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PROGRESS IN HEMATOLOGY

CAR-T cell therapy, Now the time for the next!

Development of CMV-CD19 bi-specific CAR T cells with post-infusion in vivo boost using an anti-CMV vaccine

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Abstract

Adoptive transfer of in vitro expanded, chimeric antigen receptor (CAR)-redirected CD19-specific T cells can induce dramatic disease regression in patients with leukemia and lymphomas. However, the full potential of this emerging modality is hampered in some cancer settings by a significant rate of therapeutic failure arising from the attenuated engraftment and persistence of CAR-redirected T cells, and tumor relapse following adoptive transfer. Here, we discuss an advanced strategy that facilitates post-infusion in vivo boosting of CAR T cells via CMV vaccination, to mediate durable remission of B cell malignancies by engrafting a CAR molecule onto a CMV-specific T cell. We also discuss a feasible and unique platform for the generation of the CMV-CD19CAR T cells for clinical application. This new approach would overcome multiple challenges in current CAR T cell technology including: short T cell persistence, limited duration of response, and inability to re-stimulate T cells after relapse or persistent disease.

Keywords Bi-specific CAR T cells · CMV · CD19 · CMV vaccine

Introduction

Adoptive cellular immunotherapy (ACIT) using chimeric antigen receptor-modified (CAR)-T cells or other immune effector cells such as NK cells is a rapidly growing therapeutic approach to treating patients with refractory cancers. Tisagenlecleucel (Kymriah™) is the first CAR T cell therapy approved by the U.S. Food and Drug Administration (FDA) for treatment of patients up to age 25 with relapsed/refractory acute lymphoblastic leukemia (ALL) [1, 2]. It is also approved for patients with relapsed or refractory large B-cell lymphoma including diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma based on the JULIET trial [3]. Axicabtagene ciloleucel (Yescarta™) has been approved for aggressive, relapsed and/or refractory diffuse large B cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma, and transformed follicular lymphoma based on the pivotal ZUMA-1 trial [4, 5].

However, current CAR T cell technology has multiple limitations including long manufacturing time, repeated cycles of ex vivo expansion to yield adequate cell dose (potentially promoting T cell differentiation and exhaustion), attenuated engraftment and persistence of CAR T cells, restricted capability to induce a second in vivo expansion in relapsed cases, potential risk of inducing graft-versus-host disease (GVHD) in the post-transplant setting. In particular, lack of long-term persistence of CAR T cells post infusion remains a major challenge, as it is believed to be directly linked to long-term ALL/NHL disease control, and possible cure.

To overcome these limitations, we have developed a novel combination immunotherapy, in which cytomegalovirus (CMV) pp65-specific T cells are selected and expanded for ex vivo modification with a CAR targeting CD19. Following infusion, CMV-CD19 bi-specific CAR T cells can be in vivo expanded by stimulation of the native CMV-specific T cell receptor (TCR) via a CMV vaccine, Triplex developed and clinically evaluated at City of Hope. Triplex is a multi-antigen recombinant modified vaccinia Ankara (MVA) with genes encoding 3 CMV proteins (pp65, IE1, and IE2).

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Triplex has proven safe and powerfully immunogenic in Phase I trial in CMV-seronegative and -seropositive healthy volunteers [6], and Phase II trial in recipients of allogeneic hematopoietic cell transplantation (HCT) [7].

A proof-of-principle has been demonstrated in xenograft model, showing successful redirection of CMV-specific T cells to recognize and lyse CD19 + tumor cells via CD19 CARs, while maintaining their ability to proliferate and response to CMV antigen stimulation [8]. This strategy is feasible, since upwards of 80% of adults are CMV immune in many populations; and the Triplex vaccine can induce primary immunity in CMV naïve donors. pp65-specific T cells frequency in CMV-seropositive donors is high, and there is a large bulk of clinical experience with adoptive cellular immunotherapy (ACIT) targeting the CMVpp65 antigen. Additionally, in the post-HCT setting, use of CMV selection confers a low risk of inducing GVHD due to defined TCR and the possible benefit of preventing CMV infection.

