Review Article

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Chimeric Antigen Receptor T Cell Therapy in Hematology

Hematolojik Malignitelerde Kimerik Antijen Reseptör-T Hücre Tedavisi

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Abstract:

It is well demonstrated that the immune system can control and eliminate cancer cells. Immune-mediated elimination of tumor cells has been discovered and is the basis of both cancer vaccines and cellular therapies including hematopoietic stem cell transplantation. Adoptive T cell transfer has been improved to be more specific and potent and to cause less off-target toxicity. Currently, there are two forms of engineered T cells being tested in clinical trials: T cell receptor (TCR) and chimeric antigen receptor (CAR) modified T cells. On 1 July 2014, the United States Food and Drug Administration granted 'breakthrough therapy' designation to anti-CD19 CAR T cell therapy. Many studies were conducted to evaluate the benefits of this exciting and potent new treatment modality. This review summarizes the history of adoptive immunotherapy, adoptive immunotherapy using CARs, the CAR manufacturing process, preclinical and clinical studies, and the effectiveness and drawbacks of this strategy.

Keywords: **Chimeric antigen receptor T cell, Hematological malignancies**

Öz:

İmmün sistemin kanser hücrelerini kontrol ve elimine etme özelliğine sahip olduğu gösterilmiştir. İmmün-kontrollü eliminasyonda kanser aşıları ve hematopoietik kök hücre naklini içeren sellüler terapiler bulunmaktadır. Adoptif T hücre transferi daha potent ve spesifiktir, hedef dışı toksisitesi azdır. Klinik çalışmalarda iki tür T hücresi test edilmektedir: T hücre reseptör ve kimerik antijen reseptör (KAR) modifiye T hücreleri. 1 Temmuz 2014'te Amerikan Gıda ve İlaç Dairesi anti-CD19 ŞAR modifiye T hücre tedavisini "çığır açan tedaviler" sınıfına almıştır. Bu yeni tedavi yöntemini ve etkilerini araştıran birçok çalışma yapılmıştır. Bu derleme adoptif immünoterapinin geçmişini, ŞAR modifiye T hücrelerini, üretim sürecini, klinik ve preklinik çalışmaları özetlemektedir.

Anahtar Sözcükler: **Kimerik antijen reseptör-T hücreleri, Hematolojik maligniteler**

Introduction

Poor salvage chemotherapy success rates for refractory hematological diseases have necessitated novel approaches. Adoptive T-cell transfer has gained significant interest and clinical usage in hematology because of the off target effects of allogeneic stem cell transplantation and life threatening graft versus host disease (GVHD). Therefore, research efforts have sought to generate more specific T cells with higher toxicity to tumors and not healthy targets. To achieve curative potential, T cell immunotherapy combines potency, specificity and persistence [1]. Early approaches to adoptive T cell immunotherapy were based on the graft-versus-leukemia (GVL) effect mediated by donor lymphocyte infusion (DLI) hematopoietic stem cell transplantation (HSCT) and the therapeutic infusion of ex vivo expanded tumor-infiltrating

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lymphocytes (TILs) in combination with lymphodepletion for the treatment of advanced melanoma. However, DLI is usually associated with life-threatening forms of GVHD, and TILs require time-consuming procedures with unsuccessful results [2,3]. To overcome these drawbacks, genetically modified effector T cells have been developed as an alternative approach. In hematological malignancies, engineered T cell receptors (TCRs) and chimeric antigen receptors (CARs) are new powerful T-cell based immune therapies that target specific antigens. CAR T cells have been used successfully in the treatment of solid and hematological malignancies recently. In the following sections, the history of adoptive immunotherapy, TCR gene therapy, CART cell production, and preclinical and clinical studies will be discussed.

The Role of T Cells in Cancer and T Cell Receptor Gene Therapy

In 1909, Paul Ehrlich first proposed that the immune defense system identifies and eliminates tumor cells [4]. However, recent studies revealed that the immune response may be ineffective against tumor development due to immunological tolerance and anergy [5]. Cancer immunoediting consists of three stages: elimination, equilibrium and escape. In the elimination stage, cancer is eliminated by intact innate and adaptive immunity, whereas in the equilibrium stage, variant tumor cells that develop genetic instability survive despite the immune attacks. Uncontrolled proliferation of variant tumor cells occurs in the escape stage [6].

