Additional possibilities of chimeric antigen receptor T-cells in B-cell lymphoma: combination therapy

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Abstract: B-cell non-Hodgkin's lymphoma (B-NHL) is a lymphoproliferative disorder that affects B lymphocytes. Chimeric antigen receptor (CAR) T-cell immunotherapy is a new type of immunotherapy that uses genetic engineering techniques to modify and expand the patient's autoimmune cells *in vitro*, after which these cells are reinfused into the patient. CAR-T cell immunotherapy has the potential to treat different types of B-cell lymphoma. Many clinical studies have shown that CAR-T cell therapy has significant antitumor effects on B-cell lymphoma. Although much work has been carried out to improve the efficacy of CAR-T cell therapy and to reduce associated side effects, there are still many issues to address. CAR-T cell therapy shows significant promise in treating B-NHL, but some patients still have a poor initial response to this therapy where the infused CAR-T cells show insufficient persistence. With the rapid development of immunological therapy, combination therapy has been certified to improve the efficacy of CAR-T cell therapy. Targeted drugs such as programmed death-1 (PD-1) inhibitors, programmed cell death-ligand 1 (PD-L1) inhibitors, and Bruton's tyrosine kinase (BTK) inhibitors may further enhance the efficacy and reduce the side effects of CAR-T cell treatment. This article reviews the rationale and relevant clinical research on combination therapy based on CAR-T cell therapy for B-cell lymphoma treatment.

Keywords: CAR-T; immunotherapy; B-cell lymphoma; combination therapy

Introduction

Chimeric antigen receptor (CAR) T-cell immunotherapy is a new type of immunotherapy that uses genetic engineering techniques to modify and expand the patient's autoimmune cells *in vitro*, after which these cells are reinfused into the patient (1). Compared to traditional immunotherapy, CAR-T cell immunotherapy has many advantages, such as stronger targeting, a wider range of actions, longer-lasting effects, and stronger maneuverability (2,3). It can recognize relevant antigens and act on tumor cells, killing and clearing

them (4). CAR-T cell therapy has produced encouraging results in the treatment of hematological diseases (5). Many studies have confirmed that CAR-T cell therapy shows strong antitumor effects in different types of B-cell lymphomas.

B-cell non-Hodgkin's lymphoma (NHL) is a lymphoproliferative disorder that affects B lymphocytes. The most common subtypes of B-cell NHL (B-NHL) are diffuse large B-cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma, follicular lymphoma (FL), marginal zone lymphoma, and mantle cell lymphoma (MCL). The treatment provided to

B-NHL patients is currently undergoing a revolutionary shift. Twenty years ago, rituximab revolutionized the treatment of CD20 [+] B-cell hematological malignances, and the need for conventional chemotherapy continues to decline as more immunotherapeutic approaches become available. The addition of rituximab to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) increased the percentage of cases with complete remission (CR) from 63% to 76%, 2-year event-free survival (EFS) from 38% to 57%, and overall survival (OS) from 57% to 70% (6). At present, R-CHOP (CHOP plus rituximab) is still the standard first-line treatment for many types of B-NHL: 50% to 60% of patients treated with R-CHOP achieve clinical cure. However, 30% to 40% of patients will relapse. Patients with relapsed/refractory disease are treated with second-line salvage chemotherapy. Those who respond then receive consolidation high-dose chemotherapy with autologous stem cell transplant (HDC/ASCT). Patients unsuitable for HDC/ASCT may receive subsequent lines of chemotherapy. Additionally, the introduction of targeted drugs, such as programmed death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) inhibitors, Bruton's tyrosine kinase (BTK) inhibitors, and histone deacetylases (HDACs) inhibitors, has shown exciting results in relapsed and refractory B-cell lymphoma patients (7).

Although CAR-T cell therapy shows significant promise in treating B-NHL, some patients still have a poor initial response to this therapy where the infused CAR-T cells show insufficient persistence. Multiple mechanisms may lead to CAR-T cell failure, including tumor microenvironment (TME) immunosuppression and tumor-intrinsic properties associated with antigen or inhibitory ligand expression, as well as the attributes of adoptively transferred T-cells. When CAR-T cells are infused into patients, these cells often encounter inhibitory TMEs. CAR-T cells and inhibitory ligands can bind to inhibitory receptors on T-cells and hinder the antitumor response of these cells. Some studies suggest this is mediated by the patient's immune system responding to self-reengineered T-cells through checkpoint pathway activity, resulting in loss of function or physical loss of these cells. The efficacy of CAR-T cell therapy is related to the proliferative capacity of T-cells *in vivo* and the persistence of genetically modified cells. Therefore, defining T-cell phenotypes can influence antitumor activity. Pharmacological inhibition of protein kinase B (AKT), mammalian target of rapamycin (mTOR), or glycolysis during CAR-T cell expansion can promote effector memory T-cell formation. However, inhibition

of these pathways also reduces the proliferative capacity of CAR-T cells, limiting therapeutic cell expansion. In summary, combination therapy may bring fresh hope for patients who show poor initial response to CAR-T cell therapy, as well as those with relapsed and refractory B-cell lymphoma.