Chimeric antigen receptor (CAR) biology and challenges for clinical translation

CARs are modular synthetic molecules that mimic certain T cell receptor (TCR) attributes consisting of three major functional components—the antigen-binding domain, the extracellular linker/spacer, a costimulatory domain from 4-1BB (CD137) or CD28 and an intracellular signaling domain. CAR T-cell therapy is a form of adoptive cellular treatment strategy that uses genetic engineering to graft specificity into an immune effector cell [9]. Unlike other small-molecule or antibody therapies, whose levels decrease over time, CAR-T cells are "living drugs" that undergo rapid exponential expansion and can serially kill target cancer cells. The first FDA approved "living drugs", KYMRIAH and Yescarta are CAR-T-cell immunotherapies using patient-derived T cells that have been genetically engineered in vitro, via lentiviral or retroviral transduction, to express a CD19-targeted CAR that mediates T-cell activation in a major histocompatibility complex-independent manner. CAR-expressing T cells directed to the CD19 antigen (CD19 CAR-T) have proven remarkably effective in treatment of pre-B acute lymphocytic leukemia (ALL) and other B-cell malignancies [10–13].

Unfortunately, disease relapse remains the leading cause of treatment failure in patients receiving CAR T therapy. Disease relapse could occur as a result of suboptimal CAR T cell manufacturing, including CAR design, initial T cell subsets, T cell activation, T cell exhaustion due to in vitro expansion; attenuation of in vivo CAR T cell expansion/persistence; and intrinsic patient features, such as immunity and tumor microenvironment [14].

It is important to mention that, while increasing attention focuses on enhancing potency and durability of CAR T-cell therapy, safety considerations in the clinic are equally important. Of the serious toxicities associated with CD19-CAR T-cell therapy, the most common is cytokine release syndrome (CRS), which in its most severe form is commonly managed using the anti-IL-6 receptor antibody, tocilizumab, with or without corticosteroids [15]. It is well accepted that CAR T therapy toxicities are associated with the CAR T cell dose. Hence, maximizing the efficacy with lower doses of CAR T cells is critical to increase the ratio of efficacy over toxicity.

CMV-targeting cellular immunotherapy and vaccine development

Cellular Immunotherapy for CMV

CMV is a common virus for which 75% of adults in the United States test positive [16, 17], and it was the first virus targeted by adoptive transfer strategies. Pioneering immunotherapy trials [18–20], showed that adoptive transfer of CMV-specific T cells is sufficient to reduce the incidence of CMV disease without toxicity or GVHD.

Riddell and Greenberg's pioneering studies used CMV-specific $CD8^+$ T cells after in vitro expansion of donor peripheral blood mononuclear cells (PBMCs) in the presence of CMV-infected autologous fibroblasts and depletion of $CD4^+$ T cells [21, 22]. Subsequent immunotherapy trials [18–20, 22, 23], showed that adoptive transfer of virus-specific T cells is sufficient to reduce the incidence of CMV disease without significant toxicity or increased GVHD rates.

CMV vaccine development

With the successful cloning of the pp65 gene in 1980s [24], demonstration of pp65 as an immunodominant protein [25, 26], and identification of HLA A*0201-restricted CTL epitopes from pp65 [27], the Diamond's laboratory has engineered two potent CMV vaccines, which have been evaluated in multiple Phase 1 and 2 clinical trials. The first, CMVPepVax, is composed of the HLA A*0201-restricted pp65 CD8 T-cell peptide epitope fused with the P2 peptide epitope of tetanus toxin, and administered with adjuvant Toll-like receptor (TLR) 9 agonist, PF03512676. Based on its safety profile and immunogenicity [28], CMVPepVax was tested in a pilot phase Ib trial with 36 allogeneic HCT recipients randomized to the vaccine arm ($n = 18$: vaccination on days 28 and 56 post HCT) or observation arm ($n = 18$). This first-in-HCT trial showed that CMVPepVax is safe with no adverse impact on HCT outcomes or rate of acute GVHD, and no unexpected adverse events. There was a 2.5-fold increase in CMVpp65-specific CD8 T cells during the first 100 days post HCT, reduced CMV reactivations, and usage

of pre-emptive antivirals associated with CMVPepVax compared to the observation group [29].

