Emerging Role of CAR T Cells in Non-Hodgkin's Lymphoma

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Abstract

Adoptive T-cell therapy with chimeric antigen receptor T cells (CAR-Ts) has produced impressive clinical responses among patients with B-cell malignancies, and several groups have published positive results using anti-CD19 CAR-Ts for the treatment of B-cell acute lymphoblastic leukemia. Recently, new data from clinical trials have demonstrated the benefits of CAR-T therapy in the non-Hodgkin's lymphoma (NHL) setting. This review describes some of the most recent and promising advances in engineered T-cell therapy, with particular emphasis on the clinical benefits of NHL treatment.

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Adoptive T-cell therapy with chimeric antigen receptor T cells (CAR-Ts) has emerged as an effective therapy for the treatment of malignancies, and several groups have published results using anti-CD19 CAR-Ts mostly for the treatment of B-cell acute lymphoblastic leukemia (B-ALL) and non-Hodgkin's lymphoma (NHL).^{1–4} Although clinical experience with CAR-Ts is more abundant in ALL, the benefits are being observed in other hematologic malignancies, such as NHL, chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). Lymphomas are a heterogeneous group of cancers that arise from developing lymphocytes; according to the WHO, there are >35 distinct entities.⁵ Lymphomas have been separated into NHL and Hodgkin lymphoma (HL); NHL includes a wide group of lymphoid malignancies that derive from B, T, or natural killer (NK) cells.⁶ Although malignant cells acquire genetic abnormalities, they also preserve many features of the cells from which they originated, meaning that target antigens that could potentially be used in any immunotherapy treatment are usually expressed on both lymphoma cells and their

nonmalignant counterparts. HL also differs from NHL in that the malignant Reed-Sternberg (RS) cells are relatively rare, and that nonmalignant cells that infiltrate the microenvironment play a key role in the biology of HL.⁷ It has become increasingly evident that elements of the tumor microenvironment, far from being inactive or part of an antitumor inflammatory response, actually enable cancer pathogenesis and progression. In general, B-cell NHL (B-NHL) and HL are highly sensitive to both chemotherapy and radiotherapy and, despite the presence of disseminated disease at diagnosis in most cases, remain eminently curable. However, relapse and resistance prevent the ultimate goal of achieving cure in all patients.⁸

In the past few decades, the introduction of improved chemotherapy regimens, monoclonal antibodies (mAbs), radioimmunotherapy, and targeted adoptive T-cell therapies have provided significant advances in management.⁸ Lymphomas are highly susceptible to cellular therapies, including stem cell transplant⁹ and adoptive transfer of Epstein-Barr virus (EBV)-specific T cells, which could be considered the precursor to CAR-

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T therapy.¹⁰ Recently, adoptive T-cell therapies with CAR-Ts have demonstrated wide application and significant results for the treatment of B-cell malignancies. CAR-Ts consist of an engineered extracellular domain from a single-chain variable fragment (scFv), composed of the antigen-binding regions of both heavy and light chains of mAbs, a transmembrane domain, an intracellular costimulatory domain (more commonly CD28 or 4-1BB), and a CD3 ζ .^{11,12} Unlike conventional T cells, CAR-Ts recognize unprocessed antigen, and therefore eradicate tumor cells independently of their expression of major histocompatibility complex (MHC) antigens. This circumvents some of the major mechanisms through which tumors avoid MHC-restricted T-cell recognition, such as the downregulation of human leukocyte antigen (HLA) class I molecules and defective antigen processing.^{12,13} Contrary to B-cell lymphomas, peripheral T-cell lymphomas encompass a heterogeneous group of diseases that have generally been associated with poor prognosis.⁵ Currently, T-cell lymphomas present a much more challenging treatment landscape and, due to limitations regarding antigen availability, there are very few adoptive T-cell therapies targeted against a T-cell lymphoma antigen.^{14,15}

