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CAR therapy for hematological cancers: can success seen in the treatment of B-cell acute lymphoblastic leukemia be applied to other hematological malignancies?

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Abstract

Chimeric antigen receptor (CAR) T-cell therapy has recently come into the spotlight due to impressive results in patients with B-cell acute lymphoblastic leukemia. By targeting CD19, a marker expressed most B-cell tumors, as well as normal B cells, CAR T-cell therapy has been investigated as a treatment strategy for B-cell leukemia and lymphoma. This review will discuss the successes of this therapy for the treatment of B-cell acute lymphoblastic leukemia and the challenges to this therapeutic strategy. We will also discuss application of CAR T-cell therapy to chronic lymphocytic leukemia and other B-cell malignancies including a follicular lymphoma, diffuse large B-cell lymphoma, as well as acute and plasma cell malignancies.

Keywords

autologous T-cell infusion; B-cell malignancies; CAR; chimeric antigen receptor; immunotherapy; T cells

Background

Chimeric antigen receptors (CARs) can be used to redirect T-cell specificity. CARs consist of an extracellular antigen recognition domain that is usually comprised the single chain variable fragment (scFv), derived from a monoclonal antibody (mAb). This is linked to intracellular signaling domains that provide activation (signal 1) and costimulation (signal 2) to the T cells. For activating T cells through a CAR, signal 1 is usually provided by the CD3ζ signaling domain, as in normal T-cell stimulation through the T-cell receptor (TCR). To provide costimulation (signal 2), there are several options, including but not limited to

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CD28, 4-1BB and/or OX40 signaling domains. The costimulatory signaling moieties as well as combinations of costimulatory signaling domains have been extensively reviewed elsewhere and will not be covered here [1].

An important part of CAR design is choosing an optimal tumor-associated antigen (TAA) to target. Ideally, the target antigen should only be expressed on tumor cells and not on normal cells, to ensure that there is no 'on-target-off-tumor' activity that could result in toxicity. In the best possible situation, the target antigen is a molecule that is necessary for the survival of the tumor cell. This will reduce the risk of immune editing and tumor escape variant outgrowth. However, these criteria are rarely completely fulfilled. For example, the most extensively investigated CAR in the clinical setting targets CD19, which is expressed on most B-cell malignancies, but also expressed on normal B cells. Therefore, CAR-mediated tumor destruction is accompanied by CAR-mediated destruction of normal B cells, resulting in B-cell aplasia. For these patients, this is an acceptable 'on-target off-tumor' toxicity and can be managed with infusions of immunoglobulin. In contrast, a clinical trial investigating a CAR specific for ERBB2 for the treatment of $ERBB2^{+}$ colon cancer resulted in a serious adverse event when the CAR T cells recognized low levels of ERBB2 on lung epithelial cells [2]. Unfortunately, this patient died as a result of this 'on-target, off- tumor' toxicity. Therefore, identifying an appropriate TAA is key to effective CAR T-cell therapy of cancer.

CD19-targeted CAR design

To date, CARs specific for CD19 have been the most successfully used in the clinical setting. Patient derived T cells modified to express CD19-targeted CARs have been used for the treatment of B-cell malignancies. A variety of CD19-targeted CARs have been used clinically, summarized here and in Table 1. Investigators at Memorial Sloan Kettering Cancer Center (MSKCC) have treated patients with a CD19-targeted CAR that utilizes the SJ25C1 scFv and the CD3ζ activation and CD28 costimulation signaling domains. The National Cancer Institute (NCI), Baylor College of Medicine and MD Anderson Center similarly used a CAR that contained the CD3ζ and CD28 signaling domains linked to a different CD19-specific scFv, FMC63 [3]. The University of Pennsylvania (UPenn) and Fred Hutchinson Cancer Research Center (Fred Hutch), have reported encouraging clinical trial results with T cells modified to express a CD19-targeted CAR with the same scFv as the NCI (FMC63) but with CD3ζ activation and 4-1BB costimulatory domains. Indeed even the methods of genetic modification differ between centers with most investigators employing retroviral or lentiviral transduction and one using electroporation of sleeping beauty transposons. Except for the superiority of second-generation CAR T cells over first generation CAR T cells, the clinical impact of CAR design and genetic modification strategy has not been comprehensively determined. However, all centers have seen promising results in some patient subsets, indicating that perhaps there is more than one signaling domain for effective CAR T-cell therapy.

CD19-targeted CAR T-cell therapy for the treatment of B-ALL

Patients with relapsed B-cell acute lymphoblastic leukemia (B-ALL) have a poor prognosis and expected survival of less than 6 months [4,5]. In addition to relapse, patients with refractory disease or certain chromosomal abnormalities (e.g., Philadelphia chromosome

t[9;22] positive [Ph^+]) disease also have an even worse prognosis. To date, the most successful clinical application of CAR T-cell therapy has been in the setting of relapsed B-ALL.

Investigators at MSKCC were the first to publish clinical trial results describing CD19-targeted CAR T-cell therapy of patients with relapsed B-ALL [6]. In this study, all five patients treated with CAR T cells achieved minimal residual disease negative (MRD^-) complete remissions (CR) following treatment (see Table 2). Updated results describing the treatment of 16 patients with relapsed or refractory B-ALL treated with CAR T cells were recently published [7]. The overall CR rate in this trial was 88% and 12 of 14 patients were classified as MRD^- following treatment. Seven of these patients (44%) went on to standard-of-care allogeneic hematopoietic stem cell transplant (allo-HSCT). This treatment was as effective in patients with Ph^+ disease, and in patients with relapsed disease. Patients variably experienced transient B-cell aplasias and the persistence of CAR T cells was reported to be in the range of weeks to months although persistence did not appear to affect patient outcome. Updated results reported at the American Society of Hemaotology (ASH) annual meeting in 2014 (see also Table 1) reported that the median overall survival is 9 months and five patients have relapsed, including one with $CD19^-$ disease [8]. This perhaps indicates that utilizing CAR T-cell therapy as a bridge to transplant may reduce the risk of relapse in B-ALL patients following allo-HSCT. Responding patients experienced toxicity including cytokine-mediated side effects in some consistent with large-scale T-cell activation and expansion with symptoms including fever, hypotension, fatigue and mental status changes as well as seizure-like activity. Updated results describe severe cytokine release syndrome (sCRS, those patients who required vasopressors or mechanical ventilation) in 18% of treated patients [8]. To this end, the authors have developed a guideline for determining whether medical intervention is required.

Investigators at UPenn (in conjuction with the Children's Hospital of Philadelphia) have reported on the successful treatment of two pediatric patients with relapsed, chemo-refractory B-ALL [9]. Both patients experienced morphological CR approximately 1 month following CAR T-cell infusion, a response that has been sustained in one patient. CAR T cells were shown to proliferate to up to 1000-fold and both patients experienced B-cell aplasias, consistent with CAR T-cell function. One patient experienced severe toxicity, consisting of fevers, as well as respiratory and cardiovascular compromise. This toxicity was managed with the infusion of tocilizumab, an antibody therapy that binds and blocks the interleukin (IL)-6 receptor. The second patient had previously received an umbilical cord blood (UCB) transplant, therefore the ‘autologous’ CAR T cells isolated from the patient were technically of UCB-donor origin. This patient only experienced fevers, which did not require medical intervention. This study raised an important challenge for CAR T-cell therapy, as one of the treated patients experienced disease relapse 2 months after CAR T-cell infusion. These tumor cells were determined to be CD19 negative ($CD19^-$) tumor immune escape variants. These escape tumors were related to the initial C D19 positive ($CD19^+$) tumor cells as the authors determined that the cells had the same immunoglobulin heavy locus sequence. Tumor escape variant outgrowth is an important challenge to the success of CAR T-cell therapy in the clinic. Updated results from this group describe 25 pediatric patients, with a median age of 11 years old, 88% of these patients were in at least their

second relapse [10]. They also treated five adult patients, median age of 47 years old (see Table 2). Following CAR T-cell treatment, 90% of evaluable patients were in a morphological CR at 1 month post infusion and 73% were MRD^- as determined by flow cytometry. The 6 month event-free survival was 67%. In the pediatric cohort, 6/25 patients relapsed in less than 10 months and 1/5 patients relapsed at 3 months post treatment, three patients relapsed with $CD19^-$ disease, demonstrating that the CD19 negative relapse in the initial report is a concern with this targeted therapy.