While CMVPepVax is promising, as a peptide-based vaccine, its use is limited to individuals with HLA-A*0201. CMV MVA Triplex, also developed by the Diamond's laboratory, is a multi-antigen recombinant modified vaccinia Ankara (MVA) virus vaccine with genes encoding three CMV immunogenic proteins, pp65, IE1, and IE2. Since the entire protein is expressed in the vaccine, CMV-MVA Triplex is not limited to specific HLA types. In addition, all potential CD4 and CD8 epitopes from all three CMV proteins could be available to stimulate anti-CMV immunity. MVA has an extensive history of safe delivery as a smallpox vaccine [30–34], or given to malaria patients co-infected with HIV and/or tuberculosis [35, 36], and more recently as a therapeutic vaccine in both cancer [37, 38], and HIV-AIDS patients [39, 40]. In a phase I trial (NCT01941056), safety and immunogenicity endpoints were evaluated in healthy volunteers ($n=24$), with or without prior immunity to CMV and vaccinia. Vaccinations at all dose levels (DL) were well tolerated, with only a few expected injection site reactions without any SAE or DLT [6]. Triplex vaccinations induced robust expansion of pp65-, IE1- and IE2-specific CD8 and CD4 T-cells in CMV-seropositives with no SAE or DLT at all dose levels. In a multi-center phase 2 trial, Triplex vaccine was subsequently tested in seropositive recipients of allogeneic HCT in a multi-center phase 2 trial, which demonstrated safety in this population. Triplex was associated with a twofold decrease in CMV reactivations compared with placebo, accompanied by improved CMV-specific CD4 and CD8 T cell immune reconstitution [7]. These CMV vaccine clinical trials have paved the way for clinical studies with CMV/CD19 CAR trials.

Combinatory therapy of CAR- and viral-specific T cells

Use of endogenous TCR to improve CAR T cell therapy

Adoptive T cell therapy for treatment of malignancies has been significantly more challenging and less effective than for viral diseases, primarily due to lack of persistence of the adoptively transferred tumor-specific T cells in patients [41, 42]. In contrast, the adoptive transfer of viral-specific T cells has demonstrated efficacy in preventing progressive viral infections and exhibited long-term persistence in patients, in part due to the fact that viral-specific T cells receive optimal co-stimulation after engagement of their native TCRs [18–20, 43, 44]. The mechanisms for the differential persistence of adoptively transferred virus-specific T cells in HCT recipients versus tumor-reactive T cells in cancer patients are not fully understood, but possibly reflect both the environment into which the T cells are infused and qualitative attributes of the isolated and expanded T cells for adoptive transfer.

In an attempt to improve the efficacy of CAR T cells for tumor eradication, T cells with dual specificity have been developed: isolated Epstein–Barr virus (EBV)-specific T cells modified to express GD2, CD30 CARs recognizing tumors of neural crest origin [45–47], and isolated influenza A matrix protein 1 (MP1)-specific T cells modified to express CD19 CARs recognizing B-cell malignancies [48]. These virus and CAR bispecific T cells demonstrate superior survival and antitumor activity compared to CAR T cells without virus specifics, possibly due to a more potent co-stimulation of virus-specific T cells after engagement of their native receptors. Studies demonstrate that adoptively transferred EBV or EBV × CMV × CD19CAR bi(tri)-specific T cells proliferate in patients as a result of CMV reactivation [49, 50]. Lapteva et al. reported that in patients with viral reactivation, up to 30,000-fold expansion of CD19CAR-viral-specific T cells is observed, with depletion of CD19 + B cells, and patients remain in remission at 42–60 months [53].

Development of CMV-CD19 bi-specific CAR T cells

On the basis of the clinical observation that enhanced antiviral efficacy can be achieved by stimulating the endogenous TCR, we have transduced native CMVpp65-specific T cells with a CD19CAR lentivirus to determine whether CD19CAR-redirected CMVpp65-specific T cells can respond to a CMV vaccine with rapid expansion and enhanced antitumor activity (Fig. 1). In a proof-of-concept study led by Wang et al. at City of Hope, CMV-specific T cells from CMV-seropositive healthy donors were selected after stimulation with pp65 protein and transduced with clinical-grade lentivirus expressing the CD19R:CD28:ζ/EGFRt CAR [8]. The bi-specific T cells proliferated vigorously after engagement with either endogenous CMVpp65-specific TCR or engineered CD19 CARs, exhibiting specific cytolytic activity, proliferative response, and IFNγ secretion. Upon adoptive transfer into immunodeficient mice bearing human lymphomas, the bi-specific T cells exhibited proliferative response and enhanced antitumor activity following CMVpp65 peptide vaccine administration [8] (Fig. 2). This strategy could be an improvement of CAR T cell therapies by lowering the dose of CAR- with the purpose of increasing the ratio of efficacy over toxicity. In addition, these CMV CAR T cell expansion products may play a role to control complications of CMV viral reactivation in patients. CMV selection will decrease the risk of inducing GVHD in the allogeneic HCT setting, by removing allo-reactive T cells during the CMVpp65-specific selection process.