Antigen Selection

The consistent expression of the B-cell lineage markers CD19, CD20, and CD22 across most B-cell malignancies,^{16,17} and the safety and efficacy of anti-CD19/CD20/CD22 mAbs previously described in these diseases, made them the ideal targets for CAR-Ts.^{18–21} However, normal B cells also express most lymphoma target antigens suitable for CAR recognition, leading to B-cell elimination, a relatively benign disorder that can be treated with the use of intravenous immunoglobulin. Antigens more specific and restricted for B-cell malignancies could also be used as a target, and therefore display fewer side effects compared with conventional anti-CD19/CD20/CD22 CAR-Ts. Among these alternative antigens is BCMA (B-cell maturation antigen), which is predominantly expressed by plasma cells, subsets of mature B cells, and the κ or λ light chain of malignant B cells. In the T-cell lymphoma setting, however, finding an ideal antigen to target is more challenging due to the shared expression of many targetable antigens between normal and malignant T cells.²² This shared expression

of antigens can cause fratricide in CAR-Ts, inhibiting their proliferation and viability, and in the clinic may result in eradication of normal peripheral T cells.²² Such an on-target/off-tumor effect could lead to more severe and life-threatening side effects and be less clinically manageable than the depletion of normal B cells found within the CD19, CD20, or CD22 targeted therapies.⁴ HL tumor cells have a very low expression of CD19 antigen and therefore are not eligible for anti-CD19 therapy with CAR-Ts.²³ However, despite the lack of CD19 expression, almost all Hodgkin and RS (HRS) cells overexpress the CD123 and CD30 surface molecules, which could be potentially used as a target for adoptive T-cell therapy.²⁴

CAR-Ts Against NHL

The CD19 antigen is expressed during all stages of B-cell differentiation and is still present in most B-cell lymphomas.¹⁶ Therefore, most of the clinical trials conducted with CAR-Ts in the B-cell lymphoma setting will target CD19. Different scFvs can be used to target the CD19 antigen on the cell surface, and those more commonly used in clinical trials are FMC63 or SJ25c.²⁵ Initial clinical trials for the treatment of lymphoma were conducted at the City of Hope Medical Center and used a CD19-targeted first-generation CAR-T (FMC63 19z CAR-T), without any costimulatory signaling.²⁶ Patients with relapsed/refractory (R/R) follicular lymphoma (FL) were treated with anti-CD19 first-generation CAR-Ts after lymphodepletion with fludarabine and subcutaneous interleukin-2 (IL-2) was administered concomitantly to T-cell therapy. Despite demonstrating the feasibility and safety of this new approach, anti-CD19 adoptive T-cell therapy with first-generation CAR-Ts failed to demonstrate objective antitumor effects.²⁶

Second-generation anti-CD19 CAR-Ts incorporate a costimulatory domain (CD28, 4-1BB, ICOS) and have demonstrated an enhanced antitumor effect in preclinical studies both in vitro and in vivo.²⁷ Recently, clinical trials using second-generation CD19-targeted CAR-Ts with either CD28 or 4-1BB costimulatory domains have demonstrated significant results in the B-cell lymphoma setting, more specifically for the treatment of diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma (PMBCL), FL, splenic marginal zone lymphoma (SMZL), and mantle cell lymphoma (MCL).

The first patient with NHL was treated on a phase I trial at the NCI with a second-generation CD19-targeted CAR-T (FMC63 1928z).²⁸ The patient presented with advanced R/R FL and received a lymphocyte-depleting regimen consisting of 60 mg/kg cyclophosphamide daily for 2 days followed by 5 daily doses of 25 mg/m² fludarabine. The day after the last fludarabine dose, the patient received 1x10⁸ anti-CD19 CAR-Ts intravenously, followed by 3x10⁸ anti-CD19 CAR-Ts the next day. After the second CAR-T infusion, the patient received 720,000 IU/kg IL-2 intravenously every 8 hours, for a total of 8 doses. The patient achieved partial response (PR) for 32 weeks after anti-CD19 CAR-T therapy.²⁸

