the tumors. For example, catumaxomab is administered intraperitoneally for treating malignant ascites [45-47], and rM28, a recombinant BsAb directed against high molecular weight melanoma-associated antigen and CD28 [48], is administered intratumorally against malignant melanoma.

Persistence of CAR T cells and BsAbs in vivo

Unlike BsAbs which can be administered in repeated doses over months, persistence of CAR T cells is essential especially in solid tumor because these cells are intended to last for years. Initial studies of CAR T cells documented their disappearance from peripheral blood

within two to three weeks after infusion, despite their presence when assayed by PCR techniques [49]. The current generation of CAR T cells has improved persistence up to 11 months by flow cytometry, and 2 years by PCR as a result of lymphodepleting chemotherapy (cyclophosphamide +/− fludarabine) before CAR T cell infusion [50-54]. However, intense preconditioning increases the risk of infection and major organ damage including the heart, liver, and kidney, both in the short-term and the long-term. This could be particularly problematic for prior treated patients with relapsed or refractory disease. A relatively low dose of cytoreduction chemotherapy was given in a recent clinical study and it was tolerable [52]. In pre-clinical studies, T cell pre-selection [55, 56] and resistance to Tregs [56-59] have been investigated. In T cell pre-selection, early differentiation markers are used to isolate naïve CD8(+) T cells that will persist longer in vivo, and have superior anti-tumor immunity when compared to effector memory CD8(+) T cells [55, 56]. Through the activation of the Akt pathway, T cells can be prevented from forming Tregs or can be made insensitive to Tregs [56-59].

The serum half-life of BiTE® antibodies is measured in hours because of their small size. Various strategies have been adopted to maintain their therapeutic serum level. Blinatumomab (half-life 1.25 hours [60]) and MT110, an anti-epithelial cell adhesion molecule BiTE®, (half-life 4.5 hours [61]) were administered by continuous intravenous infusion over 4 weeks. Subcutaneous administration of MT112 (anti-prostate-specific membrane antigen BiTE®) as a depot for slow release for prostate cancer was also tested in pre-clinical studies [62]. Larger size BsAbs using a multimerization platform [63], or those built like an IgG [27, 44] will have longer serum half-lives, although toxicity may also increase. Clearly, treatment cycles of BsAbs could also be repeated to achieve their maximal clinical benefit.

Ease of use, quality control and accessibility of care

Looking ahead, accessibility of care can be a limiting step for drug acceptance. CAR T cell infusion is a personalized therapy. The process from autologous T cell collection, in vitro stimulation, CAR gene viral transduction, continual in vitro expansion, to infusion of CAR T cells takes an average of 7 to 11 days [50-52, 64] and according to Citigroup estimates, the cost per patient could be substantial [65]. Since cells have finite shelf-lives, they may be difficult to distribute across the continents. At present, such treatments are performed only at comprehensive medical centers with sophisticated laboratories staffed with personnel well trained in cytotherapy techniques of autologous T cell proliferation and T cell gene modification. An active effort both academically and commercially to set up centralized cell processing and distribution logistics will help increase acceptance by the health care system.

Building on the successes of monoclonal antibody therapies, BsAbs take advantage of their standardized manufacturing methods and quality control, as well as the protocols on how these drugs are administered. For diseases with high prevalence, BsAbs have improved chances of acceptance by patients and health care systems even if they have to be administered weekly or subcutaneously, since they can be given in an outpatient clinic and their cost can be less than CAR T cells [66].

Cytokine-release syndrome (CRS) is a concern for both CAR T cells and BsAbs, since it may require critical care. Recently, standardized treatment algorithm for the management of CRS has been established in a multi-institutional group [67], which in turn should result in a broader acceptance of these modalities in the medical community. Since the severity of CRS after CAR T cell infusions correlates with tumor burden, a more intensive pre-conditioning chemotherapy could be used to reduce the disease burden [52]. In the final analysis, efficacy, cost and toxicity will determine the future of either one of these T cell retargeting strategies.