Mohty *et al.* addressed the concept of CAR T cell therapy and indicated its role in the evolving landscape of management of patients with refractory/relapsed diffuse large B cell lymphoma (8). Hopfinger *et al.* summarized the results of three large phase II CD19 CAR-T cell trials and focus on AEs (9). In this review, we summarize the principles and relevant clinical research on therapy that combines CAR-T cell therapy and multiple targeted drugs for the treatment of B-cell lymphoma.

Overview of CAR-T cell therapy

CAR-T cell structure

CARs are recombinant receptors that target specific surface molecules. CARs comprise three structural components: an extracellular antigen-binding domain; a transmembrane domain; and a intracellular signal domain (10). Different designs of these three components may affect CAR-T cell function. The extracellular domain, a single-chain variable fragment (scFv), is composed of both light and heavy chains, which are responsible for antigen recognition. This recognition involves the specific binding of antibodies to antigens. scFv designed to recognize antigens include CD19, CD20, epidermal growth factor receptor (EGFR), Her2/neu, GD2, prostate-specific membrane antigen (PSMA), and receptor tyrosine kinase-like orphan receptor 1 (ROR1). The transmembrane domain connects the intracellular and extracellular domains. It is generally composed of dimeric membrane proteins (DMC) that anchor CARs to the T-cell membrane. Different designs of the transmembrane domain affect the expression of introduced CARs. The intracellular signaling domain employs immune-receptor tyrosine-based activation motifs (ITAMs), usually TCRζ (CD-3ζ) or Fc receptor γ-chain (FcRγ), which transduces T-cell receptor (TCR)-like signals into the cell when the extracellular domain scFv binds to the target antigen (11).

CAR-T cell therapy treatment process

CAR-T cell therapy begins with the collection of mononuclear cells from the patient's peripheral blood using a blood cell separator. Then, the patient's mononuclear cells

are transferred to a cell processing center, where selective T-cells are activated in a proliferative environment. CAR-T cells are generated by transfecting CAR into T-cells using retroviral or lentiviral vectors. Expanded CAR-T cells are then returned to the treatment institution and infused into the patient, in a process that usually takes two to three weeks (12). Before the CAR-T cell expansion process, physicians often adopt bridging chemotherapy to avoid rapid disease progression and maintain the patient's general condition. This can decrease the number of T-cells (including regulatory T-cells) *in vivo*, which upregulates cytokines such as IL-7 and IL-15. These cytokines promote T-cell expansion, including CAR-T cells, and enhance the antitumor activity of CAR-T cells (13).

Progress in the development of CAR-T cell therapy

Since the first reported case of anti-CD19 CAR-T cell therapy in 2010, it has been aggressively researched and was finally approved by the US Food and Drug Administration (FDA) in 2017 (14). First-generation CAR-T cell therapy involves only one intracellular signaling region and exhibits weak antitumor effects (15). Second-generation CAR-T cell therapy enhances its antitumor effects by adding costimulatory domains CD28 (16) or 4-1BB (17,18) to first-generation CAR-T cell therapy. Third-generation CAR-T cell therapy comprises an activation domain and multiple costimulatory domains, such as CD27, CD28, and OX40 (19). The addition of these domains not only enhances the ability of CAR-T cells to recognize and bind to tumor-associated antigens (TAAs), but also significantly improves their ability to kill tumor cells. Fourth-generation CAR-T cell therapy introduces proinflammatory cytokines, such as IL-12, and costimulatory ligands (4-1BBL and CD40L) (20,21). This releases proinflammatory factors, and then recruits and activates more immune cells in the immunosuppressive microenvironment to produce more extensive antitumor immune effects (22-24). Of these four types of CAR-T cell therapy, second-generation CAR-T cell therapy is used most frequently because of its stability and controllable lateral function, and it is the most established of these therapies.

Progression of CAR-T cell therapy in the treatment of B-cell lymphoma

Despite improvements made in chemoimmunotherapy, NHL is the most common hematologic malignancy.