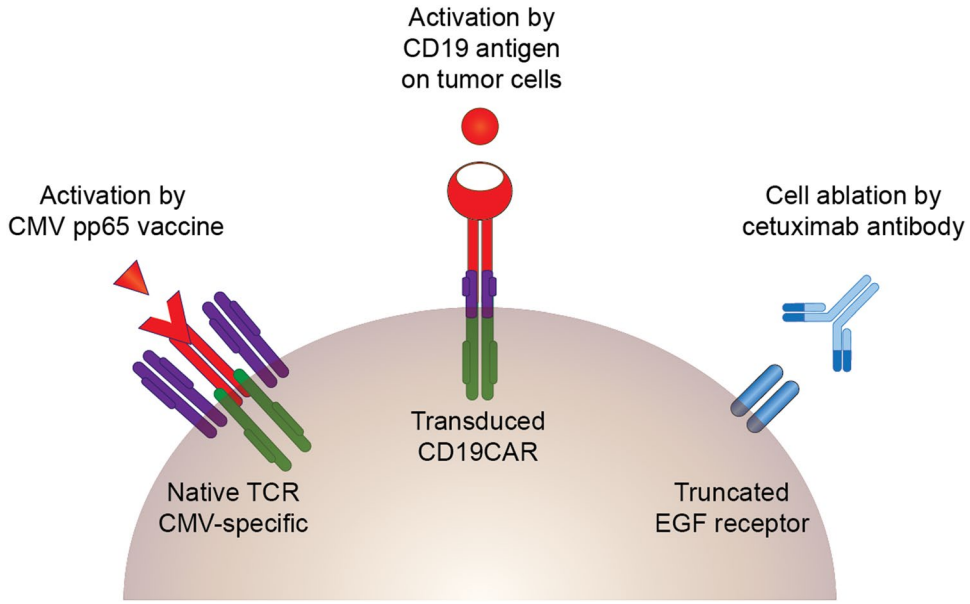

Fig. 1 Cell surface interactions of bi-specific CMV-CD19CAR T cells. *CMV* cytomegalovirus, *TCR* T cell receptor complex, *CAR* chimeric antigen receptor, *Truncated EGF* epidermal growth factor. Bi-specific T cells will be produced by first selecting CMVpp65-specific T cells from leukapheresis products based on interferon-γ-positivity, and then transducing them with a lentivirus encoding a CAR specific for the CD19 antigen. In addition to the CD19 CAR, our lentivirus construct encodes a truncated EGF receptor (EGFRt) that is recognized by the antibody cetuximab for the purpose of T cell tracking and potentially T cell ablation

Large-scale manufacturing of CMV-CD19CAR T cells for clinical application

A major challenge of the CMV/CD19 strategy is manufacturing of the CMV/CAR bispecific T cells. There was evolution in generating viral-specific T cells; previous approaches used CMV-infected fibroblasts, EBV-LCL, transfection [18, 51–54] as stimulators, while it became possible to select CMV-specific T cells [55] in one day using clinical-grade CMV antigenic peptides (i.e., pp65 overlapping peptides). Importantly, time of manufacturing process is known to inversely correlate with CAR T cell function [56–58]. To shorten the manufacturing period of the CMV/CD19CAR T cells, we developed a method for generating CMV-CD19CAR bi-specific T cells as published by Wang et al. [8]. Briefly, CMV-specific T cells from CMV-seropositive healthy donors are selected after stimulation with pp65 protein and transduced with clinical-grade lentivirus expressing the CD19R:CD28:ζ/EGFRt CAR. The resultant bispecific T cells, targeting CMV and CD19, are expanded via CD19 CAR-mediated signals using CD19-expressing cells. The clinical scale manufacturing of bi-specific polyclonal T cells is depicted in Fig. 3.

Peripheral blood mononuclear cells (PBMCs) were collected and processed in the CliniMACS Prodigy® system, in which PBMCs were first stimulated with a GMP-grade PepTivator® overlapping CMV pp65 peptide pool, then enriched for CMV-responsive IFNγ$^+$ T cells using the IFNγ Catchmatrix reagent (Miltenyi Biotec Inc.). CMV-responsive IFNγ$^+$ T cells were next transduced with a lentiviral vector encoding EGFRt/CAR, and finally expanded for approximately 15 days in vitro.