The NCI has also reported a Phase I dose escalation study investigating the treatment of B-ALL patients with CD19-targeted CAR T cells [11]. Investigators treated patients with cyclophoshphamide/fludarabine conditioning therapy and then infused fresh T-cell product, either 1e6 CAR T cells/kg or to 3e6 CAR T cells (see Table 2). Twenty young adults and children between the ages of 1 and 30 years with B-ALL were enrolled, 33% were in primary relapse. At the higher dose, three of four patients experienced grade 3 or 4 CRS, defining the maximum tolerated dose, and the remaining patients were treated at the lower dose. Four patients were treated with tocilizumab and/or corticosteroids. Following treatment, 67% of patients achieved CR, defined as having less than 5% blasts in the bone marrow. Ten patients progressed to allo-HSCT and the leukemia-free survival at 4.8 months was 79%. Two patients did not receive allo-HSCT and experienced CD19 negative disease relapse at 3 and 5 months; however, the authors saw no loss of CD19 expression in nonresponding patients.

The MD Anderson Cancer Center treated patients with CD19-targeted CAR T cells, infused as adjuvant therapy following allo-ASCT (see Tab le 2). Preliminary results were presented at ASH annual meeting, 2014 [12]. The authors generate CD19-targeted T cells using electroporation of sleeping beauty transposons into T cells, followed by stimulation on K562 artificial antigen presenting cells and the CAR utilizes CD28 and CD3ζ signaling domains. Ten patients were treated with no active disease and three of 10 patients achieved CR, maintained until at least 5 months post treatment. No patients experienced GVHD exacerbation or CRS. The CAR T cells were reported to persist at detectable levels for 3 months.

The Fred Hutchinson Cancer Research Center is also performing Phase I clinical trials for the treatment of B-ALL with CD19-targeted CAR T cells [13]. Investigators here infused patients with a 1:1 CD4:CD8 CAR T-cell ratio, which are generated from naive and central memory T cells, termed ‘defined product’ CAR T cells (see Table 2). Investigators presented preliminary results at ASH annual meeting 2014, where they describe the treatment of nine B-ALL patients. The patients were between 24 and 71 years old had a median of 27% blasts at the time of treatment. Five of seven evaluable patients achieved MDR^- CR and three patients experienced sCRS, including one patient who died as a result. The authors report persistence in T cells, though no data were available the time of publishing.

Future challenges to treating B-ALL with CAR T cells

The main challenge associated with treating B-ALL patients with CD19-targeted CAR T cells is toxicity management. Toxicities observed following CD19-tar-geted CAR therapy can be dramatic, and are associated with CAR T-cell-mediated elevations in cytokines and

generally require ICU observation and management. These toxicities are distinct from those induced by preconditioning therapy these patients receive prior to CAR T-cell infusion, and are associated with large scale T-cell activation. Characterized by increases in a panel of cytokines, this toxicity profile is termed the sCRS and can be mild (accompanied by fevers only) or severe (fevers, hypotension, hypoxia and neurological changes). Recently, C-reactive protein (CRP) has been identified as a reliable surrogate for CRS severity [7]. This has allowed for the development of guidelines for medical intervention of patients who are proposed to be at high risk for clinical complications. Of note, CRS has been reported to occur in trials performed at all centers, regardless of CAR design.

In patients that have received CD19-targeted CAR T-cell therapy, there have been cases noted of immune selection resulting in an outgrowth of $CD19^-$ tumors [9]. Treatment of patients with specifically targeted therapies always carries the risk of tumor editing and outgrowth of target antigen negative tumors. Indeed this has been seen with other targeted therapies, for example, BCR/ ABL tyrosine kinase inhibitors in chronic myelogenous leukemia patients, where mutations in the kinase protein render tumor cells resistant to therapy. The outgrowth o f C D19 - tumors has been seen in only a few patients, but warrants further investigation as we treat more patients with CD19-targeted CAR T cells. One option here is to perform a HSCT transplant following CAR T-cell therapy, this will give patients the best outcome until optimal CAR T-cell therapies can be developed. In the case of tumor escape variants it may be an option to retreat these patients with CAR T cells targeted to a different antigen. For example, another antigen that is expressed in a similar pattern to CD19 is CD22. Studies describing a CD22-specific CAR show adoptive transfer CD22-specific CAR T cells eradicated pre B-ALL in a preclinical xenogeneic model [14]. Further more, it may prove effective to treat patients with two T-cell populations expressing different CARs (e.g., one population expressing a CAR targeting CD19 and one expressing a CAR targeting CD22) to prevent immune escape.

CD19-targeted CAR T-cell therapy for the treatment of CLL

Initial studies from UPenn reported potent anti-leukemic effects of CD19 CAR T-cell therapy in three patients with refractory chronic lymphocytic leukemia (CLL), where two of three treated patients achieved MRD^- CR [15]. By extrapolation, infused CAR T cells proliferated up to 10,000-fold and persisted in the recipients for at least 6 months. Furthermore, these cells were shown to retain antitumor function *ex vivo* after 6 months. Treated patients experienced fever, rigors, dyspnea and hypotension, similar to CRS seen in relapsed B-ALL patients treated with CD19-targeted CAR T cells. Updated results were reported at the ASH annual meeting in 2012 and 2013 [16,17]. These describe more modest outcomes, where 21% of patients achieved CR and 36% of patients achieved PR [17]. They also described the severe toxicities ascribed to CRS in responding patients. These symptoms were managed with IL-6 receptor antagonist treatment, combined with corticosteroids in some patients. Updates at the ASH annual meeting 2014 describe 22% CR, 17% PR and 43% nonresponders (NR, see Table 3, [18]). Out of 26 treated patients, 14 experienced CRS and three patients required treatment with tocilizumab with or without corticosteroids. Three patients experienced disease relapse, including one patient that relapsed with CD19- disease, as seen in some B-ALL patients.

Investigators at MSKCC have reported the anti-tumor effects of CD19-targeted CAR T-cell therapy in patients with relapsed or refractory CLL in patients with bulky disease [19]. The first cohort of three patients was treated with CAR T cells alone without prior conditioning chemotherapy. All patients in this cohort developed progressive disease following treatment (see Table 3). However, three of four patients treated in the second cohort, wherein patients received preconditioning chemotherapy, experienced stable disease following CAR T-cell infusion. One patient experienced marked decrease of a peripheral lymphadenopathy. Persistence of CAR T cells was enhanced in the patients treated with preconditioning therapy, and was found to inversely correlate with tumor burden. Persistent CAR T cells were shown to retain antitumor function *ex vivo*. Elevated cytokine levels following CAR T-cell infusion were not noted in these patients. These results were updated at the ASH 2012 conference wherein treatment of two additional patients was reported [20]. These additional patients received preconditioning treatment with bendamustine prior to CAR T-cell infusion. One of these patients achieved a PR and the other achieved MRD^- CR. Toxicities reported in these two patients included fevers and mild hypoxia. The authors suggest that bendamustine preconditioning may result in improved overall antitumor response compared with cyclophosphamide preconditioning. The notion that all preparative conditioning regimes are not equal and may affect the patient's ultimate response to CAR T-cell therapy warrants further investigation.