After first-line therapy, a significant number of patients experience disease progression or relapse. Anti-CD19 CAR-T cell therapy is considered as the most promising and effective therapy for overcoming refractory and relapsed B-NHL (25). Currently, there are two US FDA-approved anti-CD19 CAR-T cell therapies for the treatment of patients with relapsed and refractory aggressive NHL: tisagenlecleucel and axicabtagene ciloleucel (axi-cel). A third product, lisocabtagene-maraleucel (liso-cel), is undergoing clinical trials, and we await the preliminary results of these trials (26-32).

Tisagenlecleucel is a second-generation CAR-T cell therapy that uses CD8 as a transmembrane domain and 4-1BB as a costimulatory domain. This therapy was developed by the University of Pennsylvania (UPenn) in collaboration with Novartis and was approved by the FDA in 2017 for pediatric B-cell acute lymphoblastic leukemia (B-ALL) (26,31). UPenn conducted a pilot study of tisagenlecleucel in patients with B-cell lymphoma. Twenty-eight patients were evaluable in this study (DLBCL, n=14; FL, n=14), and the primary study endpoint was overall response rate (ORR) at 3 months. At approximately 3 months post-infusion, the ORR was 50% (7/14) in patients with DLBCL and 79% (11/14) in patients with FL, with 57% (16/28) achieving CR. The 16 patients (DLBCL, n=6; FL, n=10) who achieved CR at 6 months post-infusion had a durable response, with a median follow-up of 29.3 months. Notably, 4 patients (DLBCL, n=1; FL, n=3) who had achieved partial response (PR) at 3 months had achieved CR at 6 months post-infusion. Subsequently, a phase II clinical trial of tisagenlecleucel was conducted in patients with relapsed and refractory DLBCL (JULIET; NCT02345248) (26,33). CAR-T cells were infused into 111 patients, of whom 93 were evaluable. The best ORR was 52% (48/93) and the CR rate was 40% (37/93). The follow-up data was presented at the 60th Annual Meeting of the American Society of Hematology (ASH) in 2018. The median duration of response and the OS of the CR patients was not reached within the median follow-up of 19.3 months (34). Recurrence-free survival was 64% at 12 and 18 months for all patients who responded to the therapy. Based on these promising results, the US FDA approved tisagenlecleucel for the treatment of relapsed and refractory DLBCL in May, 2018.

Axi-cel, which uses CD28 as the transmembrane domain and activation domain, was originally developed by the National Cancer Institute (NCI). In the ZUMA-1 trial (NCT02348216), a study of axi-cel in refractory B-NHL,

of the 119 patients enrolled, 108 patients received axi-cel infusion (35,36). There were 101 evaluable patients, who were followed up for a median of 27.1 months. Of the 101 patients, 84 (83%) had an objective response to axi-cel, 59 (58%) achieved CR, and 25 (25%) attained PR. Ten patients (10%) maintained stable disease (SD), five (5%) progressed to best response, and two (2%) were not assessable (35). A single infusion of axi-cel in many patients with relapsed and refractory B-NHL resulted in a durable response lasting more than two years without further consolidation therapy. The expected median OS with conventional chemotherapy is approximately six months and the two-year OS rate is approximately 20%. The best ORR in this study was significantly higher than that of relapsed and refractory DLBCL treated with conventional chemotherapy, the historical control (37). Axi-cel was approved by the US FDA in October, 2017, for the treatment of relapsed and refractory DLBCL when relapse occurs after at least two lines of therapy, and was approved by the European Medicines Agency (EMA) in June, 2018.

Liso-cel uses CD28 as the transmembrane domain and 4-1BB as a costimulatory domain. Juno and Celgene conducted a US multicenter phase I study of liso-cel called Transcend NHL001, which initially enrolled patients with various subtypes of aggressive B-NHL, and subsequently expanded the cohort to enroll patients with DLBCL, double-triple hit lymphoma, and transformed follicular lymphoma. Updated results of Transcend NHL001 were presented at the American Society of Clinical Oncology (ASCO) Meeting in 2018. Thirty-seven patients with relapsed and refractory DLBCL received a critical dose of liso-cel, with an ORR of 49% and a CR rate of 46% at 6 months (38). Notably, toxicities were well managed, with only 1 patient developing grade 3 cytokine release syndrome (CRS) and 13 patients experiencing grade 3 or 4 neurotoxicity.