Clinical Trials

As of January, 2015, at least 78 clinical trials using CAR T cells targeting 23 different antigens (Table I) and 42 clinical trials using 15 different types of BsAbs (Table II) have been registered to clinicaltrials.gov. Among CAR T cells, nearly half of the trials focused on CD19, and over 60% of them were designed for patients with hematological malignancies or lymphomas. Clinical studies have shown benefit among patients with B-lineage malignancies [50, 52], and their combinations with IL-2 or with dual specific T cells (e.g. EBV) have been tested (Table I). CAR T cells have also been tested in pediatric cancers (highlighted in Table I). Phase 1 clinical trials of CD19 CAR T cells in adult and pediatric patients with B cell-lineage malignancies showed safety and significant anti-tumor effects; 70-90% of patients have achieved complete remission (CR) 1 month after the CAR T cell infusion [51, 52]. Severe cytokine-release syndrome occurred in 14-27% of the patients, and they did respond to treatment with tocilizumab, a humanized anti-interleukin-6 receptor antibody (NCT01626495, NCT01029366, NCT01593696)[50, 52].

Among BsAbs, catumaxomab for malignant ascites of the epithelial cell adhesion molecule (EpCAM) positive cancers was approved by the European Medicines Agency (EMA) in 2009. Blinatumomab, the BiTE® specific for CD19 positive leukemia or lymphoma, has shown clinical benefit [45-47, 68, 69] receiving FDA approval in 2014. Furthermore, the BiTE® (e.g. EpCAM, BsAb, MT110) and various BsAb platforms targeting solid tumors, including adenocarcinoma, breast cancer, and melanoma are actively being pursued. For the pediatric populations, targeting GD2 in solid tumors have shown promise in preclinical models [27, 35, 36, 43, 70]. Clinical trials of blinatumomab in lymphoblastic leukemia are ongoing and anti-GD2 BsAbs in solid tumors will soon be open (highlighted in Table II).

T cell redirection strategies for pediatric cancers

Pediatric cancer patients face unique challenges because of their young age, the metastatic tendency of their tumors, and long-term toxicity considerations in later years. In children, certain growth factor receptors and differentiation antigens, which are the potential targets of cancer therapy, are required for normal development (e.g. insulin-like growth factor 1 receptor) [71]. Currently, several CAR T cells and BsAbs are used in preclinical or clinical trials in children including CD19, CD30, and CD33 for hematologic malignancies, GD2 for osteosarcoma and embryonal cancers including neuroblastoma and rhabdomyosarcoma, and HER2 for sarcomas and brain tumors [64, 71] with relatively short follow-up (<5 years). Even though hypogammaglobulinemia from B-cell depletion by CD19-targeted therapy is treatable with gamma globulin infusions, pan B-cell deficit and its potential long term

immune or autoimmune complications remains unknown. GD2-specific monoclonal antibodies have been used in treating pediatric neuroblastoma over the past 2 decades. Even though there were no observed late effects with anti-GD2 monoclonal antibodies or with anti-GD2 CAR T cells, careful long-term follow-up for potential damage of GD2(+) neurons is warranted.

Target discovery for redirecting T cells in pediatric cancers is continuing. ROR1 and ROR2 are examples of antigens too low in density for Fc-dependent tumor lysis. When incorporated into CAR T cell or BsAb technology, they hold anti-tumor potential. Glypican-3, glypican-5, GM2, and GD3 are other examples that deserve attention [71, 72].

Although pediatric embryonal cancers are more chemosensitive when compared to adult cancers [73], they tend to be widely metastatic to the bone, bone marrow, lymph nodes, lung, liver and the central nervous system (CNS). Here, the ability to deliver CAR T cells or BsAbs to these distinct metastatic sites can be critical, since inadequate treatment of one site will allow reseeding of cancer to the other organs. Many pediatric cancer regimens use intensive chemotherapy or radiation therapy resulting in the depletion of T cells and other immune cells. Without T cells, neither CAR T cells nor BsAb technology can be truly effective, and without other supporting immune cells, maximal tumor response cannot be achievable [27, 74]. Thus, it may be necessary to cryopreserve autologous T cells harvested before dose-intensive cytotoxic therapy. Alternatively, cytokines such as IL-7 and IL-15 may be necessary for lymphoid regeneration [75-77].