Investigators at the NCI have published on four CLL patients (aged 54–61 years) that were treated with CD19-targeted CAR T cells [21]. Patients received pre conditioning in the form of cyclophosphamide and fludarabine, and also received exogenous post-CAR T-cell infusion intravenous IL-2 until toxicity precluded additional doses. All patients had objective responses, and one patient experienced a prolonged CR in the context of B-cell aplasias, indicative of CAR T-cell function and persistence. One patient experienced a reduction in adenopathy after T-cell infusion. This patient was also shown to have long-term persistence of CAR T cells, to at least 80 days following treatment. Aside from B-cell aplasia, three of the treated CLL patients had toxicity including fever, hypotension, fatigue and renal failure, as well as altered mental status, consistent with CRS. An updated study described the treatment of 4 CLL patients with CAR T-cell infusion [22]. Here, the authors report that of four evaluable treated CLL patients, three achieved classically defined CRs and the third achieved a PR (see Table 3). The authors also demonstrate that CAR T cells were able to infiltrate a bulky tumor mass in one patient.

In different approach, investigators at the NCI treated patients with malignancies that persisted following allo-HSCT and donor lymphocyte infusion (DLI, see Tab le 3) [23]. In this study, T cells from the transplant donors were modified to express a CD19-targeted CAR and infused into the transplant recipients. Of the ten treated patients, four had CLL. These patients did not receive lymphodepleting conditioning therapy prior to T-cell infusion, which the authors hypothesized may have caused excessive activation of T cells leading to graft versus host disease (GVHD). One of these patients achieved CR durable for at least 9 months post CAR T-cell infusion. One patient had stable disease for 3 months and two patients had progressive disease. The toxicities associated with this treatment varied from symptoms indicating CRS, to more serious events like tumor lysis syndrome, cardiovascular dysfunction and pnuemonitis. None of these patients developed GVHD following CAR T-

cell infusion, although these patients were selected for low or absent GVHD scores following previous HSCT and DLI treatment. In the responding patients, CAR T cells were undetectable in the patients' peripheral blood 5 days after infusion, but the cells were subsequently detectable for up to 1 month.

Preclinical development of novel targets for CAR T-cell therapy of CLL

A strategy to improve CAR T-cell treatment of CLL is to target different TAAs. Preclinical investigation into novel CAR T-cell targets include CD23, ROR1 and IgK.

Traditionally a B-cell activation marker, CD23 is overexpressed on CLL tumor cells compared with normal B cells. A recent publication described the generation of CAR that specifically targets human CD23 and contains the CD28 and CD3ζ signaling domains [24]. The authors show that CLL-patient derived T cells can be modified to express the CD23-specific CAR and lyse autologous tumor cells. Using a preclinical xenogeneic murine model, CD23-specific CAR T cells were shown to enhance the survival of tumor bearing mice. Successful targeting of CD23 will not result in eradication of normal B cells due to low CD23 expression. In addition, CD23 is thought to contribute to CLL viability and aggressive neo-plastic phenotypes, therefore reducing the likelihood of tumor escape and making it an attractive target for a CAR [25]. Clinical studies will be required to determine the efficacy of CD23-specific CAR T-cell therapy for the treatment of CLL.

Another potential target antigen for CAR T-cell therapy of B-cell malignancies is ROR1 [26]. Expression of the ROR1-specific CAR on T cells enabled specific lysis of $ROR1^+$ tumor cells. Furthermore, patient derived $CD8^+$ T cells modified to express the ROR1-specific CAR were shown to lyse autologous CLL tumor cells. ROR1 is highly expressed on B-cell CLL, mantle cell lymphoma (MCL) cells, and developing B cells at an intermediate stage of maturation but not on mature B cells. Therefore, targeting this antigen would spare mature B cells in the peripheral blood of treated patients. However, this antigen is also expressed at very low levels on pancreatic and adipose tissues. Another benefit of targeting ROR1 is that it may be involved in the survival of malignant cells, including solid tumor cells, therefore targeting ROR1 may reduce the risk of tumor escape variants [27]. A clinical trial targeting ROR1 CAR T cells in patients with relapsed CLL will soon open at MD Anderson Center (NCT02194374).

An additional target for a CAR to treat B-cell malignancies is IgK light chain [28]. Expression IgK-specific CAR in patient T cells allowed for recognition and lysis of autologous CLL cells and proliferation of patient-derived CAR T cells. Furthermore, *in vivo* experiments demonstrated that adoptive transfer of IgK-specific T cells resulted in control of established Daudi tumor growth in a preclinical murine model. This target is attractive as eradication of tumor cells with IgK light chain will spare B cells with a lambda light chain, furthermore, IgK deficiencies are not associated with increased susceptibility to infection. Therefore, despite this ‘on target off-tumor’ toxicity IgK would be a relatively safe antigen to target with a CAR. Importantly, it was reported that free IgK caused some proliferation of the CAR T cells, though this was not uncontrolled and did not exhaust T-cell function.

Potential reasons for reduced efficacy of CAR T-cell treatment of CLL

Despite the potential of novel CAR T cell targets for the treatment of CLL as discussed above, defects in circulating T cells of CLL patients and/or the inhibitory microenvironment associated with this often bulky disease and may hamper antitumor efficacy of CAR T-cell therapy.

Unfortunately, the circulating immune cell populations in CLL patients are often defective or reduced, therefore changing the target of the CAR may not be enough to improve patient outcome following therapy. Circulating T cells were found to have dysregulation in the helper T-cell compartment, with decreases observed in TCR signaling and cytokine release [29]. Furthermore, there was an overall decrease in the number of circulating $CD4^+$ T cells in patients with indolent disease [29]. Despite these decreased numbers, T cells from CLL patients were found to secrete IL-4, which, through direct and indirect mechanisms, results in increased survival and proliferation of CLL cells [30 –33]. Other studies have described that T cells from CLL patients have an exhausted phenotype [34]. This was accompanied by functional evidence of exhaustion, where patient T cells had a reduced capacity to proliferate and mediated reduced lysis of target cells compared with T cells isolated from health donors. The impact of these defects may extend to patient T cells that are isolated and modified to express a CAR, potentially resulting in dysfunctional CAR T cells. In additional to dysfunctional effector T cells, patients with CLL have increased levels of regulatory T cells (Tregs) compared with healthy age-matched controls [35]. The absolute Treg count was increased in patients with advanced stage CLL and these authors suggest that absolute Treg count may be used a prognositc marker in CLL. Methods to restore T-cell function or deplete Tregs/relieve Treg suppression may allow re-establishment of T-cell function and allow for effective CAR T-cell therapy.