A follow-up trial from the NCI group was conducted in patients with FL or SMZL.²⁹ In this trial, patients were preconditioned with cyclophosphamide, 60 mg/kg for 2 days and fludarabine, 25 mg/m² for 5 days and treated on day 0 with a single infusion of CAR-transduced T cells. IL-2 was also administered intravenously 3 hours after the CAR-T infusion at a dose of 720,000 IU/kg every 8 hours; doses of CAR-Ts ranged from 0.3x10⁷ to 3.0x10⁷ CAR-Ts/kg bodyweight. Results from this trial showed that 3 of 4 patients with FL achieved PR, with a follow-up between 8 and 17 months, and 1 of 1 patient with MZL achieved PR, with a follow-up of 12 months.²⁹

Another clinical trial from the NCI group used CD19-targeted CAR-T for the treatment of R/R DLBCL or indolent B-cell lymphomas (SMZL and PMBCL).³⁰ The preconditioning chemotherapy consisted of cyclophosphamide at a total dose of either 120 or 60 mg/kg, followed by 5 daily doses of fludarabine, 25 mg/m², and the dose of CAR-Ts used was 1x10⁶ cells/kg. Exogenous IL-2 was not administered in this study. Of the 7 evaluable patients with DLBCL, 4 achieved complete response (CR), 2 obtained PR, and 1 had stable disease (SD) after infusion of CAR-Ts. All 6 patients with indolent B-cell malignancies obtained either a PR or CR. Interestingly, this study also demonstrated that CAR-Ts were actually capable of infiltrating malignant lymph nodes and eradicating tumor cells. Anti-CD19 CAR-T gene detection with qPCR collected at multiple time points after CAR-T infusion determined that this level of CAR-positive blood cells varied considerably among patients and peaked between 7 and 17 days after infusion. In addition, CAR-T phenotype analysis demonstrated a decrease in the percentage

of CAR-Ts with a central memory phenotype and an increase in the percentage of CAR-Ts with either an effector memory phenotype or a CD8+ effector memory phenotype and with PD-1 expression.³⁰

A follow-up study from the NCI group in the DLBCL setting demonstrated that anti-CD19 CAR-Ts were capable of inducing CR in a high percentage of patients.³¹ Of the 7 evaluable patients, 5 achieved CR, 4 of whom had long-term durability with CR durations of 56, 51, 44, and 38 months, none of whom have experienced relapse at time of publication. Importantly, CR continued after recovery of nonmalignant polyclonal B cells in 3 of the 4 patients.³¹

Another trial from Baylor College of Medicine treated patients with R/R DLBCL and analyzed the safety and efficacy of both second- (1928z) and third-generation (1928BBz) CAR-Ts.³² Patients were preconditioned with cyclophosphamide (500 mg/m²/d) and fludarabine (30 mg/m²/d) for 3 days and were simultaneously infused with 1 to 20x10⁶ of both second- and third-generation CAR-Ts/m² 48 to 72 hours after lymphodepletion. Of the 5 patients evaluable for response, 2 experienced CR, 1 had continued CR after autologous stem cell transplantation, 1 had a PR, and 1 had disease progression.³² Collectively, all of these phase I clinical trials demonstrated the feasibility and safety of this therapy and its efficacy against NHL.