Future directions

As single modalities, CAR T cells and BsAbs have shown anti-tumor responses. By combining them with other targeted drugs with non-overlapping toxicities, their efficacy can be enhanced and the emergence of resistant cells can be prevented. For example, in neuroblastoma, CAR T cells or BsAbs directed at GD2 can be combined with other monoclonal antibodies to induce cell death (anti-DR4 or anti-DR5) [78], to remove immune checkpoint blockade (anti-CTLA4, anti-PD1, anti-PD-L1) [79], to disable growth factor pathways (anti-IGF1 and anti-IGF-2, anti-IGF-1R, anti-HGF-R, anti-EGF-R, anti-TrkB) [80, 81], to inhibit angiogenesis (anti-VEGF) [82], or to combine with cytokine that enhance CD8(+) T cells (e.g. IL-15) [77]. In the CD19-CAR T cell therapy of pre-B cell ALL, the escape of CD19(−) clones can pose substantial obstacles to cure. This can potentially be surmounted by using combination strategies such as the additional targeting through CD22 [83]. Small molecules targeting antigens involving tumor growth, invasion, metastasis, and resistance to cell death can also combine with T-cell redirection, as long as they do not interfere with T cell functions [84, 85].

CAR-modified leukemia-redirected autologous T cells may provide an alternative to allogeneic stem cell transplantation. In allogeneic transplantation, cytoreduction with intensive chemotherapy and/or radiation is required prior to stem cell infusion to prevent graft failure, followed by immunosuppression to prevent graft versus host disease (GVHD). The accompanying toxicities from GVHD and opportunistic infections have compromised quality of life and survival. It is conceivable that CAR-modified autologous T cells alone or

in combination with autologous stem cell transplant, which have less acute and long-term toxicity compared to allogeneic transplant, could achieve similar anti-tumor benefits. Furthermore, autologous stem cell harvests can also be purged ex vivo using CAR-modified autologous T cells or BsAb to reduce tumor cell contamination before cryopreservation or before reinfusion.

Conclusion

CAR T cells and BsAbs are novel T-cell based therapies; each has its unique advantages and challenges. Both have shown impressive clinical efficacy in leukemia and lymphoma. As these platforms are fine-tuned, their relevance for solid tumors should become more obvious. CAR T cells require sophisticated cytotherapy, and intense supportive care pre- and post-injection. Similar to other monoclonal antibodies, BsAbs are likely to be more acceptable in a non-specialized clinic setting for the treatment of the more prevalent cancers, be it liquid or solid tumors, especially for patients who require immediate therapy, or those with suboptimal clinical conditions likely to be imperiled by additional high dose chemotherapy. In children, late effects are critical considerations. As T-cell based therapies for pediatric cancer enter the ongoing accelerated phase of development, exciting possibilities will likely become available in not too distant future.

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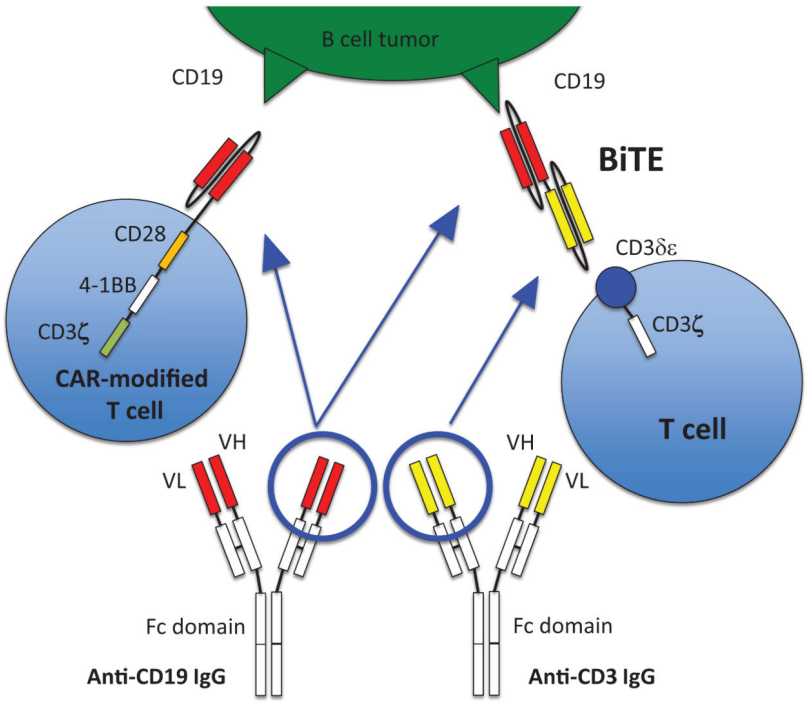

Figure 1.