In 1890, William B Coley observed that patients with malignancies respond to the intratumoral inoculation of live bacterial organisms or bacterial toxins that cause tumors to express unique proteins that could trigger an immune response [7]. Since the beginning of the 20th century, research has shown that most cancer cells carry overexpressed tumor-associated or tumor-specific antigens that are not present on healthy cells; this feature has led to the successful application of adoptive T-cell transfer. The discovery of T-cell growth factor, in vitro T-cell culture and the role of lymphodepletion have led to T-cell based therapy studies [8]. The first successful study on T-cell transfer immunotherapy using autologous TILs was performed in advanced melanoma in 1990 [9]. Since tumor infiltrating lymphocyte isolation was first attempted, in vitro expansion and re-infusion have been shown to be time-consuming and produce transient anti-tumor effects, and genetic engineering methods have been applied to create specific T cell-generated TCRs.

The TCR is a heterodimer that carries information for defined tumor antigens and is formed by alpha and beta chains associated with a CD3 complex (Figure 1) [10]. TCR technology has advantages as a redirected T-cell therapy. Ideal effector T cells match with selected tumor target antigens through HLA recognition. The natural mechanism of T-cell immunity is associated with a low risk of cytokine release syndrome. The major difficulties that need to be overcome are the low surface expression of TCRs, HLA dependency, the and short persistence of transferred T-cells in vivo [11]. In thymic selection during the development of T cells, a few mutated proteins are encoded by cancer-causing genetic mutations (driver mutations), the large proportion of tumor antigens are self antigens, and T cells have low affinity for self antigens [12]. To create a higher avidity, selected TCRs from immunized human HLA transgenic mice with relevant epitopes are used along with insertion of targeted mutations in the complementary-determining region 2 or 3 (CDR2 or 3) in the variable regions of the TCR alpha/beta chains. These modified TCRs interact with the HLA/epitope complex [13]. However, TCRs can create unwanted alpha/beta heterodimers between the new and endogenous TCR alpha/beta chains in a process called mispairing, which results in low avidity [14]. TCR-modified T cells adapted for solid tumors have not been successful in most studies (Table 1) [10].

Chimeric Antigen Receptors

The genetic modification of T cells with CARs represents a breakthrough for gene engineering in hematological malignancies. The first CAR concept originated from the cloning of the TCR CD3 ζ-chain that was found to activate T cells independently [18]. First-generation CARs included only a single-chain variable fragment (scFv) that was constructed from the variable heavy and variable light sequences of a monoclonal antibody (mAb) specific for a tumor cell surface molecule and the cytoplasmic CD3 ζ-chain signaling domain. The initial studies were conducted in patients with HIV infection with prolonged survival [19]. In the first-generation

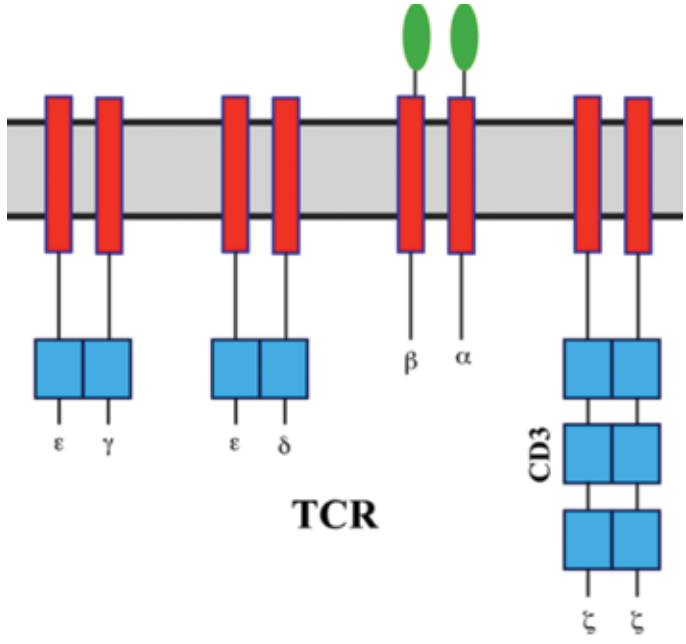


Figure 1. T cell receptor (adapted from Wieczorek and Uharek [10]).