Reports from a Fred Hutchinson Cancer Research Center (FHCRC) study used a defined T-cell subset administration of 1:1 CD4+/CD8+ ratio of CAR-Ts for the treatment of NHL.³³ The 32 patients who proceeded to lymphodepletion chemotherapy and CD19 CAR-T infusion had previously received a median of 5 treatment regimens for de novo large B-cell lymphoma (LBCL; n=11), LBCL that had transformed from indolent disease (n=11), MCL (n=4), or FL (n=6); 16 patients had experienced relapse after autologous (n=14) or allogeneic (n=4) hematopoietic stem cell transplantation (HSCT).³³ Lymphodepletion with cyclophosphamide, cyclophosphamide/etoposide, or cyclophosphamide/fludarabine was administered before CAR-T infusion. Between 36 and 96 hours after completion of chemotherapy, CAR-Ts were infused at 1 of 3 cell dose levels (2x10⁵, 2x10⁶, or 2x10⁷ CAR-Ts/kg). Results obtained after therapy demonstrated that CD19-targeted CAR-Ts were capable of inducing CR in 2 of 11 patients (18%) with de novo aggressive B-cell lymphoma, 6 of 10 patients (60%) with transformed LBCL, 2 of 5 patients (40%) with FL, and

0 of 4 patients (0%) with MCL.³³ Interestingly, this study demonstrated that consistent with the increase in CAR-T expansion and persistence observed with cyclophosphamide/fludarabine lymphodepletion, the addition of fludarabine to the lymphodepletion regimen was associated with improvement in the depth of response. Intensification of lymphodepletion through addition of fludarabine to cyclophosphamide, as in our study, increased the peak of expansion and long-term persistence of infused CAR-Ts and improved the CR rate, overall survival, and progression-free survival.³³ An additional first-in-human trial was reported by the NCI group for the treatment of NHL using a fully human CAR-T as a means to avoid immunogenicity against the murine scFv of the chimeric receptor, and therefore enhance CAR-T expansion and persistence.³⁴ This novel humanized CD19-targeted CAR-T used a CD28 costimulatory domain and achieved an 86% response rate (n=9).³⁴

A study from University of Pennsylvania treated patients with double-hit NHL using (FMC63) 19BBz CAR-Ts.³⁵ Double-hit DLBCL (DHL) is defined by chromosomal breakpoints affecting the MYC/8q24 locus and BCL2/18q21 and/or BCL6/3q27 loci and arise either from transformation of FL (tFL) or de novo and have no standard effective therapy in the relapsed setting. This study treated R/R germinal center (GC) or nongerminal center (NGC) DLBCL, DHL, and tFL; 13 patients with DLBCL were enrolled and evaluable for response (7 GC, 5 NGC, 1 undetermined). Lymphodepleting chemotherapy regimens were bendamustine (90 mg/m² x 2; n=1); cyclophosphamide (1 g/m²; n=2); radiation and cyclophosphamide (4,000 cGy and 750 mg/m²; n=1); modified EPOCH (n=3); and hyperfractionated cyclophosphamide (300 mg/m² every 12 hours x 6; n=6). A total of 12 patients received 5.00E+08 (range, 5.10–6.75E+06 cells/kg) CAR-Ts; 1 patient received 1.93E+08 (3.10E+06 cells/kg). At 3 months post-CAR T-cell therapy, overall response rate (ORR) was 52% for all patients (7/13); ORR at 3 months was 71% (5/7) for GC and 40% (2/5) for NGC. The CR rate at 3 months was 38% (5/13); 43% (3/7) for GC; and 40% (2/5) for NGC. Best response for all patients is CR in 6 of 13 (46%); 57% (4/7) for GC; and 40% (2/5) for NGC. Of 7 patients with GC DLBCL, 3 had tFL and all 3 achieved a CR; 2 of 7 patients with GC DLBCL had DHL, and both achieved a CR. At the time of study publica-

tion, no patient who achieved a CR had experienced relapse.³⁵

Multicenter clinical trials recently demonstrated and confirmed the efficacy of anti-CD19 CAR-Ts against R/R NHL. The first trial (ZUMA-1) used the (FMC63) 1928z CAR-T and treated 101 patients with DLBCL, tFL, or PMBCL³⁶ using one single infusion of 2x10⁶ CAR-Ts/kg. The ORR was 82% (CR, 54%), and at 8.7 months median follow-up, 44% remained in response and 39% in CR.³⁶ Another phase II multicenter clinical trial (JULIET) used a (FMC63) 19BBz CAR-T for the treatment of DLBCL.³⁷ This global, pivotal study showed a 3-month ORR of 45% (23 of 51 patients), with 37% achieving a CR and 8% achieving a PR.³⁷ Another multicenter trial used the (FMC63) 19BBz CAR-T for the treatment of 14 patients with either DLBCL (n=13) or MCL (n=1). Two deaths were seen in the DLBCL arm due to disease progression. Results demonstrated an ORR of 82% (9/11) and a CR rate of 73% (8/11) for DLBCL and a CR rate of 0% (0/1) for MCL.³⁸