Combination of CAR-T cell therapy and targeted agents in the treatment of lymphoma

CAR-T cell therapy in combination with PD-1 inhibitors

PD-1 is an inhibitory receptor expressed by activated T-cells, activated B-cells, natural killer (NK) cells, and myeloid cells (39,40). PD-L1 is widely expressed in many somatic cells exposed to pro-inflammatory cytokines, and intratumoral inflammation-induced PD-L1 expression leads to PD-1-mediated T-cell exhaustion, which suppresses the antitumor immunity of cytotoxic T-cells (41-43). Engagement of the

PD-1/PD-L1 pathway leads to the phosphorylation of the tyrosine motif in the cytoplasmic tail of the PD-1 inhibitory receptor. Due to the resultant dephosphorylation of phosphatidylinositol 3-kinase (PI3K), this also promotes the recruitment of tyrosine protein phosphatase non-receptor type 11 (PTPN11). The inhibition of PI3K leads to downstream activation of the Rac-serine threonine protein kinase, which reduces T-cell activation, proliferation, and survival (44). CAR-T cell therapy is a promising approach for the treatment of refractory hematologic malignancies, as CAR-T cells can increase the expression of exhaustion markers, such as PD-1, Tim-3, and Lag-3 (39,45). Blocking the activation of the PD-1/PD-L1 pathway has therefore been suggested as a therapeutic strategy for enhancing the antitumor efficacy of CAR-T cell therapy (46,47).

One expected effect of CAR-T cell therapy is a significant decrease in B-cells post-therapy. The blockade or inhibition of these checkpoints by naturally occurring checkpoint mechanisms in the human immune system can prolong the action of CAR-T cells, and patients with persistent B-cell aplasia who are not treated with transplantation are at high risk of relapse (48). At the 2018 ASH Meeting, Dr. Maude presented the results of a small single-center study that included 14 children and adolescents with B-ALL who received pembrolizumab or nivolumab 2 to 7 weeks after an infusion of tisagenlecleucel. The B-cell counts of three patients recovered soon after the tisagenlecleucel infusion and fell again after the addition of PD-1 blockade, a marker of CAR-T cell function recovery. CR was later attained by all three of these patients. PD-1/PD-L1 inhibition helped seven patients in this study re-establish their initial response to tisagenlecleucel, and of four patients whose disease had spread beyond the bone marrow, two attained CR and two attained PR. Four patients who did not respond to CAR-T cell therapy did not attain CR when treated with pembrolizumab, but PR was observed. Dr. Maude and her team continue to follow these patients and explore strategies to improve health outcomes using CAR-T cell therapy in combination with immune checkpoint inhibitors (9).

In a small study conducted in China, researchers designed four experimental groups to investigate combination therapy for PD-1 overexpressing malignant lymphoma. The first group contained anti-CD19 CAR-T cells prepared from the peripheral blood of patients with PD-1 overexpressing malignant lymphoma. The second group also contained anti-CD19 CAR-T cells prepared from the peripheral blood of patients with PD-1 overexpressing malignant lymphoma, but nivolumab was added at final

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concentrations of 72, 36, and 18 µg/mL on day 8 of cell expansion. The third group contained T-cells from patients with high peripheral blood PD-1 expression combined with 72 µg/mL of nivolumab; and the fourth group contained anti-CD19 CAR-T cells prepared from five healthy donors (HDs). The results of this study show that the transfection rate of anti-CD19 CAR-T cells in patients with high PD-1 expression was close to that of HDs (32.80%±7.22% *vs.* 35.10%±5.84%, P=0.593). The combination of 72 µg/mL of nivolumab and anti-CD19 CAR-T cells showed better killing rates than either the combination of 72 µg/mL of nivolumab with patient-derived T-cells or the anti-CD19 CAR-T cells alone (P<0.001 for both). The killing rate of the combination of 72 µg/mL of nivolumab and anti-CD19 CAR-T cells at 48 hours was 71.61%±9.50%; the killing rate of the combination of 72 µg/mL of nivolumab with patient-derived T-cells was 6.77%±1.26%; and the killing rate of anti-CD19 CAR-T cells alone was 15.33%±4.11%. Different doses of nivolumab in combination with patient-derived anti-CD19 CAR-T cells did not affect the levels of inflammatory cytokines IFN-γ and TNF-α (P>0.05 for all). This study showed that nivolumab at a final concentration of 36 µg/mL combined with anti-CD19 CAR-T cells reduced side effects of the drug while enhancing the killing activity of anti-CD19 CAR-T cells (49); however, studies with larger sample sizes are needed to confirm these results.

Schuster, Riedell, and Joseph McGuirk from the University of Kansas Cancer Center are in the process of testing the safety and effectiveness of pembrolizumab administered to patients with DLBCL several weeks after treatment with tisagenlecleucel, rather than in response to patients relapsing or failing to respond to CAR-T cell therapy (9). We await the results of this ongoing study.