As summarized in Table 1, using this isolation and expansion method, we were able to consistently recover $4.8\% \pm 1.4 \times 10^6$ CMV-specific T cells with $78.3\% \pm 2.9$ purity from 1×10^9 PBMC input. This platform is able to generate $20–60 \times 10^6$ bi-specific CMV-CAR19CAR T cells from one leukapheresis product based on CMVpp65-specific IFNγ intracellular cytokine (ICC) and EGFRt positivity, which exceeds the dose level used in the clinical trial with VZVxGD2 bi-specific T cells ($1 \times 10^6/m^2$) (NCT01953900). Characterization of the final product, manufactured in a large scale, demonstrated bi-functionality of the CMV-CD19 CAR T cells against CD19 tumor with reduced expression of exhaustion markers (i.e., PD1) [59]. When CMV-CD19 bi-specific T cells were stimulated with pp65 antigen or CD19 + tumor, their gene expression on gated IFNg + CAR + cells analyzed with the PrimeFlow™ RNA technology, demonstrated that CMV-CD19 bi-specific T cells maintained more favorable memory phenotype and persistence after pp65 TCR stimulation than CAR stimulation with CD19 + tumor [59].

Clinical applications

We are currently developing our CMV-CD19 bi-specific CAR T cell platform for CD19 + hematologic malignancies—NHL and ALL, while this platform can be also applied in other CAR T cells for a range of hematologic/non-hematologic cancers if found to be successful in NHL/ALL.