The results obtained from the phase I and II clinical trials in NHL are significantly better than those seen in the benchmark historical SCHOLAR-1 trial, with ORR and CR rates of 26% and 7%, respectively, obtained with standard therapies.³⁹

Other Targets Against NHL

The disappearance of CD19 from the surface of malignant lymphocytes, or mutations in this target antigen, is a well-documented escape phenomenon in patients refractory to CD19-targeted CAR-T therapy.^{3,40} Despite the frequent expression of CD19 in B-cell malignancies, it may be downregulated or mutated in tumor cells, allowing these cells to become resistant to CD19-directed therapy.^{40,41} According to recent estimates, epitope loss accounts for up to 40% of reported relapses.^{3,40,42,43} Alternative markers, such as CD20 and CD22, are also frequently expressed in B-NHL and B-ALL, and therefore could potentially be used as a target for adoptive T-cell therapies.^{44,45} A CAR-T that targets CD20 and uses CD137 (4-1BB) as a costimulatory domain (CAR.20-137z) was used in the clinical setting for the treatment of NHL in a Chinese PLA General Hospital trial,⁴⁶ which enrolled 7 patients with R/R DLBCL and preconditioned them with distinctive combinations of multiple different chemotherapy regimens (eto-

poside, vincristine, dexamethasone, cyclophosphamide, carboplatin, and high-dose cytosine arabinoside). Results showed that 1 of the 2 patients with no bulky tumor achieved a 14-month durable and ongoing CR by cell infusion only, and the other attained a 6-month tumor regression; 4 of 5 patients with bulky tumor burden were evaluable for clinical efficacy, in 3 of whom a 3- to 6-month tumor regression was observed.⁴⁶ In a follow-up early-phase IIa clinical trial of anti-CD20 CAR-Ts⁴⁷ that included 8 patients with DLBCL, 1 with MCL, 1 with FL, and 1 with PCMZL, 7 patients received cytoreductive chemotherapy, including cyclophosphamide for debulking and lymphocyte depletion, before the infusion, whereas the other 4 were not treated due to a smaller tumor burden and lower level of lymphocytes. The ORR was 81.8%, with 54.5% of the patients (6/11) achieving a CR and 27.3% (3/11) achieving a PR; the other 2 patients had SD.⁴⁷

A third-generation anti-CD20-specific CAR-T with both CD28 and 4-1BB domains was used in an FHCRC phase I trial for the treatment of indolent B-cell lymphomas and MCLs; 4 patients were enrolled, and 3 received T-cell infusions after cyclophosphamide lymphodepletion.⁴⁸ Treatment was well tolerated, although 1 patient developed transient infusion symptoms; 2 patients without evaluable disease remained progression-free for 12 and 24 months, respectively, and the third patient had an objective PR and experienced relapse at 12 months after CAR-T therapy.⁴⁸

A study from Baylor College of Medicine used CAR-Ts that target the κ light chain (κ .CAR) with the intention to treat a broad range of B-cell tumors (CLL, NHL, and MM) and to spare CD19+ endogenous B cells from CD19-targeted CAR-Ts.⁴⁹ Patients on chemotherapy at the time of treatment could receive κ .CAR no sooner than 4 days after finishing their last cycle of chemotherapy, and patients received no or limited lymphodepleting chemotherapy (12.5 mg/kg cyclophosphamide) 4 days before CAR-T therapy. Otherwise, no chemotherapy was given to patients before or after κ .CAR infusion. Three dose levels of κ .CARs were administered: 2×10^7 , 1×10^8 , and 2×10^8 cells/ m^2 . Among 4 patients with DLBCL treated with κ .CARs, 2 developed CR and 2 had no response.⁴⁹