CAR-T cell therapy in combination with BTK inhibitors

BTK is a Tec family kinase present in the cell membrane and nucleus, and it is an essential component of the B-cell receptor (BCR) signaling pathway. BTK plays an important role in B-cell maturation and regulation of cellular processes, such as cell differentiation, cell division, and signal transduction, and this kinase is highly expressed in B-cell malignancies (50). Ibrutinib is a covalent irreversible inhibitor of BTK that is highly selective for this kinase and has been approved by the US FDA and the EMA for the treatment of CLL (51). BTK inhibitors can theoretically increase the efficiency of CAR-T cell therapy, enhancing its overall antitumor effect by reducing cell terminal

differentiation through the inhibition of AKT signaling, which in turn increases the proportion of memory CAR-T cells (52). In addition, ibrutinib effectively mobilizes tumor-infiltrating B-cells to infiltrate peripheral blood so they can be destroyed by circulating CAR-T cells.

A recent study combined ibrutinib with anti-CD19 CAR-T cell therapy (tisagenlecleucel), anticipating an improved response in MCL (53). Combining MCL cell lines, primary MCL samples, autologous or normal donor-derived anti-CD19 CAR-T cells, and ibrutinib, these researchers tested the effect of this combination *in vitro* and in a mouse xenograft model. The *in vitro* investigation showed that MCL cells strongly activate multiple effector functions of tisagenlecleucel, and that tisagenlecleucel kills MCL cells more effectively in the presence of ibrutinib. In the xenograft MCL model, mice treated with tisagenlecleucel showed a statistically significant improvement in lymphoma control compared to mice treated with ibrutinib. All mice treated with ibrutinib monotherapy died before day 100, whereas tisagenlecleucel fostered long-term survival in recipient mice, which suggests that tisagenlecleucel is therapeutically more effective than ibrutinib in this model. When ibrutinib and tisagenlecleucel were administered in combination, 80% of the mice maintained long-term remission (P<0.05). This study demonstrated that when used in combination, these two therapies improve antitumor activity and exhibit a lower recurrence rate compared to monotherapy.

Gill *et al.* presented the results of a trial that introduced anti-CD19 CAR-T cell therapy into conventional CLL therapy for patients who did not achieve CR after six months of ibrutinib treatment at the 2017 ASCO Meeting (54). All patients received bridging chemotherapy, comprising bendamustine or fludarabine and cyclophosphamide, approximately one week before treatment. Subsequently, the researchers administered CAR-T cells and ibrutinib therapy to the patients. They later examined the patients' bone marrow CLL burden by flow cytometry and IGH gene rearrangements and used computed tomography imaging to evaluate the lymph nodes and spleen. After a median six-month follow-up, eight of nine evaluable patients achieved minimal residual disease (MRD) negativity as demonstrated by flow cytometry, and all patients had maintained bone marrow CR at their last follow-up. The nine evaluable patients displayed CRS at different levels: two patients with grade 1; six patients with grade 2; and one patient with grade 3. Overall, patients tolerated this regimen well and did not require corresponding treatment for side effects.

As demonstrated by computer tomography, five patients attained CR, two patients attained PR, and two patients attained SD. However, the researchers indicated that long-term follow-up is needed to confirm the robustness of these results, and that further trials are required to investigate whether this combination therapy, when used as a first-line regimen for CLL, can spare patients from long-term treatment.

CAR-T cell therapy in combination with PI3K inhibitors

While the results of these CAR-T cell therapy studies are encouraging, there remain some lymphoma patients who do not respond. It has been shown that this phenomenon is associated with the infusion of low numbers of early memory T-cells (55). A phase II clinical trial of second-generation anti-CD19 CAR-T cell therapy containing CD28 costimulatory domains found that the frequency of CD62L-expressing central memory T (T_{CM}) cells in the infused product were significantly correlated with the volume of CARs in the peripheral blood, and that the frequency of these T_{CM} cells was directly related to improved ORR and antitumor effects of the therapy (56). Therefore, a major goal for improving CAR-T cell therapy is generating T_{CM}-phenotype cells for patient infusion (57). T-cell expansion and differentiation is depended on the integration of TCRs, costimulatory molecules, and cytokine receptors (58). These signals converge to activate two major signal transduction networks within T-cells: the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR pathways. These pathways are critical for T-cell activation, survival, expansion, and cytokine production, as well as T_{CM} cell formation and differentiation (59). The genetic and pharmacological disruption of extracellular signal-regulated kinases (ERKs), a distal component of the MAPK pathway, significantly impairs T-cell proliferation in both mice (60) and humans (61). In contrast, the proliferation and survival of murine CD8 T-cells is not impaired by the deletion of PI3CD, the gene that encodes the p110 catalytic subunit of PI3K, or the inhibition of AKT (62). Therefore, pharmacological interference of AKT signaling results in T-cell activation and expansion in human peripheral blood, as well as efficient retroviral transduction by means of CAR or TCR and promotion of CD62L expression (63). Additionally, glycolysis can enhance T-cell differentiation and lead to impaired antitumor effects *in vivo*. Therefore, the inhibition of AKT in CD19 CAR-T cell therapy can inhibit glycolysis and increase T-cell activity by interrupting the expression of key metabolic enzymes (55).