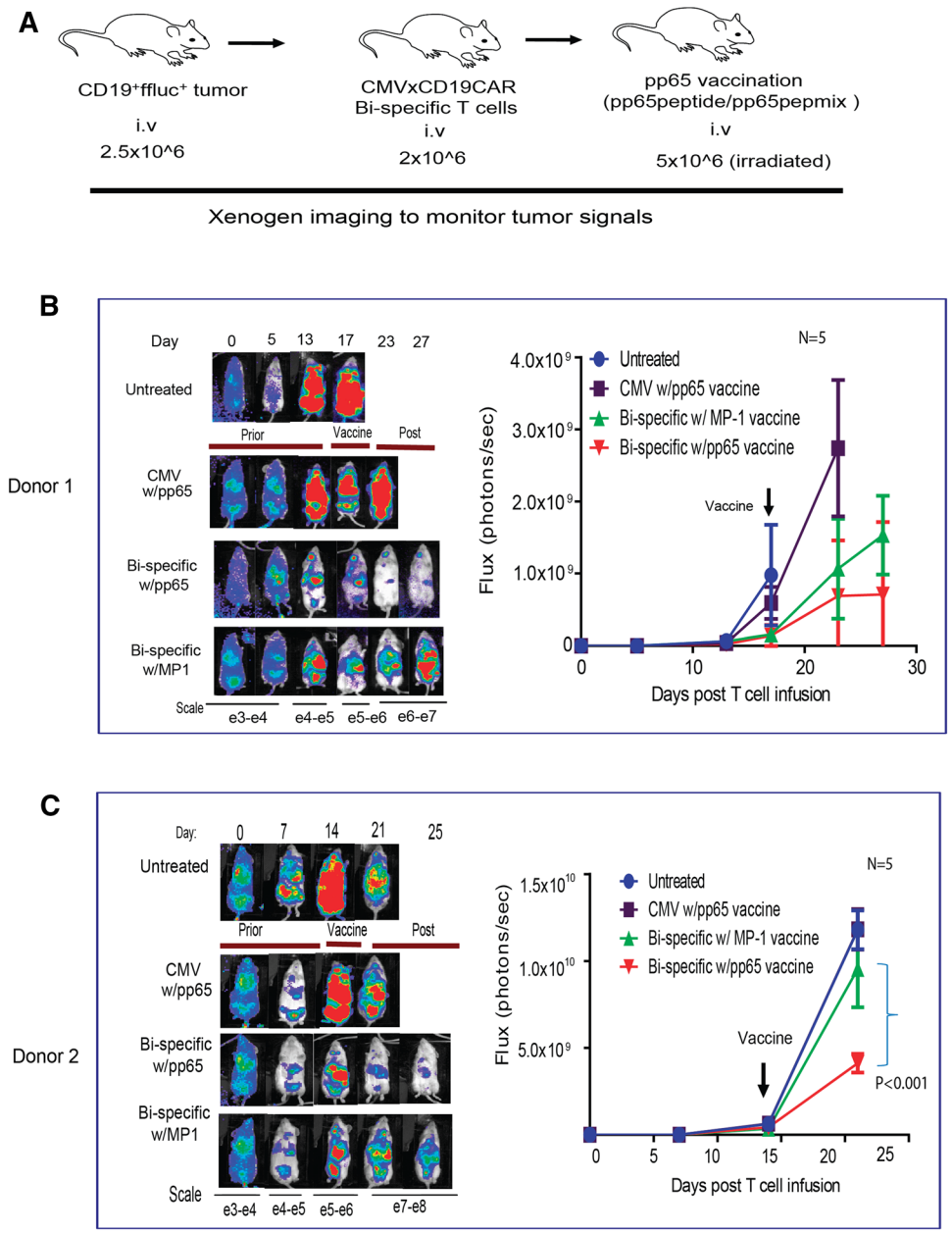

Fig. 2 Anti-tumor activity of adoptively transferred bispecific T cells is enhanced by CMVpp65 vaccination (**A**) NSG mice were injected i.v. on day 0 with 2.5 × 106 GFPffluc + LCL cells. Three days after tumor inoculation, recipient mice were injected i.v. with 2 × 106 bi-specific cells that underwent 2 rounds of CD19 stimulation. Vaccine was given by i.v. injection of peptide pulsed autologous T cells. Fourteen to seventeen days post T cell infusion, 5 × 106 pp65pepmix (**B**) or pp65 peptide (**C**) (or MP1) loaded autologous T cells were irradiated and injected (iv) into T-cell-engrafted mice as vaccine. pp65 vaccine was also supplemented to the mice that were treated with 10 × 10^6 CMV-specific T cells from the same donor and untreated mice were used as another type of control. Tumor growth was evaluated by Xenogen® imaging. $n = 5$ for each group in the experiments. The Mann Whitney test was used for statistical analysis. Adopted from Wang et al. 2015 [8]

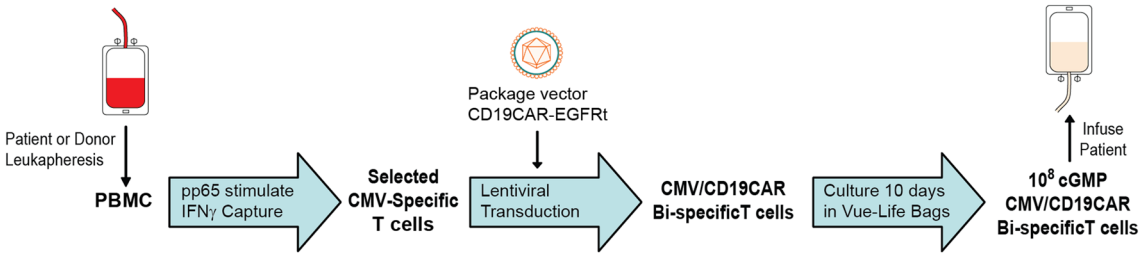

Fig. 3 cGMP Manufacturing Methodology. PBMCs are collected and processed in the CliniMACS Prodigy® system, in which PBMCs were first stimulated with a GMP-grade PepTivator® overlapping CMV pp65 peptide pool, then enriched for CMV-responsive IFNγ + T cells using the IFNγ Catchmatrix reagent (Miltenyi Biotec Inc.). CMV-responsive IFNγ + T cells are next transduced with a lentiviral vector encoding EGFRt/CAR, and finally expanded in vitro. PBMC-peripheral blood mononuclear cells, CMV cytomegalovirus, IFN-interferon

Table 1 Summary of PD runs for CMV-CAR bispecific T cells

Healthy donar	PBMC	Input PBMC × 10^6	Recovery of IFNr$^+$ cells × 10^6	Purity of IFNr + cell	CD4$^+$ IFNγ^+	CD8$^+$ IFNγ^+
1	Frozen	1	1.4	72%	69%	31%
2	Fresh	1	2.8	78%	47%	52%
3	Fresh	2	8.7	83%	36%	67%
4	Fresh	1	2.1	75%	81%	11%
5	Fresh	1	4.59	72%	38%	36%
6	Fresh	1	9.25	90%	39%	43%
Average		1.2 ± 0.2	4.8 ± 1.4	78.3 ± 2.9	51.7 ± 7.7	40 ± 7.8

B cell non-Hodgkin lymphoma (NHL)

According to the data from the Surveillance, Epidemiology and End Results program (SEER), over 70,000 new cases of NHL are diagnosed each year in the United States with about 20,000 deaths each year, representing the 5th leading cause of cancer deaths. Efforts to improve the survival of patients with recurrent lymphoma have focused primarily on the use of autologous HCT [60], which is curative in approximately 46% of good-risk patients, but confers a less than 15% 5 year event-free survival in patients with poor prognostic features [61]. With the improved outcome of patients receiving rituximab-based induction therapy for NHL, those who relapse early have a poor prognosis even with auto HCT [62]. Allogeneic HCT provides a tumor-free stem cell graft, containing cells that have not been damaged by prior chemotherapy and the opportunity for a graft-versus-lymphoma (GVL) effect, and has been applied in patients with relapsed NHL [63]. However, allogeneic HCT is associated with significant risk of transplant-related complications, such as GVHD and infection, offsetting the potential benefit from GVL [64, 65]. In addition, as a significantly greater proportion of high-risk patients are selected for allogeneic HCT, the rate of relapse observed after this treatment (25–41% at 3–5 years) has been similar to that in patients undergoing autologous HCT [66–71].