Anti-CD19 CAR T cell (the third generation) recognizes CD19 antigen on a tumor cell via an anti-CD19 single chain Fv (scFv) binding domain. Anti-CD19 × anti-CD3 BiTE® is composed of two scFvs joined in tandem, i.e. anti-CD19 scFv and anti-CD3 scFv, recognizing CD19 on tumor cells and CD3 on T cells.

Table I

Clinical trials of CARs (Pediatric studies highlighted in grey) (as of Jan. 2015)

CAR and target	Tumor type	Phase	Clinical trial	Result
CD19 CAR (1st vs 2nd)	CD19+ leukemia, B-cell NHL	1	NCT00586391	Active, No recruit
CD19 CAR (1st vs 2nd 4-1BB)	CD19+ leukemia, B-cell NHL	1	NCT01029366	Completed
CD19 CAR	Relapsed or Refractory CD19+ CLL	2	NCT01747486	Completed
CD19 CAR	Relapsed or Refractory B-cell NHL	1	NCT01840566	Recruiting
CD19 CAR	Relapsed or Refractory B-cell NHL	1, 2	NCT02134262	Recruiting
CD19 CAR	Relapsed or Refractory CD19+ leukemia, B-cell NHL	1, 2	NCT01865617	Recruiting
CD19 CAR	CD19+ leukemia, B-cell NHL post-HSCT	1	NCT01497184	Recruiting
CD19 CAR	Relapsed CD19+ ALL (1-26 years old)	1	NCT01683279	Recruiting
CD19 CAR	Relapsed or Refractory CD19+ ALL (up to 26 years old)	1	NCT01860937	Recruiting
CD19 CAR	CD19+ leukemia, B-cell NHL (1-30 years old)	1	NCT01593696	Recruiting
CD19 CAR	Relapsed or Refractory CD19+ mantle cell lymphoma	1, 2	NCT02081937	Recruiting
CD19 CAR 2nd 28	CD19+ leukemia, B-cell NHL post-HSCT	1	NCT02050347	Recruiting
CD19 CAR 2nd 4-1BB	Relapsed or Refractory CD19+ ALL	2	NCT02030847	Active, No recruit
CD19 CAR 2nd 4-1BB	Relapsed or Refractory B-cell NHL	2	NCT02030834	Recruiting
CD19 CAR 2nd 4-1BB	Relapsed or Refractory CD19+ leukemia, B-cell NHL	--	NCT01864889	Recruiting
CD19 CAR 2nd 4-1BB	Relapsed or Refractory CD19+ leukemia, B-cell NHL (1-24years old)	1	NCT01626495	Recruiting
CD19 CAR 2nd 4-1BB expressing	Relapsed or Refractory CD19+ CLL (1-26 years old)	1, 2	NCT02028455	Recruiting
CD19 CAR (2nd 28 vs 4-1BB)	CD19+ ALL	1	NCT01044069	Recruiting
CD19 CAR (2nd 28 vs 4-1BB)	Relapsed or Refractory CD19+ CLL, B Cell NHL	1, 2	NCT00466531	Recruiting
CD19 CAR (2nd 28 vs 28 and 4-1BB)	Relapsed or Refractory CD19+ CLL, B Cell NHL	1	NCT01853631	Recruiting
CD19 CAR 3rd (28 and 4-1BB)	Relapsed or Refractory CD19+ leukemia, B-cell NHL	1, 2	NCT02132624	Recruiting
CD19 CAR 3rd (28 and 4-1BB)	Relapsed or Refractory CD19+ ALL	1	NCT02186860	Not yet recruiting
CD19 CAR + IL-2	CD19+ leukemia, B-cell NHL	1, 2	NCT00924326	Recruiting
CD19 CAR + IL-2	B-cell NHL	1	NCT01493453	Recruiting
CD19 CAR +/− IL-2	CD19+ leukemia, B-cell NHL post-HSCT	1	NCT00968760	Recruiting
CD19 CAR allo T cells	CD19+ leukemia, B-cell NHL recurrent or persistent after HSCT	1	NCT01087294	Recruiting
CD19 CAR, EBV T cells	CD19+ CLL, B-cell NHL	1	NCT00709033	Active, No recruit
CD19 CAR, allo EBV T cells	CD19+ ALL post-HSCT	1	NCT01430390	Recruiting
CD19 CAR, EBV T cells + EBV cell	CD19+ leukemia, B-cell NHL post-HSCT	1, 2	NCT01195480	Recruiting
CD19 CAR, adeno virus/EBV/CMV T	CD19+ leukemia, B-cell NHL post HSCT	1. 2	NCT00840853	Recruiting
CD19 CAR, allo CMV or EBV T cells	CD19+ leukemia, B-cell NHL post-HSCT	1, 2	NCT01475058	Active, No recruit