Idelalisib is a PI3K inhibitor that has been approved for the treatment of CLL and FL. As well as being able to eliminate malignant B-cells, idelalisib can exhaust regulatory T-cells, therefore reversing immune tolerance (64). In a previous study of CLL treatment, researchers combined idelalisib with anti-CD19 CAR-T cells. Peripheral blood samples were collected from 10 HDs and 9 CLL patients (65). Their results demonstrated that, when cultivated with idelalisib, CD19-CAR expression increased significantly in both HDs (59%±12% *vs.* 64%±13%, P=0.002) and CLL patients (65%±13% *vs.* 73%±11%, P=0.02). On day 10, malignant B-cells were markedly decreased in CLL patient samples and reached similar levels to HD-derived CAR-T cells. Idelalisib had no significant effect on the B-cells of HDs. Cytotoxic T-cells (CD3+/CD8+) and T helper cells (CD3+/CD4+) derived from HDs and CLL patients expanded in different ways during cell cultivation. In HDs, T helper cell numbers decreased and cytotoxic T-cell numbers increased after introducing idelalisib. In contrast, CAR-T cells derived from CLL patients where idelalisib was not introduced contained more T helper cells and fewer cytotoxic T-cells. CD45RA + CCR7 + naive-like T (TN) cells positive for the memory-associated marker CD95 can be recognized by T memory stem cells (TSCM) in CAR-T cell products. The fraction of TN cells within CAR-T cells cultivated with idelalisib expressed higher levels of CD62L in both HDs and CLL patients; more than 95% of HD-derived cells expressed CD62L. Researchers used a xenograft NSG mouse model with human ALL cells (Nalm-6) to analyze the antitumor activity of anti-CD19 CAR-T cells in the presence or absence of idelalisib. This study ultimately showed that the antitumor activity of CAR-T cells was enhanced after idelalisib was introduced.

Decreased T-cell counts and an abnormal T-cell phenotype involving significantly increased proportions of CD27⁻ CD28⁻ T-cells, which is a hallmark of senescence, have been observed in heavily pretreated DLBCL patients. This may lead to insufficient yield or poor CAR-T cell quality during cell production (66). In another study, low concentrations of idelalisib and VIPhyb, a VIP pathway antagonist, were introduced into a mouse model. This significantly improved viable T-cell counts and delayed terminal differentiation (67). Conversely, the cytotoxicity of CAR-T cells against lymphomas was enhanced, which suggests that PI3K and VIP signal interruption is a promising method for limiting T-cell depletion during

the expansion of CAR-T cells and improving the clinical efficacy of genetically modified cells.

CAR-T cell therapy in combination with HDAC inhibitors

HDACs are enzymes that deacetylate the acetyl group from ε-N-acetyl lysine amino acids to produce histone, which leads to the tight wrapping of DNA. This enzyme can promote chromatin compaction and repress transcription of their related genes (68). HDAC inhibitors (HDACis) have been shown to enhance the levels of CD20-expressing protein and mRNA in Burkitt's lymphoma, which in turn enhances the expression of CD20 antigens on the surface of malignant B-cells (69). Pretreatment with HDACis before CD20 CAR-T cell therapy can enhance cytotoxic activity, and increased toxicity toward tumor cells and prolonged overall survival in a mouse cell model of Burkitt's lymphoma (70).

Another study showed that romidepsin combined with anti-CD20 CAR modified expanded peripheral blood NK (anti-CD20 CAR exPBNK) cells significantly induced cell death in rituximab sensitive and resistant Burkitt's lymphoma cells *in vitro*, and reduced tumor burden in mice xenografted with human Burkitt's lymphoma, improving their progress (71). This study demonstrated that romidepsin could enhance the antitumor activity of NK cells and anti-CD20 CAR exPBNK in rituximab sensitive and resistant Burkitt's lymphoma.