cancer studies, CAR T cells did not proliferate in vivo and persistence was transient or the T cells were present at very low frequencies [20]. Based on a second genetic modification, CARs possess an antibody-based extracellular receptor structure that binds to target cells along with intracellular activating domains. Costimulatory protein receptors (e.g., CD28, CD137 (4-1BB), ICOS, CD134 (OX40), CD27, or CD244) were added to the cytoplasmic tail of the CAR in the second- and third-generation CARs [21] (Figure 2). Second-generation CARs are constructed with one costimulatory molecule while third-generation CARs contain more than one additional costimulatory molecule. The antitumor effect of CAR-T cells varies due to differences in the cytoplasmic domain and the extracellular domain's ability to recognize a different epitope of the same antigen with different affinities for each CAR construct [22]. Whether the addition of secondary costimulation as in third-generation CARs obtains more efficacy is still an unanswered question [23]. CARs have several advantages: initiation of reliable high-potency signals, HLA independency, no requirement for antigen processing, and no competition for CD3. The number of target molecules on tumor cells that bind to CARs is greater than the number of major histocompatibility complex (MHC)/peptide complexes, and the scFv has a higher binding affinity for antigens than the TCRs [24]. Recently, Oren et al. compared the functional properties of engineered T cells expressing native low-affinity αβ-TCR chains with high-affinity TCR-like Ab-based CARs targeting the same specificity and suggested that the upper affinity threshold should be used to mediate effective functional outcomes of engineered T cells [25]. The major disadvantage of CARs is the massive cytokine release induced by binding and the immunogenicity of the mouse-derived scFv portion of the CAR complex, which may result in immune responses and the clearance of CAR T cells. In addition to that, intracellular molecules cannot be recognized [26].

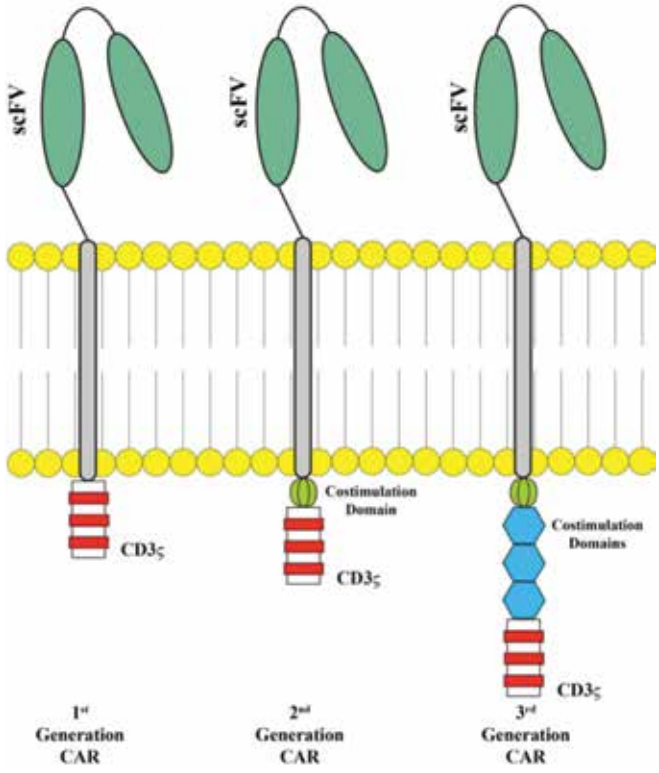


Figure 2. Generations of CART cells (adapted from Porter et al. [55]).

Table 1. T cell receptor clinical studies.

Antigen	Tumor	Effectiveness	Toxicities	Reference
MART 1	Melanoma	6 PRs in 20 patients	Erythematous skin rash grade 1-2 (14/20), hearing loss (10/20), uveitis (11/20)	2009, [15]
gp100	Melanoma	3 PRs in 16 patients	Erythematous skin rash grade 1-2 (15/16), hearing loss (5/16), uveitis (4/16)	2009, [15]
MAGE-A3	Melanoma multiple myeloma	Not evaluable	Acute cardiac failure (2/2), cytokine release syndrome, death (2/2)	2013, [16]
MAGE-A3/A9/A12	Melanoma, synovial sarcoma, esophageal cancer	4 PRs, 1 CR in 9 patients	Neurological toxicity (4/9), death (2/9)	2012, [17]

CR: Complete remission, PR: partial remission.