Patients with CLL often present with bulky tumors, with lymph nodes being major sites of disease and tumor cell proliferation [36]. The tumor cells have been documented to grow in bulky aggregates, known as pseudo-follicular structures [37]. The micro-environment of these tumors is strongly supported by stromal cells that are present within the normal B-cell development niches. These supporting cells lend the CLL cell resistance to apoptosis [38]. CLL tumors have been reported to secrete CCL12 and CXCL12 chemo-kines that may recruit inhibitory macrophages [39 – 41]. In addition, CXCL12 secreted from stromal cells may contribute to CLL cell resistance to apoptosis [42]. Recent studies have demonstrated that CLL patients have increased levels of myeloid-derived suppressor cells (MDSCs), potentially resulting from skewed myeloid cell differentiation [43]. These MDSCs were shown to be suppressive to effector T cells and recruit or induce Tregs. The increased levels of MDSCs, tumor associated macrophages and Tregs may contribute to the overall inhibitory microenvironment in CLL patients. This solid-type tumor and inhibitory microenvironment may hinder the trafficking and also function of CAR T cells, rendering them less effective against CLL tumors.

Preclinical development of strategies to protect CAR T cells from the inhibitory tumor microenvironment

Strategies to modulate the inhibitory tumor micro-environment may allow enhanced CAR T-cell mediated antitumor efficacy. Here, we will discuss two strategies to improve the anti tumor function of CAR T cells and enhance patient outcomes following therapy.

CAR T cells may be further modified to express immune stimulatory proteins to increase their antitumor activity and protect them from inhibition within the tumor microenvironment. Preclinical studies investigating CAR T cells further modified to secrete the pro-inflammatory cytokine IL-12 have demonstrated promising preclinical results. IL-12 secreting CAR T cells eradicated tumor without prior conditioning in a syngeneic murine B-cell tumor model and were resistant to Treg-mediated suppression *in vitro* [44]. IL-12 secreted from tumor-targeted T cells may modify the MDSC and dendritic cell populations in the tumor microenvironment decreasing their suppressive capacity [45,46]. Armoring CAR T cells with mechanisms to avoid the suppressive microenvironment may improve the overall antitumor efficacy of CAR T-cell therapy and ultimately improve patient outcome. The antitumor effects of IL-12 secreting tumor infiltrating lymphocytes is being investigated in the clinical setting (NCT01236573), however, CAR/IL-12 T-cell therapy currently awaits translation to the clinic.

Adoptively transferred T cells are also subject to regulation mediated by inhibitory receptors expression on normal healthy T cells. One such inhibitory receptor is programmed death -1 (PD-1), which is upregulated following T-cell activation and when stimulated by its cognate ligands, PD-L1 and PD-L2, downregulates T-cell function. It has been shown that adoptively transferred T cells that persist express high levels of PD-1 receptor [47]. Checkpoint inhibitors are a class of therapeutics that serve to block these inhibitory receptors. Combining checkpoint inhibitors with CAR T-cell therapy may render CAR T cells more responsive to CLL tumor cells. Common targets for checkpoint blockade include PD-1 as well as CTLA-4, receptors expressed on activated T cells designed to dampen T-cell function upon interaction with the respective ligands. Preclinical murine studies demonstrated combination of CAR T cells with a blocking antibody to PD-1 increased the overall antitumor efficacy of therapy [48]. This study utilized a CAR T cells targeted to erbB2, over expressed on some breast cancers, however, this approach may be applicable to CD19-targeted CAR T-cell therapy as well. Significantly, a current clinical trial at Baylor College of Medicine is investigating CD19-targeted CAR T-cell therapy in combination with ipilimumab, a mAb that blocks the inhibitory CTLA-4 T-cell receptor (NCT00586391). This ongoing trial will recruit patients with ALL, CLL and B-cell non-Hodgkin lymphoma (NHL).

CD19-targeted CAR T-cell therapy for the treatment of lymphoma

As for leukemia, the most promising clinical trials investigating CAR T-cell therapy of lymphoma have utilized CARs specific for CD19. Indeed, the first trial investigating CAR T-cell therapy was performed in patients with NHL (see Tab le 4). Many of the studies investigating CD19-targeted CAR T cells investigate the treatment of patients with low grade (follicular lymphoma, FL) or intermediate grade (diffuse large B-cell lymphoma,

DLBCL) lymphomas. The results in these trials have been largely encouraging, but are also accompanied by toxicities akin to the CRS seen in leukemia patients treated with the CD19-targeted CAR T cells.

An early clinical trial performed at Baylor College of Medicine compared the clinical efficacy of first and secondary generation CAR T cells in patients with active FL or DLBCL [49]. Herein, six patients were treated with two types of autologous CD19-targeted CAR T cells, first-generation (CD3ζ signaling domain) and second-generation (CD3ζ and CD28 signaling domains) CAR T cells, in the absence of preconditioning. There was no toxicity seen in these patients following infusion of CAR T cells. These authors demonstrated that second-generation CAR T cells persisted longer and at higher levels that T cells expressing the first-generation CAR. Unfortunately, none of these patients showed any evidence of sustained tumor regression, however, this study demonstrated the importance of the inclusion of a costimulatory signaling domain in the CAR construct with respect to CAR T cell *in vivo* persistence.

More recently, Investigators at the NCI have treated four FL patients with CD19-targeted CAR T cells [21]. These patients all had progressive disease at the time of treatment and received preconditioning consisting of cyclophosphamide and fludarabine (see Tab le 4). In the three evaluable treated patients, CAR T-cell infusion led to PRs. Interestingly, one patient developed progressive $CD19^+$ lymphoma 7 months following treatment. This patient was retreated with CAR T cells with the same regimen and achieved a second PR. These patients experienced less CRS-like toxicity following CAR T-cell infusion, though the reasons for this remain unclear. This study also described one patient who had splenic marginal zone lymphoma. This patient had progressive disease at treatment and was treated with CD19-targeted CAR T cells and IL-2. Following treatment, this patient experienced B-cell aplasia and achieved a PR. More recently, the NCI reported the successful treatment of relapsed DLCBL with the CD19-targeted CAR T cells, where, of three evaluable DLBCL patients, two patients achieved a CR and one achieved a PR [22]. Of two evaulable patients with primary mediastinal Large B-cell lymphoma (PMBCL), one patient achieved a CR and one a PR. One patient with PMBCL experienced severe toxicity, including tachycardia and vascular leak syndrome, and died on study. Again, CAR T-cell infusions were associated with CRS toxicities greater than grade 3 in 8/9 patients, including fever, hypotension and neurological toxicities that resolved by three weeks post CAR T-cell infusion (see Table 4). The dose of CAR T cells used in this study was dropped from 5e6 CAR T/kg to 1e6 CAR T/kg due to the severe toxicity observed following treatment.

At the ASH annual meeting 2014, the NCI group reported a new strategy, involing low dose chemotherapy preconditioning prior to CAR T-cell infusion [50]. Nine patients were treated with 300 mg/m^2 cyclophosphamide and 30 mg/m^2 fludarabine and then received 1×10^6 fresh CAR^+ T cells/kg. Grade 3–4 toxicity was seen in 76% of treated patients, however, no patients required mechanical ventilation or vasopressors. Of nine patients with DLBCL, one achieved a CR, and four achieved PR, while three patients had progressive disease. One patient with FL experienced a PR.

Investigators at UPenn reported preliminary finding of a trial where patients with active NHL were treated with CAR T cells [52]. Patients received CAR T cells four days after preconditioning therapy, in a median dose of 5.8e6 CAR T cells/kg. Of six evaluable DLBCL lymphoma patients, two achieved CR and four experienced PD. Of two evaluable patients with FL, one achieved a CR and one a PR. Severe CRS was noted in one patient, and treated with tocilizumab/steroids, and neurotoxicity was noted in two patients. CAR T cells were detected at peak levels 2 weeks post infusion, indicating expansion of infused cells.