CAR and target	Tumor type	Phase	Clinical trial	Result
CD19 CAR, CM T cells	CD19+ leukemia, B-cell NHL post-HSCT	1, 2	NCT01318317	Active, No recruit
CD19 CAR + HyTK (selection/ suicide)	B-cell NHL (16-70 years old)	1	NCT00182650	Completed
CD19 CAR, self-withdrawal mechanism	B-cell NHL	1, 2	NCT02247609	Recruiting
CD19 CAR, TCRζ 4-1BB	HL	pilot	NCT02277522	Not yet recruiting
CD19 CAR, EGFRt	CD19+ leukemia,	1	NCT02146924	Recruiting
CD20 CAR + IL-2	Relapsed or Refractory B-cell NHL	1	NCT00012207	Completed
CD20 CAR 2nd 4-1BB vs TCR zeta	Relapsed or Refractory CD20+ leukemia	--	NCT01735604	Recruiting
CD20 CAR 3rd + IL-2	CD20+ leukemia, B-cell NHL	1	NCT00621452	Completed
CD22 CAR	CD22+ ALL, NHL, follicular, large cell lymphoma (1-30 years old)	1	NCT02315612	Recruiting
CD30 CAR	CD30 + NHL, CD30+ HL (16-80 years old)	1, 2	NCT02259556	Recruiting
CD30 CAR 2nd	CD30 + NHL, CD30+ HL	1	NCT01316146	Recruiting
CD30 CAR, EBV T cells	CD30 + NHL, CD30+ HL	1	NCT01192464	Recruiting
CD30 CAR, self-withdrawal mechanism	CD30+ HL, anaplastic large cell lymphoma	1, 2	NCT02274584	Recruiting
CD33 CAR 2nd 137	CD33+ AML (5-90 years old)	1, 2	NCT01864902	Recruiting
CD123 CAR 2nd 28	Relapsed or Refractory CD123+ AML	1	NCT02159495	Not yet recruiting
CD138 CAR 2nd 4-1BB	CD138 + multiple myeloma	1, 2	NCT01886976	Recruiting
LewisY CAR 2nd 28	LewisY+ myeloma, AML or high risk MDS	1	NCT01716364	Unknown
Kappa CAR 2nd 28	CLL, recurrent or Refractory B-cell NHL, multiple myeloma	1	NCT00881920	Recruiting
NKG2D-Ligands CAR	AML, MDS, multiple myeloma	1	NCT02203825	Not yet recruiting
HER-2 CAR 2nd 28	HER-2+ sarcoma	1	NCT00902044	Recruiting
HER-2 CAR 2nd 4-1BB	HER-2+ solid tumors	1, 2	NCT01935843	Recruiting
HER-2 CAR, 3rd 28 and 4-1BB + IL-2	HER-2+ metastatic cancer	1	NCT00924287	Terminated
HER-2 CAR, CMV T cells	HER-2+ glioblastoma	1,2	NCT01109095	Recruiting
HER-2 CAR, TGFbeta DNR EBV T cells	HER-2+ metastatic or advanced stage cancer	1	NCT00889954	Recruiting
GD2 CAR, EBV T cells	Relapsed or Refractory neuroblastoma	1	NCT00085930	Active, No recruit
GD2 CAR multivirus specific	Relapsed or Refractory neuroblastoma (18 months to 17 years old)	1	NCT01460901	Recruiting
GD2 CAR 3rd and iCaspase suicide	Relapsed or Refractory neuroblastoma	1	NCT01822652	Recruiting
GD2 CAR 3rd VZV and iCaspase suicide	Refractory or metastatic GD2+ sarcoma	1	NCT01953900	Recruiting
GD2 CAR 3rd	Non-neuroblastoma, GD2+ solid tumors (1-35 years old)	1	NCT02107963	Recruiting
CD171 CAR 2nd	Refractory neuroblastoma, ganglioneuroblastoma (up to 18 yearsold)	1	NCT02311621	Recruiting
CEA CAR	Adenocarcinoma	1	NCT00004178	Completed
CEA CAR	CEA+ tumor	1	NCT01212887	Terminated
CEA CAR 2nd 28	Colorectal cancer	1	NCT00673322	Terminated