Chimeric Antigen Receptor T Cell Manufacturing

Gene transfer technology has rapidly developed; however, the clinical production of CARs for therapy is restricted to specialized, licensed manufacturing facilities with stringent rules (Good Manufacturing Process). In vitro culture systems for T cell expansion are used to manufacture large quantities of engineered T cells. The average production time to generate large numbers of unselected CD4 and CD8 T cells required for therapy is 10-14 days in clean rooms. First, peripheral blood mononuclear cells are isolated from the patient using leukapheresis, and T cells are selected by anti-CD3/anti-CD28 paramagnetic beads. Recent studies have demonstrated that less differentiated T cells have superior engraftment and antitumor activity [27]. In particular, CD8 T central memory cells can be modified with tumor-specific CARs [28]. T

Table 2. Chimeric antigen receptor T cell trials in hematological malignancies [55].

scFv/Signaling Domain	Vector	Dose	Number of Patients	Responses	Reference
CD20/CD3	Electroporation	$1x10^8/m^2$ to $3.3x10^9/m^2$	7 (indolent and MCL)	2 CR, 1 PR, 4 SD	2008 [46]
CD20 or CD19/CD3	Electroporation	$10^8/m^2$ to $2x10^9/m^2$	4 (2 FL, 2 DLBCL)	2 CR after autologous stem cell transplantation	2010 [52]
CD19/CD3 and CD28-CD3	Gammaretrovirus	$2x10^7/m^2$ to $2x10^9/m^2$	6 NHL	2 SD	2011 [53]
CD19/CD28 and CD3	Gammaretrovirus	$0.4\text{-}3.3x10^7$ CAR cells/kg	8 CLL and 1 ALL	1 death, (ALL) B cell aplasia, 1 reduction in lymphadenopathy	2011 [54]
CD19/4-1BB and CD3	Lentivirus	$1.46x10^5$ to $1.6x10^7$ CAR cells/kg	3 CLL	2 CR, 1 PR, 3 B cell aplasia	2011 [55]
CD19/CD28 and CD3	Gammaretrovirus	$0.3\text{-}3x10^7$ CAR cells/kg	8 (3 FL, 4 CLL, 1 MZL)	6 objective remissions (4 B cell aplasia)	2010 [56]
CD20/CD28 and 4-1BB and CD3	Electroporation	$1x10^8$ to $3.3x10^9/m^2$	3 (2 MZL, 1 FL)	No progression in 2 patients, 1 patient PR	2012 [57]
CD19/CD28 and CD3	Gammaretrovirus	$1.5\text{-}3x10^6$ CAR cells/kg	5 ALL	All 5 converted to MRD, 1 relapsed, 1 B cell aplasia	2013 [58]
CD19/4-1BB and CD3	Lentivirus	$1.4x10^6$ and $1.2x10^7$ CAR cells/kg	2 ALL	2 CR, both B cell aplasia	2013 [59]
Lewis Y/CD28 and CD3	Gammaretrovirus	$1.4\text{-}9.2x10^6$ CAR cells/kg	4 AML	2 SD, 1 transient cytogenetic remission	2013 [48]
CD19/CD28 and CD3	Gammaretrovirus	$1.5x10^7$ to $1.2x10^8$ total T cells/m^2	8 ALL	4 of 8 patients with decreased B cell counts	2013 [60]
CD19/CD28 and CD3	Gammaretrovirus	$0.4\text{-}7.8x10^6$ CAR cells/kg	4 CLL, 2 DLBCL, 4 MCL	2 PD, 6 SD, 1 PR, 1 CR	2013 [61]

AML: Acute myeloid leukemia, ALL: acute lymphoblastic leukemia, CLL: chronic lymphocytic lymphoma, CR: complete remission, DLBCL: diffuse large B cell lymphoma, FL: follicular lymphoma, MCL: mantle cell lymphoma, MRD: minimal residual disease, MZL: marginal zone lymphoma, NHL: non-Hodgkin lymphoma, PD: progressive disease, PR: partial remission, SD: stable disease.