Similar to their studies for the treatment of ALL, investigators presented preliminary findings on their treatment of patients with relapsed/refractory NHL with a ‘defined composition’ CAR T-cell infusion at the ASH annual meeting 2014 [13]. Thirteen patients received CAR T cells at doses ranging from 2e5/kg to 2e7/kg, and no patient experienced sCRS although some neurotoxicity was noted. Six of ten patient who received the ideal defined composition dose (1:1 CD4:CD8 generated from Tnaive or Tcentral memory) achieved objective responses, including one CR. Persistence of CAR T cells was seen in both CD4 and CD8 subsets and correlated with clinical responses.

The NCI investigators report the outcome of treating patients with allo-donor-derived CAR T cells (see Table 4). Two patients with DBLCL and four patients with mantle cell lymphoma (MCL) were treated [23]. These patients responded well to therapy, both DBLCL patients and three of four MCL patients experienced stable disease following CAR T-cell infusion. One patient with MCL experienced a partial remission following therapy, involving a dramatic regression of a mediastinal mass. Of these patients, two with MCL experienced increased levels of serum cytokines associated with toxicity and one of these patients was reported to experience grades 3–4 toxicities consisting of hypotension and headaches.

Investigators at MSKCC reported preliminary findings at the ASH 2014 annual meeting where patients were treated with autologous SCT followed by infusion of CAR T cells [51]. Following treatment with pegfilgrastim, patients received 0.5–1e7 CAR T cells/kg in two doses. Two patients has transformed FL, three had relapsed DLBCL and one had marginal zone lymphoma (MZL), and all patients achieved CR, which was durable, with a median follow-up of 9 months post treatment.

Investigators from the MD Anderson center reported preliminary findings a the ASH annual meeting 2014, where they treated patients following auto-SCT using CAR T cells as adjuvant therapy [12]. Five patients with DLBCL or FL received 5e7–5e8 CAR T cells without preconditioning chemotherapy following HSCT, these patients experienced no toxicity and no CRS. Four patients achieved CR, median follow-up of 12 months and one patient achieved a PR with relapse at 6 months. These patients had transient persistence of CAR T cells, with detectable CAR up to 3 months post infusion.

CD20-targeted CAR T-cell therapy for the treatment of lymphoma

Other strategies to treat indolent lymphomas and NHL, include targeting CD20 with a CAR. Investigations at the Fred Hutchinson Cancer Research Center published a clinical trial in patients with MCL or FL [53]. A third-generation CD20-targeted CAR, containing CD3ζ,

CD28 and 4-1BB signaling domains, was utilized. Patients with MCL or FL were pretreated with cyclophosphamide and received IL-2 infusions following CAR T-cell therapy. The three evaluable patients had no evidence of disease at the time of CAR T-cell infusion. The two MCL patients remained disease free for 1 and 2 years, respectively, and the FL patient experienced partial remission prior to disease progression. The authors note that there was only toxicity in one patient, consisting of fevers, hypotension, hypoxemia and no B-cell aplasias were observed.

Clinical studies investigating CAR T-cell therapy for the treatment of acute myeloid leukemia

Acute myeloid leukemia (AML) is a disease comprised of a heterogeneous group of cancers that all originate from cells of myeloid origin. AML is the most common type of acute leukemia in adults, where patients over 60 years of age and with complex cytogenetic features have a poor 5-year survival rate and greater than 70% relapse rate [54,55]. Frontline therapy for AML consists of induction chemotherapy and post-remission therapy, which may include allo-HSCT. However, the outcome for AML patients remains poor and prognosis is much worse in the setting of disease relapse.

Immunotherapy using immune cells engineered with CARs against AML represents a novel therapy for this disease. However, given the wide range of myeloid cells from which AML can originate, specific antigen candidates are difficult to identify. The challenge in target antigen selection lies in determining a TAA will allow full eradication of tumor while sparing hematopoietic progenitor cells in turn allowing for regeneration of a healthy myeloid cell compartment. Studies with mAb therapy in AML have preceded the use of CAR T cells in the clinic, with mAb therapy targeting CD33 and CD123. However, it is now abundantly clear that targeting an antigen with an antibody is markedly different to targeting the same antigen with a CAR T cell. Differences including trafficking, bioavailablity and persistence of cellular therapies compared with antibodies need to be considered. While antibodies are cleared from the host, a cellular therapy may proliferate and/or persist for a longer duration of time. In this case, methods may need to be developed to remove the transferred cells to allow regeneration of a healthy myeloid cell compartment. There have been a number of preclinical and clinical studies that have explored these options and show potential for utilizing CAR T cells for the treatment of AML.

One clinical trial has investigated CAR T-cell therapy for the treatment of AML. In this study, T cells were engineered with CARs against Lewis Y (LeY). LeY is over expressed on AML cells and has limited expression on normal tissues [56]. Preclinical studies show that T cells modified to express the LeY-specific CAR were able to lyse AML targets and produce IFN-γ [57]. Anti-LeY CAR T cells were studied in Phase I trial of AML at Peter MacCallum Cancer Centre in Australia (NCT01716364) [58]. In this study, patients received fludarabine conditioning chemotherapy followed by CAR T-cell infusion. Of four evaluable treated patients, one patient achieved a transient cytogenetic remission (5 months), one patient experienced a reduction in peripheral blood blasts but ultimately progressed and another experienced stable disease for 23 months. No high-grade toxicities or CRS was

observed. The anti-LeY T cells persisted up to 10 months in patients, however, all patients eventually relapsed.

Preclinical development of novel CAR targets for the treatment of AML

The most exploited antigen in AML immunotherapy is CD33. CD33 is highly expressed on normal myeloid progenitor cells and leukemia cells [59]. CD33 was first targeted therapeutically for AML using a humanized anti-CD33 antibody, lintuzumab, which showed modest efficacy in patients [60]. Subsequently, CD33 was targeted in AML patients with gemtuzumab ozogamicin (GO), a mAb conjugated to an immunotoxin that is a derivative of calicheamicin-γ1 [61]. Despite the limited success in targeting CD33 with antibodies in AML, preclinical data with CARs targeting CD33 have been promising. First- and third-generation CARs (utilizing CD3ζ or CD3ζ, CD28 and OX40 signaling domains, respectively) targeted to CD33 have been studied *in vitro* in cytokine-induced killer (CIK) cells [62]. CIK cells are a mixed population of *ex vivo* expanded $CD3^+/CD56^+$ cells with NK cell-like cytotoxicity [63]. The CD33-directed CIKs released immunostimulatory cytokines and were directly cytotoxic to $CD33^+$ AML target cells [62]. Curiously, in this study, the anti-CD33 CAR CIK cells did not disrupt the colony-forming capacity of normal hematopoietic progenitors despite the expression of CD33 on progenitor cells. In another study, Epstein Barr virus-specific cytotoxic T cells (EBV-CTL) were modified to express a CD33- specific CAR. These cells had myeloblative activity and impaired normal $CD34^+$ hematopoietic progenitors [64]. However, these studies have not addressed the potentially harmful effects of long-term persistence of CD33-targeted CAR T cells. Of note, there is one currently recruiting clinical trial investigating CD33 targeted CAR T-cell therapy for the treatment of AML is being conducted by the Chinese PLA General Hospital. This trial investigates a CD33-targeted CAR, utilizing CD3ζ activation and 4-1BB costimulatory signaling domains (NCT01864902). Recruitment is ongoing and the results of this trial are yet to be published or reported.