CAR and target	Tumor type	Phase	Clinical trial	Result
CEA CAR 2nd 28	CEA+ Adenocarcinoma with liver metastasis	1	NCT01373047	Completed
CEA CAR 2nd 28 +/− IL-2	Breast cancer	1	NCT00673829	Active, No recruit
CEA CAR 2nd 28 + IL-2	CEA+ Adenocarcinoma	2	NCT01723306	Active, No recruit
EGFR CAR 2nd 137	Relapsed or Refractory EGFR+ non-small cell lung cancer, colorectal	1, 2	NCT01869166	Recruiting
EGFR CAR	Glioblastoma	1	NCT02331693	Recruiting
EGFRvIII CAR	Glioblastoma	1	NCT02209376	Recruiting
EGFRvIII CAR 3rd +/− IL-2	Glioblastoma	1, 2	NCT01454596	Recruiting
PSMA CAR 2nd	Castration-resistant metastatic prostate cancer	1	NCT01140373	Recruiting
Folate receptor CAR +/− IL-2	Ovarian cancer	1	NCT00019136	Completed
Mesothelin CAR 2nd	Ovarian cancer	1	NCT02159176	Recruiting
IL-13 zetakine CAR	CNS tumors	1	NCT00730613	Completed
IL13 receptor α2 CAR	Malignant glioma	1	NCT02208362	Not yet recruiting
ErbB T4+ CAR 2nd 28 (intra tumoral)	Squamous cell cancer of the head and neck	1	NCT01818323	Not yet recruiting
FAP CAR (intra pleural)	Malignant pleural mesothelioma	1	NCT01722149	Recruiting

1st, 1st generation; 2nd, 2nd generation; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukemia; CM T cells, central memory T cells; CMV, cytomegalovirus; CNS, central nervous system; EBV, Epstein-Barr virus; EGFR, epidermal growth factor receptor; EGFRt, truncated epidermal growth factor receptor; FAP, fibroblast activation protein; GD2, disialoganglioside; HER-2, human epidermal growth factor receptor 2; HL, Hodgkin lymphoma; HSCT, hematopoietic stem cell transplant; HyTK, hydromycin-thymidine kinase; IL-2, interleukin-2; IL-13, interleukin-13; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; NKG2D, natural-killer group 2, member D; PSMA, prostate-specific membrane antigen; TCR, T cell receptor; TGFbeta DNR, transforming growth factor beta dominant negative receptor; VZV, varicella zoster virus

Table II

Clinical trials of BsAbs (Pediatric studies highlighted) (as of Jan. 2015)