CAR-Ts After HSCT for the Treatment of B-NHL

The use of CAR-Ts after autologous HSCT (autoHSCT) in the NHL setting was also conducted in 2 phase I dose escalation clinical trials. Investigators at Memorial Sloan Kettering Cancer Center used an anti-CD19 (SJ25c) 1928z CAR-T for the treatment of tFL, DLBCL, transformed SMZL, and Burkitt lymphoma.⁵⁰ Patients underwent BEAM (carmustine, etoposide, cytarabine, melphalan) conditioning before autoHSCT, and CAR-Ts were administered on days +2 and +3; 7 patients were treated at dose level of 5×10^6 1928z CAR-Ts/kg, with 1 dose-limiting toxicity reported involving prolonged grade III/IV cytopenia and 1 non-relapse mortality due to mucormycosis pneumonia. One patient treated at a dose level of 1×10^7 CAR-Ts/kg experienced a dose-limiting toxicity related to severe cytokine-release syndrome (sCRS) and fully recovered. All patients achieved neutrophil engraftment post-autoHSCT. Results showed that 5 of the 8 patients achieved CR (between 10 and 18 months of follow-up), 2 patients had disease progression, and there was 1 case of non-relapse mortality.⁵⁰

Another study from City of Hope investigated the combination of anti-CD19 CAR-Ts and autologous bone marrow transplant for the treatment of NHL and took a slightly different approach by using a subpopulation of central memory T cells (T_{CM}) CAR-Ts.⁵¹ Previous preclinical studies have demonstrated the enhanced capacity of T_{CM} cells to persist after adoptive transfer and to repopulate functional memory niches, as well as their self-renewal capacity and multipotency.^{52–54} The City of Hope trial used either the first-generation 19z (cohort 1) or second-generation 1928z (cohort 2) CAR-Ts for the treatment of DLBCL or MCL. The HSCT conditioning regimen was not specified; however, all patients received bis-chloroethylnitrosourea, etoposide, cytarabine, and melphalan, and CD19 CAR-Ts were infused 2 to 3 days after stem cell infusion.⁵¹ The CAR-T dose ranged from 25×10^6 to 100×10^6 CAR/injection (cohort 1) and 50×10^6 to 200×10^6 CAR/injection (cohort 2). In cohort 1, 4 of 8 patients were progression-free at both 1 and 2 years; and in cohort 2, 6 of 8 patients were progression free at 1 year. The T_{CM} -derived anti-CD19 1928z CAR-Ts exhibited improvement in expansion; however, persistence was around 28 days, similar to that seen by others using other anti-CD19 second-generation CAR-Ts.

CAR-Ts Against HL and T-Cell Lymphomas

Despite the relatively high rates of success with the current therapies against HL, 10% to 15% of patients with localized disease and 20% to 40% of those with advanced-stage disease will experience relapse,^{55,56} and an additional 10% to 15% are refractory to first-line therapy.⁵⁷ Thus, alternative therapeutic strategies are required to treat patients with resistant/relapsed disease, and to reduce the morbidity attributable to chemotherapy/radiotherapy. HL tumor cells have a very low expression of CD19 antigen and therefore are not eligible for anti-CD19 therapy with CAR-Ts.²³ However, despite the lack of CD19 expression, almost all HRS cells overexpress the CD123 and CD30 surface molecules, which could potentially be used as a target for adoptive T-cell therapy.²⁴