CD123 is another TAA that has been studied in CAR therapy for AML. CD123 is overexpressed in AML cells compared with normal hematopoietic stem/progenitor cells [65]. CARs against CD123 in CIK cells have been compared with CD33-directed CIK cells and both of the CAR CIK cells showed cytotoxicity against AML but the anti-CD123 CIK had a safer profile against progenitors both *in vitro* and in an xenogeneic mouse model [66,67]. However, even with the CD123-specific CIK cells, ‘on-target off-tumor’ effects were observed in endothelial cells and normal monocytes, which are known to express lower levels of CD123 [68]. CD123-targeted CARs have also been tested in T cells. Second-generation CD123-targeted CAR T cells with a 4-1BB costimulatory domain prolong the survival of mice engrafted with either an AML cell line or primary samples [69]. However, these CD123-specific T cells also ablated nearly all normal human bone marrow cells, despite the fact that previous studies with CD123-specific antibodies did not reveal toxicities [70]. The toxicity to bone marrow cells may be due to the longevity of the CAR T cells [6,71]. CD123-targeted CARs specific for two different CD123 epitopes have been investigated [72]. AML patient-derived T cells were modified with either CAR and both CARS were shown to mediate antitumor efficacy against autologous AML cells regardless of relapsed or refractory states of disease. There were no differences seen between targeting

either CD123 epitope *in vitro* or *in vivo.* Furthermore, the myeloid and erythroid colony-forming ability was not diminished by anti-CD123 CAR T cells [72].

CD44 is broadly overexpressed on hematologic and epithelial cancers. CD44 is a marker of cancer stem cells and functionally contributes to cancer initiation, homing and engraftment [73–75]. CD44 is expressed on a wide range of normal tissues, however, a spliced iso-form variant has been identified and expression of the variant, CD44v6, is relatively tumor restricted [76]. A CAR specific for CD44v6 was designed for the treatment of AML [77]. Second-generation CAR T cells targeted against CD44v6 were able to lyse autologous primary AML samples *in vitro* and *in vivo* in a pre-clinical murine model. However, upon investigating the toxicity profile of CD44v6 targeted T cells, it was found that CD44v6-CAR T cells were directly toxic to monocytes.

An additional target is Wilms tumor antigen-1 (WT-1), a zinc finger transcription factor that is over expressed by AML tumor cells. This antigen is expressed on all leukemic cells and may promote proliferation and oncogenicity of tumor cells. This TA A is attractive as development of a CAR specific for WT-1 may be useful for the treatment of leukemia as well as other tumors, including some solid tumors. To utilize this intracellular antigen as a target, it is necessary to target WT-1-derived peptide epitopes bound to HLA molecules. Naturally occurring WT-1 specific $CD8^+$ T cells can be isolated and expanded, or alternatively, $CD8^+$ T cells can be engineered to express TCR α and β chains that are specific for WT-1 in HLA complexes [78]. A clinical trial tested the antitumor efficacy of donor derived WT-1 specific CD8 T cells in patients with leukemia [79]. These T-cell infusions were shown to be safe and mediated antitumor effects in some patients. Further investigations into this approach are underway in ongoing clinical trials at MSKCC for the treatment of leukemia, myeloma and solid tumors (NCT00620633, NCT01758328, NCT00562640) and at the Fred Hutchinson Cancer Research Center for the treatment of leukemia (NCT01640301). Clinical application of the WT-1 TCR approach is being investigated in a trial at University College of London (NCT01621724), where patients will be treated patient T cells expanded *ex vivo* and modified to express a TCR that targets HLA-A*0201 restricted WT-1 antigen. This study is currently ongoing with no results yet to be published or reported.

A CAR can be designed to specifically bind a WT-derived epitope bound to a HLA molecule, resulting in a CAR that functions in an HLA-restricted fashion, similar to the mechanism for antigen recognition by the endogenous TCR. TCR-like monoclonal antibodies that specifically recognize a WT-1 derived peptide epitope bound to HLA-0201, RMF-peptide/HLA-A2, have been developed and may be used to design CARs [80]. Early studies of WT-1 specific CAR T cells were presented this year at the 2014 BMT tandem meeting [81]. This study demonstrated that T cells can be engineered to express a CAR derived from a TCR-like mAb, and expression of this CAR can allow T-cell-mediated lysis of solid tumor and B-cell malignancy lines, *in vitro*. Future studies investigating the utilization of WT-1-specific CAR T cells for the treatment of hematological malignancies and other solid tumors are highly anticipated.

CAR T-cell therapy for the treatment of plasma cell malignancies

Multiple myeloma (MM) and Waldenstrom's macro-globulinemia (WM) are two related malignancies that are derived from plasma cells and lymphoplasmasitic cells, respectively. Tremendous progress has recently been made with novel proteasome inhibitors in both diseases, immunomodulatory drugs (IMiDs) in MM and Bruton's tyrosine kinase (BTK) inhibitors for WM. However, it is still generally accepted that current therapies are unable to completely eradicate the malignant clone in either disease. Additionally, in both diseases, there are cohorts of patients with high-risk disease genetics, where the time to recurrence is Significantly shorter [82]. Therefore, novel treatments are needed to treat these high-risk patients.

MM is particularly challenging with respect identifying surface antigens with ubiquitous expression on malignant plasma cells but limited expression on essential normal cells. Common markers expressed on MM, including CD138, CD38 and CD56, are expressed on normal cells and are therefore likely to induce ‘on-target off-tumor’ toxicity [83]. MM is generally CD19 negative, and in the approximately 2% of patients that are CD19 positive, CD19 is not expressed on all the malignant plasma cells. However, it is a possibility, although unproven, that CD19 may be expressed on the putative MM stem cell or MM precursor cell. Investigators at UPenn are testing the hypothesis that a CD19-targeted CAR may still benefit MM patients in a clinical trial (NCT02135406).

Clinical trials now open testing CAR T-cell therapy in MM include those directed toward the LeY antigen (NCT01716364), IgK (NCT00881920), CD138 (NCT01886976) and B-cell maturation antigen (BCMA; NCT02215967). Using IgK-specific CARs is not ideal, as plasma cells secrete their light chain, therefore it is no longer presented on the cell surface. As for the CD138 directed CAR, there is a great concern regarding ‘off-tumor on-tar-get’ toxicity as this antigen is also expressed at high levels on bronchial epithelial cells, amongst other essential normal tissues. The newly opened trial targeting BCMA is the most exciting CAR T-cell trial for the treatment of MM to date. This antigen is expressed on most MM cells and its expression is restricted to B cells [84]. However, BCMA is secreted and it is unclear how secreted BCMA may impact CAR T-cell efficacy in humans. Other preclinical studies have been conducted using T or NK cells with CARs directed to CD38 and CD56 and show promise in preclinical studies but have concerns for potential ‘on-target off-tumor’ toxicities as described above [85,86]. Other targets such as CD70, CD44v6 or CS1 may be more promising [87–90].

Preclinical development of CAR T-cell therapy for WM

Identification of TA A is imperative for the successful application of CAR T-cell therapy to MM and WM. As a less differentiated B-cell malignancy, it is fortunate that WM cells typically express CD19. This means that the current CD19 directed CAR vectors, which are already under investigation in clinical trials for CLL, ALL and other NHLs may be tested in this malignancy. Another strategy that has begun preclinical exploration is targeting the MYD88-L265P mutation that is present in approximately 90% of WM. Similar to WT-1, MYD88 is intracellular, thus the strategy to target MYD88-L265P may involve the

generation of a TCR-like mAb that binds to HLA-bound peptides derived from the mutated protein, which will be used to design an scFv to use in a WM targeted CAR.