CAR-T cell therapy in combination with rituximab

Rituximab has greatly improved the outcomes of most B-NHL patients (72,73). However, it is reported that B-NHL cells may lose CD20 expression after rituximab treatment, and this potential absence may result in resistance to rituximab (74,75). Conversely, anti-CD20 CAR-T cells can provide alternative targets that can be treated sequentially with rituximab, or else rituximab can be used with anti-CD19 CAR-T cells to target multiple antigens simultaneously, therefore reducing the risk of immune escape (76). B-cell exhaustion after rituximab treatment is beneficial, in that it can maintain anti-CD20 CAR-T cell survival (77) and allow T-cells to efficiently transfer to B-NHL patients and remain at the lesion location. In another study, researchers attempted to investigate the cytotoxic effects of rituximab in combination with either anti-CD19 CAR-T or anti-CD38 CAR-T in xenografted B-NHL mice, and ultimately found that synergistic tumor

inhibition continued for more than two months in these mice (78). We anticipate further encouraging results as investigations into combined rituximab and CAR-T cell therapy for different types of lymphoma continue.

A theoretical limitation of rituximab use in combination with CAR-T cell therapy is that residual serum rituximab may hinder CAR binding to CD20, which would impede T-cell mediated antitumor responses (76). Researchers identified a range of rituximab serum levels in CAR-T cell assays and ultimately found that, in the majority of patients, rituximab concentration was 100 mg/mL or less with a median concentration of more than 40 mg/mL. In this concentration range, anti-CD20 CAR-T cells retain effective activity both *in vitro* and *in vivo*. Rituximab concentrations are significantly higher in CAR-T cell assays in mouse models than in most human patients. The results of mouse studies have indicated better health outcomes in mice that receive combination therapy compared to mice treated with CAR-T cells alone. Moreover, mice that receive combination therapy are not impaired by the rituximab. These experiments also clarify a point of clinical relevance: CAR-T cells may be effective in the treatment of rituximab-resistant tumors.

CAR-T cell therapy in combination with lenalidomide

Multiple myeloma (MM) is a hematological malignancy characterized by the clonal expansion of terminally differentiated B-cells (plasma cells) in the bone marrow. Its clinical characteristics include osteolytic bone disease, infection, and abnormal renal function. There are several ongoing clinical studies on CAR-T cell therapy in MM (79). In one such study, an MM patient was given a tisagenlecleucel infusion after a standard autologous transplantation. An important finding of this study was that tisagenlecleucel cells were still detectable in the blood and bone marrow up to several days post-infusion (80). The patient commenced lenalidomide maintenance therapy three months after infusion, and CR was still observed at the one-year follow-up. No signs of fever or other CRS were reported after tisagenlecleucel infusion. This study is ongoing, and we await the results of future follow-ups.

Summary

In recent years, CAR-T cell therapy for the treatment of both hematologic malignancies and solid tumors has

advanced (81). However, it still faces many challenges: both T-cell exhaustion and immunological barriers limit the effectiveness of CAR-T cell therapy; and adverse events, including CRS and neurotoxicity, negatively impact treatment safety (82). Combination therapy based on CAR-T cell therapy may be a viable solution for these problems. Many targeted drugs have demonstrated excellent efficacy in the treatment of a variety of B-cell lymphomas. Combining CAR-T cell therapy with targeted drugs is therefore a promising strategy for the treatment of B-cell lymphoma. We summarized some trials *in vivo* and *in vitro* mentioned in this article of CAR-T cell therapy combination with targeted agents for the treatment of B-cell lymphoma in the *Table 1*.

Table 1 *In vivo* and *in vitro* trials of CAR-T cell therapy in combination with targeted agents for the treatment of B-cell lymphoma

Combination target	Drug	CAR-T name/ design/target	Disease	Intervention	Result
PD-1	Pembrolizumab or nivolumab	Tisagenlecleucel/ 4-1BB/CD19	B-ALL	14 children and adolescents with B-ALL received pembrolizumab or nivolumab within 2 to 7 weeks after the infusion of tisagenlecleucel	3 patients CR 7 patients re-established their initial response to tisagenlecleucel
PD-1	Nivolumab	Anti-CD19 CAR-T cell	*In vitro*	Arm 1: anti-CD19 CAR-T cells are prepared from peripheral blood PD-1 overexpressing malignant lymphoma	1. The transfection rate of anti-CD19 CAR-T cells in patients with high PD-1 expression was close to that in the HDs (32.80%±7.22% *vs.* 35.10%±5.84%, P=0.593)
				Arm 2: nivolumab was added at final concentrations of 72, 36, and 18 µg/mL on day 8 of the cell expansion to the anti-CD19 CAR-T cells are prepared from peripheral blood PD-1 overexpressing malignant lymphoma patients	2. The combination of 72 µg/mL nivolumab and anti-CD19 CAR-T cells had better killing rates than the combination of 72 µg/mL nivolumab with patient-derived T-cells, as well as the anti-CD19 CAR-T cells alone
				Arm 3: T-cells from patients with high peripheral blood PD-1 expression combined with 72 µg/mL nivolumab	3. The combination of nivolumab at a final concentration of 36 µg/mL with anti-CD19 CAR-T cells reduced the side effects of the drug while enhancing the killing activity of anti-CD19 CAR-T cells
				Arm 4: anti-CD19 CAR-T cells prepared from 5 healthy donors (HDs)	
BTK	Ibrutinib	Anti-CD19 CAR-T cell	*In vitro*		MCL cells strongly activated the function of multiple tisagenlecleucel effectors, and the killing of MCL by tisagenlecleucel was further enhanced by ibrutinib