Finding an ideal target in the T-cell lymphoma setting poses a much more challenging task due to shared expression of most targetable surface antigens between normal and malignant T cells, potentially leading to fratricide of CAR-Ts or profound T cell–related immunodeficiency. Therefore, options for targeted therapy of T-cell malignancies remain scarce. In the preclinical setting, CD5-targeted CAR-Ts have been shown to be capable of specifically recognizing and killing malignant T-cell lines and primary T-cell ALL (T-ALL) blasts. Although expansion of CD5 CAR-Ts was preceded by transient fratricide, the extent of self-killing was limited. Interestingly, anti-CD5 CAR-Ts were capable of expanding in vitro, eradicating CD5+ malignant T cells and efficiently enhancing survival in xenograft mouse models.²² More recently, a novel anti-CD7 CAR-T was developed; CD7 is a transmembrane protein highly expressed in T-ALL and in a subset of peripheral T-cell lymphomas. Normal expression of CD7 is largely confined to T and NK cells, reducing the risk of off-target-organ toxicity. However, CAR-Ts were shown to display high expression of CD7 on their surface that led to massive fratricide. To overcome this limitation, CD7-targeted CAR-Ts modified with genomic editing to disrupt CD7 expression (CD7KO anti-CD7 CAR-T) demonstrated robust expansion and antitumor effect in the preclinical setting, with minimal fratricide.⁵⁸

Preclinical studies have validated the potential benefits of using anti-CD30 or anti-CD123 CAR-Ts for the treatment of HL.^{59,60} Recently, a clinical

trial using a CD30-targeted second-generation CAR-T (CD30-CAR-T) was conducted in patients with R/R EBV-negative, CD30+ HL or T-cell NHL (ie, anaplastic large cell lymphoma).⁶¹ In this trial, 9 patients (HL, n=7; NHL, n=2) received CD30-CAR-Ts; 2 were treated on dose level 1 (2×10^7 CD30-CAR-Ts/m²), 2 patients on dose level 2 (1×10^8), and 5 patients on dose level 3 (2×10^8). None of the patients received any conditioning regimen before CAR-T infusion. At 6 weeks follow-up, 1 patient presented with a CR, 1 achieved a very good PR, and 4 had SD, whereas 3 patients had disease progression.⁶¹

Side Effects

Toxicity rates have varied significantly across studies, likely reflecting the differences in various factors, such as type of scFv, costimulatory molecule, vector, preconditioning regimen, disease burden, and CAR-T dose. In addition, different groups use distinctive criteria to define sCRS, which makes the comparison confusing. Overall, the side effects encountered with CAR-T therapy for NHL are very similar to those encountered in the B-ALL setting.^{1,3,4} The most common CAR-T–related side effects observed in the above-mentioned clinical trials were hypotension, hypoxia, acute renal failure, and neurotoxicity (confusion, aphasia, seizure/seizure-like events) (Table 1).

Conclusions

In the past 10 years, the field of cancer immunotherapy has seen remarkable advances, and the significant benefits observed with the use of CAR-Ts has brought new hope for the treatment of diseases once considered incurable. The initial success using CD19-targeted CAR-Ts against B-ALL has not only paved the way for the use of this treatment in a much broader spectrum of malignancies, but has also provided vital evidence toward understanding the side effects associated with this novel therapy. In the past few years, a significant number of clinical trials have shown unprecedented success in the treatment of NHL using T cells, mostly with CD19 as a target. The comparison between different costimulatory domains (CD28 vs 4-1BB) remains unanswered. Based on the results presented herein, CAR-Ts using either CD28 or 4-1BB costimulatory