Conclusion & future perspective

Initial investigations into CAR T-cell therapy were slowly transitioned to the clinic. Regulatory hurdles, expenses and unknowns regarding infusion of CAR T cells into patients slowed the clinical experimentation of this therapeutic strategy. As scientists, doctors and regulatory bodies become more experienced with personalized biological therapies, rapid progress and clinical experience is expected. The successes seen today with CAR T-cell therapies in B-cell malignancies will be more broadly expected as we better identify patient subsets that will most likely benefit from CAR T-cell therapy. Identification and validation of new targets will allow application of CAR T-cell therapy to the treatment of more hematological malignancies, including malignancies discussed in this review. In addition, as better targets are identified, toxicities associated with treatments will be, at best, eliminated entirely, or at least, managed with increased efficiency resulting in a safer, more effective treatment. Strategies to manage toxicity currently include administration of steroids or tociliuzimab, an IL-6 receptor mAb [9]. In the future, use of suicide/elimination genes may be used to eliminate CAR T cells in the case of severe toxicity, for example, inducible caspase 9, or truncated EGFR [91,92]. CAR T-cell therapy for many hematological malignancies is expected to be highly successful in the next 10 years Significantly improving patient survival. Moving forward, the most significant challenge facing investigators in this field will be to apply successes seen with hematological malignancies to the treatment of more heterogenous solid tumors.

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Executive summary

- A majority of patients with relapsed B-cell acute lymphocytic leukemia achieve complete remission following CD19-targeted chimeric antigen receptor (CAR) T-cell therapy. However, toxicity and tumor escape variant outgrowth remains a challenge.
- Chronic lymphocytic leukemia (CLL) may also be responsive to CD19-targeted CAR T-cell therapy, although this often bulky disease has some unique challenges to effective CAR T-cell therapy. These include a potentially inhibitory tumor microenvironment and dysfunctional CLL patient-derived T cells. Novel strategies to increase the antitumor efficacy of CAR T-cell treatment for CLL include alternative CAR target antigens, and additionally modification strategies to modulate the tumor microenvironment.
- CAR T-cell therapy might be useful for the treatment of AML; however, careful target antigen selection is important for the success of this strategy. Novel target antigens for CAR T-cell therapy are in preclinical development.
- CD19-targeted CAR T-cell therapy can be used to treat follicular lymphoma, and diffuse large B-cell lymphoma.
- Multiple myeloma and Waldenstrom's macroglobulinemia may be amenable to CAR T-cell therapy.

Table 1
CD19-targeted chimeric antigen receptor design

Institute	scFv clone name	Spacer regions	Signaling domains	Genetic modification method
Baylor	FMC63	Human IgG_1-CH2CH3 domain	CD28 and CD3z Or CD3z alone	Retroviral transduction
NCI	FMC63	No hinge	CD28 costimulation CD3z activatory	Retroviral transduction
MD Anderson	FMC63	Modified human IgG_4	CD28 costimulation CD3z activatory	Sleeping beauty transposon Electroporation
MSKCC	SJ25C1	No hinge	CD28 costimulation CD3z activatory	Retroviral transduction
UPenn	FMC63	Human CD8a hinge	4-1BB costimulation CD3z activatory	Lentiviral transduction
Fred Hutch	FMC63	Modified human IgG	4-1BB costimulation CD3z activatory	Lentiviral transduction

Baylor: Baylor college of Medicine; Fred Hutch: Fred Hutchinson Cancer Research Center; MD Anderson: MD Anderson Center; MSKCC: Memorial Sloan Kettering Cancer Center; NCI: National Cancer Center; UPenn: The University of Pennsylvania.

Table 2

CD19-targeted trials for the treatment of B-cell acute lymphoblastic leukemia

Institution/CAR	Conditioning and dosing	Patient population	Clinical responses	CAR T-cell persistence	Ref.
MSKCC SJ25-28Z	Physicians choice salvage Tx,1 dose 1.5-3 g/m^2 Cy 3 × 10^6 CAR T/kg, fractionated (1/3, 2/3)	Adult R/R medians: 50 y/o 54% morph dis 46% MRD$^+$ n = 27 evaluable	88% CR postT-cell infusion 75% molecular CR (deep sequencing) 70% progression to allo-SCT No persistent B-cell aplasias 5 pts relapse, incl. 1 CD19 relapse 18%sCRS	1-2 weeks until peak T-cell levels, undetectable 2-3 mo byqPCR	[7,8]
UPenn CHOP FMC-63-BBz	Physicians choice interim Tx, n = 27 None, n = 3 0.76-17.3 × 10^6 CAR$^+$cells/kg	Pediatric R/R, n = 25 Median: 11 y/o 72% postASCT 12% primary relapse 88% <2 relapses 5% no MRD at Tx Adult R/R, n = 5, median: 47 y/o 3/5 primary R 40% first relapse 20% morph. remission	90% morph. remission at 1 mo 73% MRD - by flow cytometry 67% EFS at 6 mo Peds: 6/25 relapse Adults: 1/5 relapse 3 pts with CD19 relapse 27% sCRS B-cell aplasia for up to 1 year following CAR T-cell disappearance from blood	Detectable up to 11 mo by qPCR	[9,10]
NCI FMC63-28Z	1 dose Cy (900 mg/m^2) 3 doses Flu (25 mg/m^2) 1-3 × 10^6 CAR T/kg	Young adults (1-30 y/o) n = 20 B-ALL 33% primary refractory	70% CR (<5% blasts) 10 pts to allo-SCT 70% LFSat4.8mo 2 pts, CD19 relapse 14% Scrs No persistent B-cell aplasias	CAR T detectable to day 28, no CAR T detected at day 68 by flow cytometry and qPCR	[11]
MD Anderson FMC63-28Z	1 × 10^6-5× 10^{8}CART/m^2 Donor/pt derived T cells as adjuvant therapy post SCT no conditioning prior to CAR T Tx	Post SCT, no active disease, n = 10	NoGVHDorsCRS 3/10 CR at 5 mo	CAR T detectable to 3 mo	[12]
Fred Hutch FMC63-BBZ	Cy conditioning 2× 10^5-2× 10^7 CAR T/kg (1:1 CD4:CD8)	n = 9 24-71 y/o mean 27% blasts at Tx	5/7 MRD CR 3 sCRS, incl. 1 death	Persistence in responding patients, data unavailable	[13]

Allo-SCT: Allogeneic stem cell transplant: B-ALL: B-cell acute lymphocytic leukemia: CAR: Chimeric antigen receptor: CR: Complete response: CRS: Cytokine release syndrome: Cy: Cyclophosphamide: EFS: Event-free survival: Flu: Fludarabine: Fred Hutch: Fred Hutchinson Cancer Research Center: GVHD: Graft versus host disease: incl.: Including: LFS: Leukemia-free survival: MD Anderson: MD Anderson Center: Mo: Months: Morph. dis.: Morphological disease: MRD: Minimal residual disease: MSKCC: Memorial Sloan Kettering Cancer Center: NCI: National Cancer Center: NHL: Non-Hodgkin lymphoma: Pts: Patients: qPCR: Quantitative PCR: R/R: Relapsed refractory: sCRS: Severe cytokine release syndrome: Tx: Treatment: UPenn: The University of Pennsylvania: y/o: Years old.