More recently, the advances in adoptive cellular immunotherapy using CAR T-cells have led to a dramatic improvement in outcomes of patients with relapsed and refractory large B-cell NHL [3, 4]. In Phase I studies designed to improve long-term remission rates in patients with B cell NHL by administration of central memory-derived CD19 CAR T cells after autologous HCT, our team demonstrated safety and feasibility of CD19 CAR Tcm therapy in two separate trials (#NCT01318317: NHL1 and #NCT01815749: NHL2), with promising efficacy (4 of 8 in NHL1 and 6 of 8 in NHL2 are progression-free at 1 year) [72]. However, CAR T cells persistent in these trials were limited to ≤ 28 days [72]. Data from JULIET trial showed comparable tisagenlecleucel exposure in peripheral blood by quantitative polymerase chain reaction (qPCR; quantification of CAR transgene levels) in responders and non-responders, with longer persistence in patients with sustained response [5].

B cell ALL

As another form of B cell malignancies, an estimated 6000 new cases of ALL are being diagnosed in the United States annually, of which about 25% ($n = 1470$) die, according to the data from the SEER. Despite high induction remission rates (80–90%), the overall survival (OS) rate is low in adults with ALL (30–40%), [73] and < 10% in those patients whose disease progresses or recurs within 1 year of induction therapy [74, 75]. Disease relapse is a leading cause of treatment failure after allogeneic HCT for ALL patients with the current 3 year survival rate of only 25–28% in advanced cases (CIBMTR). Thus, there is an urgent need for the development of new therapies that can consolidate the tumor cytoreduction achieved with auto- or allogeneic HCT by eradicating the limited number of tumor cells surviving after conditioning chemo-radiotherapy.

Tisagenlecleucel (Kymriah™) is the first CAR T cell therapy approved by the FDA for treatment of patients up to age 25 with relapsed/refractory acute lymphoblastic leukemia (ALL) [1, 2]. At City of Hope, Khaled and colleagues conducted a phase I trial (NCT02146924), in which CD19-specific, CD28-costimulatory CAR (CD19:28z-CAR) T cells were infused for treatment of adult patients with relapse/refractory ALL. In this trial, all patients achieved complete remission or complete remission with incomplete hematopoietic recovery, with a low incidence of severe CRS and neurotoxicity [76]. Although the numbers are small, the unanimous response, combined with a tolerable and reversible toxicity profile in patients with both low and high disease burden is remarkable in this trial, and suggests promise for this naïve and memory T cell (Tn/mem) manufacturing platform for CD19 and other CAR targets.

Proposed clinical trials—pre-emptive infusions with vaccine boost in the setting of HCT

We are developing several protocols using CMV-CD19 bi-specific CAR T cells after lymphodepletion or autologous/allogeneic HCT. This treatment will be followed by Triplex vaccination 28 days after bispecific CAR T cell infusion for in vivo expansion of bi-specific T cells. The primary objectives of these trials are to examine safety and persistence/expansion of CMV-CD19CAR T cell before and after Triplex vaccine boost.

For NHL, our first trial will use CMV-CD19 bi-specific CAR T cells in the setting of autologous HCT, built upon our previous experience in the use of CAR T cells immediately after autologous HCT. The CAR T cells will be manufactured from patient autologous T cells harvested prior to G-CSF-mobilization of peripheral blood stem cells. These bi-specific CAR T cells (10×10^6) will be infused to patients on day + 2 post-transplant, which will then be followed by a CMV vaccine, Triplex, on day 28 and day 56 (Fig. 4a).

For ALL, our trial will use the setting of allogeneic HCT with delayed donor leukocyte infusion (DLI) using the engineered CMV-CD19 bi-specific CAR T cells. In this pre-emptive approach, we plan to infuse these cells on day 28, followed by Triplex boost on day 56 and 84 post HCT (Fig. 4b).

These studies serve as proof of principle for a method of enhancing CAR effectiveness and controlling T cell expansion and can be applied to multiple diseases in both transplant and non-transplant settings.