cells are then transduced with a CAR-encoding viral vector. Two vector systems, retroviral or lentiviral vectors, can be used to transfer CAR-coding genes into T cells. Retroviral vectors have permanent gene expression; however, the transduction can be performed only on efficiently dividing T cells. Lentiviral vectors can also integrate into nondividing cells. The disadvantages of viral vectors are the expense and experience required for production. Transposon systems such as Sleeping Beauty 100X (SB100X) or PiggyBac (PB) are new methods for genetic modification of T cells with high gene expression; they are simple and inexpensive and have large cargo capacity and low immunogenicity [10]. T cells are expanded in culture by stimulating them using the anti-CD3 clone OKT3 with cytokines like IL-2, IL-7, and IL-15. Moreover, in vivo persistence can be achieved by the overexpression of antiapoptotic proteins such as Bcl-2 or Bcl-xL. An adequate number of CAR T cells, which remains unknown, are then transferred to the patient using host preparative lymphodepletion regimens based on drugs and techniques to deplete Tregs, such as cyclophosphamide, fludarabine, low-dose irradiation, gemcitabine, denileukin diftitox, azacitidine, or decitabine [10,29,30]. CARs on T cells bind to their antigen on the tumor, and activation is controlled by the intracytoplasmic domains within the CAR. Tumor killing can be mediated by the direct cytotoxicity of the $CD8^+$ CAR T cells with granzyme and perforin or cytokines released by $CD4^+$ CAR T cells that bypass the MHC. Long-term eradication and prevention can be achieved by memory CAR T cells from a single infusion [31].

Studies Involving Chimeric Antigen Receptor T Therapy

TThe ideal targets for CAR-modified T cells are expressed on tumor cells but are not expressed on normal cells. CD19 and CD20 are attractive targets due to their specificity for the B cell linage [32]. The first-generation CARs were not sufficient to produce a durable immune response; they rapidly underwent apoptosis after stimulation [33]. 19z CAR T cells were expanded on CD19+CD80+IL15+ cells and eradicated established systemic Raji tumors in 50% of SCID-beige mice [34]. Second-generation CARs that express CD28-containing costimulation in the CD19+CD80/CD86-ALL SCID-beige tumor model showed superior in vivo tumor activity and T cell function. CD22 is also under investigation and shows potential [35]. Imai et al. showed that in vivo anti-CD19 chimeric receptors containing the 4-1BB signal transduction domain had powerful antileukemic activity, destroying CD19+ acute lymphoblastic leukemia (ALL) cell lines in an in vivo microenvironment [33]. Target discovery for T cell leukemias and myeloid leukemias is problematic because blasts express the same antigens as normal hematopoietic stem cells [36]. For myeloid leukemias, CARs directed against CD123 have demonstrated efficacy in preclinical models; however, vascular endothelial cells also express CD123, which requires more investigation before clinical application [37]. Kenderian et al. stated that anti-CD33-specific CAR T cells exhibited significant effector functions in vitro and resulted in eradication of leukemia and prolonged survival in acute myeloid leukemia (AML) xenografts [38]. In multiple myeloma, CAR-engineered natural killer cells that targeted CS-1 protein displayed enhanced cytolysis in vitro [39].

The translation of this therapy to clinical settings involves various antigens and malignancies, and most trials have focused on B cell malignancies with B cell antigens CD19 and CD20 as the targets [40]. The first case report of CD19+ CAR T cells was published in 2011 by Porter et al. in relapsed refractory chronic lymphoid leukemia [41]. In that study, 3x108 T cells were transduced using a lentiviral vector, and the patient exhibited complete remission after 10 months. The largest dose-optimization trial involved 27 chronic lymphocytic leukemia (CLL) patients and found no difference between two doses of CAR T cells ($<5x10^7$ versus $>5x10^7$) with a complete response rate of 40% of patients [42]. In another study, CAR-modified T cells were shown to persist for more than 3 years with an initial response rate of 57% and complete remission of 29%, which was more favorable as compared to ibrutinib (an overall response rate of 71% but a complete remission rate of 2.4%) [43]. In B cell ALL (B-ALL), Davila et al. reported on 16 relapsed or refractory cases that were treated with 19-28z-expressing CAR T cells with an overall complete response rate of 88%, as compared to 44% with salvage chemotherapy. CAR T cells persisted for 2-3 months, and almost half of the patients proceeded to allogeneic stem cell transplantation [44]. In 30 ALL patients treated with CD19 CAR T cells, a 6-month event-free survival of 67% and overall survival of 78% were achieved, and ongoing remission for up to 2 years was possible without transplantation [45]. The underlying causes of the limited clinical efficacy of the CAR T cells in patients with CLL compared to B-ALL include the limited persistence of CAR T cells in CLL patients, the inhibitory effect of the tumor microenvironment in CLL, the lymph node-based nature of CLL, and the lower tumor burden at treatment in patients with B-ALL [40].