Table 3

CD19-targeted trials for the treatment of chronic lymphocytic leukemia

Institution/CAR	Conditioning and dosing	Patient population	Clinical responses	CAR T-cell persistence	Ref.
MSKCC SJ25-28z	None, n = 3 1 dose Cy, n = 4 1 dose bendamustine, n = 2 0.4-3.0 × 10^7 CAR T cells/kg	n = 9, R/R CLL 44–66 y/o	No preTx: 3/3 PD Cy preTx: – D bendamustine preTx: 1 MRD CR and 1 PR no Scrs	Persistence out to 30 days detected by qPCR	[19, 20]
UPenn CHOP FMC63-BBz	Pre Tx 3-5 days pre 5 × 10^7-5 × 10^8 CAR T cells/kg	n = 26, R/R CLL Median 62 y/o	22% CR, 17% PR 43% NR 3 relapse (incl. CD19) 14/26 CRS, n = 3 sCRS (intervention)	Persistence by flow cytometry Time unknown	[15–18]
NCI FM63-28z	2 doses Cy 60 mg/kg, 5 doses Flu 25 mg/m^2 0.3–2.8 × 10^7 CAR T cells/kg +IL-2 (720000 IU/kg/8 h)	n = 4 R/R CLL, aged 54 – 61 y/o	n = 1 CR, n = 2 PR, n = 1 SD IL-2 toxicity and CRs	Persistence up to 181 days by qPCR	[21]
	1 dose Cy 60 –120 mg/kg, 5 doses Flu 25 mg/m^2 1–5 × 10^6 CAR T cells/kg	n = 4, R/R CLL Aged 48 = 68 y/o	3/4 classically defined CR, CR > grade 3, n = 4	Persistence up to 65 days by qPCR	[22]
	0.4-2.4 × 10^6 CAR T cells/kg Post allo-SCT, no preTx	n = 4, R/R CLL Aged 44–66 y/o	n = 1 CR (9 mo), n = 1 PR, n = 2 PD No GVHD exacerbation Tumor lysis syndrome	Peristence to 30 days detected by qPCR	[23]

Allo-SCT: Allogeneic stem cell transplant; CLL: Chronic lymphocytic leukemia; CAR: Chimeric antigen receptor; CR: Complete response; CRS: Cytokine release syndrome; Cy: Cyclophosphamide; Flu: Fludarabine; GVHD: Graft versus host disease; incl.: Including; Mo: Months; MRD: Minimal residual disease; MSKCC: Memorial Sloan Kettering Cancer Center; NCI: National Cancer Center; NR: No response; PR: Partial response; qPCR: Quantitative PCR; R/R: Relapsed refractory; sCRS: Severe cytokine release syndrome; SD: Stable disease; Tx: Treatment; UPenn: The University of Pennsylvania; y/o: Years old.

Table 4

CD19-targeted trials for the treatment of B-cell lymphoma

Institution/CAR	Conditioning and dosing	Patient population	Clinical responses	CAR T-cell persistence	Ref.
Baylor FMC63-28z	No PreTx 2–20 × 10^7 CAR T/m^2 of each first and second generation	n = 6 Refractory NHL 46–59 y/o	No responses	Second-generation CAR persist longer (up to 9 mo) qPCR on PB samples	[49]
FMC63-z NCI FM63-28z	2 doses Cy 60 mg/kg, 5 doses Flu 25 mg/m^2 0.3–2.8 × 10^7 CAR T cells/kg + IL-2 (720000 IU/kg/8 h)	n = 4 FL, 47–63 y/o n = 1 MZL, 55 y/o	FL: n = 3 PR (7–18 mo) MZL = PR (12 mo)	Persistence up to 181 days by qPCR	[21]
	0.7-4.6 × 10^6 CAR T cells/kg Post allo-SCT, no preTx	DLBCL, n = 2 MCL, n = 4	DLBCL: SD (1 and 11 mo) MCL: SD, n = 4 (2–3 mo) PR, n= (3 mo) CRS > grade 3, n = 1	Persistence to 30 days Detected by qPCR	[23]
	1 dose Cy 60–120 mg/kg, 5 doses Flu 25 mg/m^2 1.5 × 10^6 CAR T cells/kg	DLBCL, n = 4 PMBCL, n = 3 Low-grade NHL, n = 1 SMZL, n = 1	DLBCL: CR, n = 2 (6–12 mo) PR, n = 1 (6 mo), NE, n = 1 PMBCL: CR (22 mo), SD (1 mo), NE CRS > grade 3, n = 8/9, 1 Death	Persistence up to 75 days Detected by qPCR	[22]
	3 low doses Cy (300 mg/m^2), 3 doses Flu (30 mg/m^2) 1 × 10^6 CAR T/kg	Refractory DLBCL, n = 8 FL, n = 1	DLBCL: 1 CR, 4 PR, 3 PD FL: 1 PR No CRS, some neurotoxicity	CAR T detectable to 6 mo by qPCR	[50]
MD Anderson FMC63-28z	5-50 × 10^7 CAR T/m^2 Post auto-SCT No conditioning IL-2 infusion	DLBCL, FL n = 5 Evaluable pts, infused post auto-SCT	No GVHD or sCRS CR, n = 4 (12 mo) PD, n = 1 (6 mo)	CAR T detectable to 3 mo	[12]
MSKCC SJ25-28z	Post auto-SCT Pegfilgrastim prior to 0.5-1 × 10^7 CAR T cells/kg	R/R DLBCL, n = 3 Transformed FL, n = 2 MZL, n = 1 Median 61 y/o	PFS 100% 9 mo mean follow-up sCRS, n = 1 at 1 × 10^7 dose	No data available	[51]
UPenn FMC63-BBz	EPOCH, n = 1 Bendamustine, n = 5 Cy/Flu, n = 1 median: 5.8 × 10^6 CAR T/kg	DLBCL, n = 6 FL, n = 2	CR, n = 2 DLBCL, 1 FL PR, n = 1 FL PD, n = 4 DLBCL sCRS, n = 1 (intervention)	Peak expansion at 2 weeks, flow cytometry	[52]
Fred Hutch FMC63-BBz	Cy conditioning 2 × 10^5-2 × 10^7 CAR T/kg (1:1 CD4:CD8)	R/R NHL n = 9	CR, n = 1 PR, n = 5 No sCRS, some neurotoxicity	Persistence in responding patients, data unavailable	[13]

Allo-SCT: Allogeneic stem cell transplant; Auto-SCT: Autologous stem cell transplant; B-ALL: B-cell acute lymphocytic leukemia; Baylor: Baylor college of Medicine; CAR: Chimeric antigen receptor; CR: Complete response; CRS: Cytokine release syndrome; Cy: Cyclophosphamide; DLBCL: Diffuse large B-cell lymphoma; FL: Follicular lymphoma; Flu: Fludarabine; Fred Hutch: Fred Hutchinson Cancer Research Center; GVHD: Graft versus host disease; MCL: Mantle cell lymphoma; MD Anderson: MD Anderson Center; Mo: Months; MSKCC: Memorial Sloan Kettering Cancer Center; MZL: Marginal zone lymphoma; NCI: National Cancer Center; NHL: Non-Hodgkin lymphoma; PD: Progressive disease; PMBCL: Primary mediastinal B-cell lymphoma; PR: Partial response; Pts: Patients; qPCR: Quantitative PCR; R/R: Relapsed refractory; sCRS: Severe cytokine release syndrome; SD: Stable disease; Tx: Treatment; UPenn: The University of Pennsylvania; y/o: Years old.