BsAb; Target	Tumor type	Phase	Clinical trial	Result
Catumaxomab (Removab); Trifunctional BsAb; EpCAM × CD3	Epithelial ovarian cancer	2	NCT00563836	Completed
	Epithelial ovarian cancer	2	NCT01815528	Recruiting
	Epithelial ovarian cancer	1	NCT01320020	Terminated
	Epithelial ovarian cancer, fallopian tube, or primary peritoneal cancer	2	NCT00189345	Completed
	Epithelial ovarian cancer, fallopian tube, or primary peritoneal cancer	2	NCT00377429	Completed
	Epithelial ovarian cancer, fallopian tube, or primary peritoneal cancer	2	NCT01246440	Unknown
	Malignant ascites (epithelial ovarian cancer)	2	NCT01065246	Completed
	Malignant ascites (epithelial ovarian cancer)	3	NCT00822809	Completed
	Malignant ascites (epithelial ovarian cancer, fallopian tube cancer, or peritoneal cancer)	2	NCT00326885	Completed
	Malignant ascites (EpCAM positive tumor; e.g. ovarian, gastric, colon, breast)	2, 3	NCT00836654	Completed
	Gastric adenocarcinoma with macroscopic peritoneal carcinomatosis	2	NCT01784900	Recruiting
	Gastric adenocarcinoma or adenocarcinoma of the esophagogastric junction	2	NCT00352833	Completed
	Gastric adenocarcinoma or adenocarcinoma of the esophagogastric junction	2	NCT00464893	Completed
	Gastric adenocarcinoma or adenocarcinoma of the esophagogastric junction with macroscopic peritoneal carcinomatosis	2	NCT01504256	Recruiting
Ertumaxomab trifunctional BsAb; HER-2/neu × CD3	HER-2/neu expressing (1+ or 2+/FISH negative) hormone therapy refractory or metastatic breast cancer	2	NCT00452140	Terminated
	HER-2/neu expressing (1+ or 2+) hormone therapy refractory or metastatic breast cancer	2	NCT00351858	Terminated
	HER-2/neu expressing (1+/FISH positive, 2+ or 3+) solid tumors	1, 2	NCT01569412	Recruiting
	HER-2/neu overexpressing (2+/FISH positive or 3+) metastatic breast cancer	2	NCT00522457	Terminated
Blinatumomab (BiTE®); (MT103,AMG103); CD19 × CD3 **FDA approved in 2014 (Blincyto™)**	Relapsed or refractory B-precursor ALL	2	NCT01209286	Active but not recruiting
	Relapsed or refractory B-precursor ALL (up to 17 years old)	1, 2	NCT01471782	Active but not recruiting
	Relapsed or refractory B-precursor ALL	3	NCT02013167	Recruiting
	Relapsed B-precursor ALL (1 year old to 30 years old)	3	NCT02101853	Recruiting
	Relapsed or refractory Philadelphia-chromosome(−) B-precursor ALL	2	NCT01466179	Active but not recruiting
	Relapsed or refractory Philadelphia-chromosome(+) B-precursor ALL	2	NCT02000427	Recruiting
	Minimal residual disease of B-precursor ALL	2	NCT01207388	Active but not recruiting

BsAb; Target	Tumor type	Phase	Clinical trial	Result
	Minimal residual disease of B-precursor ALL	2	NCT00560794	Completed
	Relapsed or refractory diffuse large B-cell lymphoma	2	NCT01741792	Active but not recruiting
	Relapsed non-Hodgkin lymphoma	1	NCT00274742	Completed
	New diagnosis of B-precursor ALL	3	NCT02003222	Recruiting
	New diagnosis of B-precursor ALL (≥ 65 years old)	2	NCT02143414	Not yet recruiting
FBTA05 trifunctional BsAb; CD20 × CD3	Relapsed or refractory CLL, low grade or high grade non-Hodgkin lymphoma with positive CD20 after allogeneic transplantation	1, 2	NCT01138579	Recruiting
REGN1979 CD20 × CD3	Refractory CLL, non- Hodgkin lymphoma	1	NCT02290951	Recruiting
Rituximab × OXT3; CD20 × CD3	Multiple myeloma, plasma neoplasm	1	NCT00938626	Completed
MGD006; CD123 × CD3	Relapsed or refractory AML	1	NCT02152956	Recruiting
MT110 (BiTE®); EpCAM × CD3	EpCAM positive tumor; e.g. ovarian, small cell lung, adenocarcinoma of the lung, gastric, colon, breast, prostate cancer	1	NCT00635596	Completed
BAY2010112 (BiTE®); (MT112,AMG212); PSMA × CD3	Advanced castration-resistant prostate cancer	1	NCT01723475	Recruiting
IMCgp100; gp100 × CD3	Stage III unresectable or IV malignant melanoma	1	NCT01211262	Recruiting
rM28; HMV-MAA × CD28 *	Stage III or IV malignant melanoma with injectable soft tissue metastasis	1, 2	NCT00204594	Completed
BAY2010112 (BiTE®); (MT112,AMG212); PSMA × CD3	Advanced castration-resistant prostate cancer	1	NCT01723475	Recruiting
MOR209/ES414 PSMA × CD3	Progressive prostate cancer	1	NCT02262910	Recruiting
MGD007: gpA33 × CD3	Metastatic colorectal adenocarcinoma	1	NCT02248805	Recruiting
RO6958688 CEA × CD3	Locally advanced/metastatic sold tumors	1	NCT00352833	Recruiting

* CD28 is a cell surface costimulatory receptor on T cells; CD28 is required to activate T cell in addition to TCR-mediated signaling. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; EpCAM, epitherial cell adhesion molecule; FISH, fluorescence in situ hybridization; gp100, glycoprotein 100; HER-2, human epidermal growth factor receptor 2; hu3F8, humanized 3F8; PSMA, prostate-specific membrane antigen