Patients with B cell malignancy were first treated with modified autologous CD20-specific T cells in 2008 by investigators from the Fred Hutchinson Cancer Research Center and the City of Hope National Medical Center. T cells persisted for up to 9 weeks with 7 patients with indolent or mantle cell lymphoma achieving partial response (1 patient), stable disease (4 patients), or complete response (2 patients) [46]. In 2014, an anti-CD19 chimeric antigen receptor trial for chemotherapy-refractory diffuse large B cell lymphoma and indolent B cell malignancies was published

by Kochenderfer et al.; they demonstrated that 8 of 15 patients had complete response with 1-5x106 CAR T cells transduced by gammaretrovirus [47]. The targets for CAR therapy in multiple myeloma can be CD138, CD38, CD56, and CS1. Unlike CD19, these targets are coexpressed on other important cell types and result in unacceptable on-target, off-tumor toxicity. The first AML trial targeted the LeY antigen, and only 1 of 4 patients had 23 months of stable disease following therapy [48]. Contrary to preclinical studies, the CD33 antigen as a target was not proven to be safe due to the high level of toxicity against normal hematopoietic cells [49]. Phase I clinical trials involving CD123 targeting by mAbs and immunotoxins have produced only minor clinical responses, suggesting the need to develop more powerful AML strategies [50]. Table 2 shows the CAR T cell therapies in hematological malignancies [51].

Adverse Effects of Chimeric Antigen Receptor T Cell Therapy

AAs with all therapies, the toxicity from CAR T cells may be classified as on-target or off-target. The most common toxicity is cytokine release syndrome (CRS). In most cases, CRS is correlated with antitumor activity, and patients exhibit a range of symptoms from high fever, hypoxia, and hypotension to mild flu symptoms. The increased cytokines, particularly IL-6 and TNF-α, are produced by dying B cells, tumor cells, or macrophages [51]. Grupp et al. reported that the IL-6 receptor-blocking monoclonal antibody tocilizumab may ameliorate CRS in steroid-refractory circumstances without compromising T cell efficacy [59]. CRS was reported to occur in 6/13 patients with high complete response rates with tocilizumab as an alternative treatment option. The C-reactive protein level has been shown to be an indicator of severe CRS [45]. Another off-target adverse effect is tumor lysis syndrome, which is due to rapid and massive destruction of tumor cells. Macrophage activation syndrome is another life-threatening off-target effect of systemic inflammatory symptoms and pancytopenia, although the mechanisms are still unknown [42]. Several patients in CD19-CAR trials experienced reversible obtundation, seizures, aphasia, and mental status changes, possibly due to systemic cytokines crossing the blood-brain barrier [51]. B cell aplasia is an expected result of CD19-directed therapies and can be managed by γ-globulin replacement therapy. Persistent B cell aplasia results in an increased risk of infections [52].

Future Directions

AAdoptive T cell transfer has been used for the treatment of malignant diseases and may be regarded as an anticancer biopharmaceutical. A biopharmaceutical is defined as a product that is originally natural or derived from biological sources with industrial additions [62]. The main goals of T cell engineering are tumor antigen targeting and an increase in antitumor functions [1]. CAR T cell therapies are powerful breakthrough therapies, but several challenges need to be addressed. The optimal design of CARs remains an area of investigation. To be useful in other disease types, tumor-specific targets must be identified in solid tumors. T cell trafficking to the tumor microenvironment is critical in the moderate success against solid cancers [63]. To minimize severe toxicity, standardized approaches to the management of CRS should be applied [64]. B cell aplasia is still a problem with long-term exposure and may have an economic impact on health care. Once the B cell malignancy has been eradicated, anti-CD19-CAR T cells should be ablated to maintain normal B cell activity. A suicide system has been developed to eliminate gene-modified T cells when they display unwanted toxicities, such as the thymidine kinase gene of the herpes simplex virus [65]. Relapse remains a challenge and may be prevented with optimization of CAR design. Finally, in order for the therapy to become routinely used, automation and robotic culture technologies should be performed during the manufacturing process instead of manual cell culture technologies [66].

The induction of adoptive immunotherapy using CAR T cells has been successful in clinical trials, and the final goal is to induce durable immunity against disease progression without severe adverse effects. Whether this treatment option will replace HSCT or be used as a bridge to HSCT in the near future is still an unanswered question.

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