Conclusion

Choosing CMV-specific T cells for engineering with a CD19 CAR, endows the potent and persistent virus-specific T cells with a second specificity for CD19 tumor antigen, which enables the bi-specific T cells to persist and numerically expand in vivo via stimulation of the CD19 CAR by tumor cells, as well as via the endogenous TCR by viral antigens (from CMV virus or Triplex vaccine). The discussed manufacturing platform would result in high quality of CAR T cells by avoiding T cell exhaustion by CD3/CD28 stimulation that is required for the generation of conventional CAR T cells. The native pp65 antigen stimulation followed by transduction would maintain better function and less differentiation. In addition, re-expansion of CAR T cells by vaccine administration will allow to augment in vivo rather than ex vivo expansion of CD19CAR T cells, avoiding excessive T cell exhaustion from prolonged growth ex vivo. The expected higher potency and persistence of bi-specific T cells should improve disease responses to CAR T cell therapy in CD19 + NHL and ALL. We also suspect that

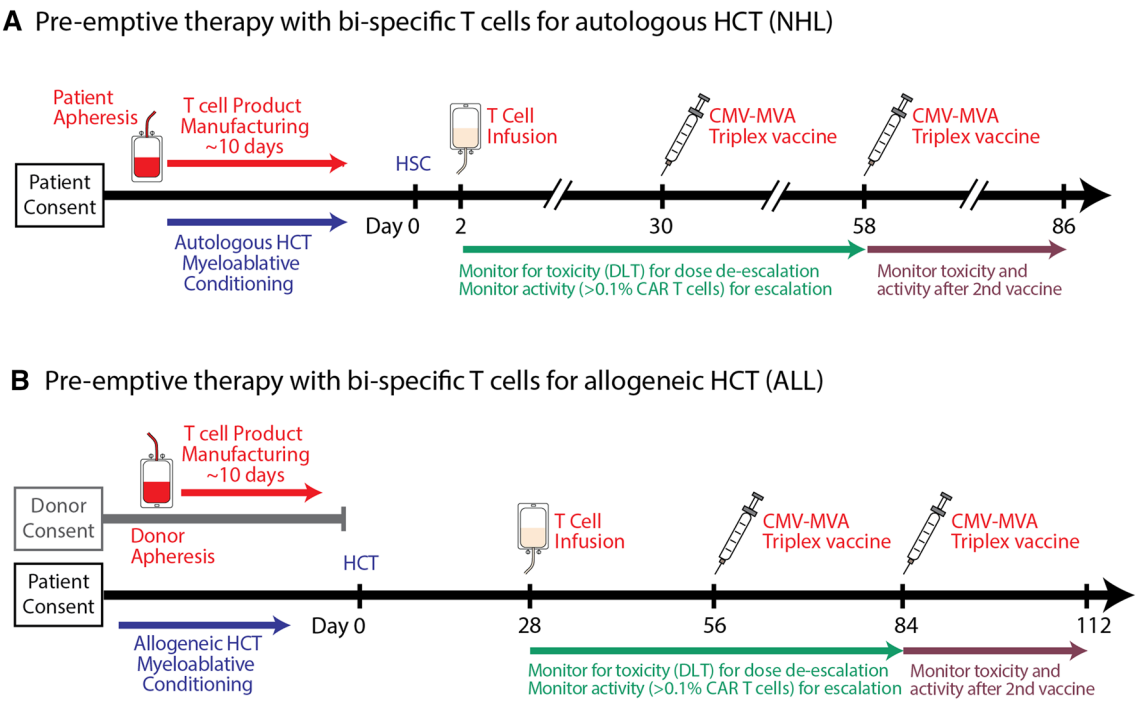

Fig. 4 **a** Pre-emptive therapy with bi-specific T cells for autologous HCT (NHL). **b** Pre-emptive therapy with bi-specific T cells for allogeneic HCT (ALL)

using virus-specific memory T cells may enhance homing to lymph nodes, allowing better lymphoma targeting. Furthermore, our EGFRt system will also allow us to efficiently monitor, select, and ablate CMV-CD19CAR T cells in vivo. Taken together, the significance of the approach is that for the first time we have in vivo control of adoptively infused CMV-CD19CAR T cells with regard to both expansion/persistence and ablation. We have completed IND-enabling studies and the IND application is underway. We plan to initiate our first clinical trial in the fourth quarter of 2021 for patients with intermediate/advanced-grade B cell NHL and relapsed/refractory B cell ALL.

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Declarations

Conflict of interest Xiuli Wang: No conflict of interest relevant to this work. Don J. Diamond: Helocyte for royalties and research support. Stephen J. Forman: No conflict of interest relevant to this work. Ryotaro Nakamura: Helocyte for research support.

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