Table 1 (*continued*)

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Table 1 (*continued*)

Combination target	Drug	CAR-T name/ design/target	Disease	Intervention	Result
BTK	Ibrutinib	Anti-CD19 CAR-T cell	Mouse xenograft model		All mice treated with ibrutinib monotherapy died before day 100, whereas tisagenlecleucel fostered long-term survival of the recipient mice. With the combination of ibrutinib and tisagenlecleucel, 80% of mice maintained long-term remission (P<0.05)
BTK	Ibrutinib	Anti-CD19 CAR-T cell	9 CLL patients who did not achieve CR after 6 months of Ibrutinib treatment		Minimal residual disease (MRD) negativity was achieved on flow cytometry, and all patients retained bone marrow CR at last follow-up
					CRS: grade 1 for 2 patients; grade 2 for 6 patients; and grade 3 for 1 patient
					CR in 5 patients, PR in 2 patients, and SD in 2 patients
PI3K	Idelalisib	Anti CD19 CAR-T	*In vitro*	Arm 1: peripheral blood samples were collected from 10 HDs	The results showed that cultivation with idelalisib significantly increased CD19-CAR expression in both the HDs (59%±12% *vs.* 64%±13%, P=0.002) and the CLL patients (65%±13% *vs.* 73%±11%, P=0.02)
				Arm 2: peripheral blood samples were collected from 9 CLL patients	
PI3K	Idelalisib and VIPhyb	Anti CD19 CAR-T	Mouse xenograft model	Low concentrations of idelalisib and VIPhyb were added to the mouse model	The numbers of viable T-cells were significantly improved and the terminal differentiation was delayed
HDAC	Romidepsin	Anti-CD20 CAR-T	A mouse cell model of Burkitt's lymphoma	Pretreatment with HDACis before CD20 CAR-T treatment	The cytotoxic activity was enhanced, the toxicity to tumor cells was increased, and overall survival was prolonged
HDAC	Romidepsin	Anti-CD20 CAR exPBNK	A mouse xenograft humanized Burkitt's lymphoma model		The antitumor activity of natural NK cells and anti-CD20 CAR exPBNK in rituximab-sensitive and resistant Burkitt's lymphoma was enhanced
CD20	Rituximab	Anti-CD19 CAR-T or anti-CD38 CAR-T	A xenograft B-NHL mouse model		Synergistic tumor inhibition continued in mice for more than 2 months

CAR-T cells frequently encounter inhibitory TMEs when infused into patients, in which cells and inhibitory ligands can bind to inhibitory receptors on T-cells and hinder their antitumor response. Checkpoint blockade therapies against PD-1, PD-L1, and CTL-4 use antibodies to disrupt interactions with inhibitory receptors on T-cells. This strategy of administering checkpoint blockade therapy after CAR-T cell treatment enhances the efficacy of CAR-T cell therapy in tumors with immunosuppressive TMEs.

The attributes of adoptively transferred T-cells themselves are also an important factor that affects the therapeutic effect of CAR-T cells. Ibrutinib primarily interferes with BCR signaling pathways by inhibiting BTK; however, it can affect multiple parts of the hematopoietic system, including T-cells. The theoretical basis for the binding of ibrutinib to CAR-T cells is its direct effect on T-cells and its ability to destroy immunosuppressive TMEs. PI3K delta has a role in both B-cell and T-cell function, and inhibition of this signaling pathway has direct antitumor activity in NHL and CLL. PI3K signaling is also important for the proliferation and function of T-cells.

At present, preclinical and limited clinical data shows that some binding of small molecules and monoclonal antibodies to CAR-T cells can overcome many drug resistance mechanisms and further improve the effectiveness of CAR-T cells. Although many combination approaches are mentioned in this paper, an exhaustive list is not included and many relevant clinical trials are ongoing; we eagerly anticipate future results. This new combination immunotherapy regimen merits further investigation to enhance its antitumor efficacy and minimize side effects.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-72). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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