Role of CAR T Cells in NHL

Table 1. Most Significant Side Effects Observed

Year	Population	scFv/ Costimulatory Domain	Vector	Preconditioning Chemotherapy	CAR T Cell Dose	CRS/Neurotoxicity
2012 ²⁹	Adults: B-NHL or CLL: 4 CLL 3 FL 1 SMZL	FMC63/CD28	RV	Flu: 25 mg/m ² x 5 Cy: 60 mg x 2	3x10 ⁶ to 3x10 ⁷ /kg	Gr >3 hypotension: n=2 Gr >3 hypoxemia: n=2 Gr >3 acute renal failure: n=3
2016 ³⁵	B-NHL (n=27): 13 DLBCL 14 FL	FMC63/4-1BB	LV	Investigator's choice	<u>DLBCL</u> 3.1x10 ⁶ to 6.75x10 ⁶ /kg <u>FL</u> 3x10 ⁶ to 8.8x10 ⁶ /kg	<u>DLBCL</u> Gr >3 CRS: n=1 Gr >3 neurotoxicity: n=1 <u>FL</u> Gr >3 CRS: n=2 Gr 5 encephalitis: n=1
2017 ³⁶	Adults: B-NHL (n=111): DLBCL PMBCL FL	FMC63/CD28	RV	Flu: 30 mg/m ² x 3 Cy: 500 mg/m ² x 3	2x10 ⁶ /kg	Gr >3 encephalopathy: 24% Gr >3 CRS: 20% Gr >3 neurotoxicity: 29% Gr 5 hemophagocytic lymphohistiocytosis: n=1
2016 ³²	B-NHL: 6 DLBCL	FMC63/CD28	RV	Flu: 30 mg/m ² x 3 Cy: 500 mg/m ² x 3	1 to 20x10 ⁶ /m ²	Gr 2 CRS: n=2
2016 ³³	B-NHL (n=32): 21 DLBCL 5 FL 4 MCL	FMC63/4-1BB	LV	Cy: 2–4 g/m ² (d1) Cy/Eto: Cy, 2–4 g/m ² (d1) and Eto, 100–200 mg/m ² (d1–3) Cy/Flu: Cy, 60 mg/kg (d1) and Flu, 25 mg/m ² per either d2–4 or d2–6	2x10 ⁵ to 2x10 ⁷ /kg (defined 1:1 CD4+/ CD8+ ratio)	sCRS: 4/32 Gr >3 neurotoxicity: 9/32
2017 ³⁷	B-NHL (n=51): DLBCL	FMC63/4-1BB	LV	Not available	0.1 to 6.0x10 ⁸ cells/ infusion	Any gr CRS: 57% Gr 3/4 CRS: 26% Gr 3/4 neurotoxicity: 13%

Abbreviations: B-NHL, B-cell non-Hodgkin's lymphoma; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CRS, cytokine-release syndrome; Cy, cyclophosphamide; DLBCL, diffuse large B-cell lymphoma; Eto, etoposide; FL, follicular lymphoma; Flu, fludarabine; Gr, grade; LV, lentivirus; MCL, mantle cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; RV, retrovirus; scFv, single-chain variable fragment; sCRS, severe CRS (requiring intensive care unit management and treatment with tocilizumab and/or corticosteroids); SMZL, splenic marginal zone lymphoma.

domains have demonstrated similar clinical benefits and side effect patterns, and therefore which of the costimulatory domains is safer or displays better clinical outcomes is unknown. Another conclusion that can be drawn from the NHL trials is the absolute need for preconditioning chemotherapy, especially combination cyclophosphamide and fludarabine, to enable optimal function of the CAR-Ts—confirming what was previously observed in B-ALL trials. However, the outcomes and side effects observed with the 1:1 CD4+/CD8+ ratio of infused CAR-Ts was similar to those observed with unselected products. More results are needed before any conclusion can be drawn.

The clinical results observed with the use of CAR-Ts represent the beginning of this exciting and extremely promising new technology. New generations of CAR-Ts currently under development have demonstrated exciting preclinical results.^{11,62} Further, the combination of CAR-Ts and other drugs, such as checkpoint blockade inhibitors, could potentially significantly enhance the CAR-T antitumor therapeutic effect.^{63–65}

In summary, adoptive T-cell therapy holds great potential to revolutionize cancer treatment, and with the advent of next-generation CAR-Ts, this therapy will likely be applied to a much broader spectrum of hematologic and solid malignancies.

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