Hematological Oncology
Hematol Oncol 2015; 33: 67–69
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/hon.2220

Supplement Article

XII. Therapeutic exploitation of autologous T-cell activation in B-cell lymphoma

Anas Younes*

Lymphoma Service, Memorial Sloan Kettering Cancer Center, New York 10065, NY, USA

**Correspondence to: Anas Younes, Lymphoma Service, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA. E-mail: younesa@mskcc.org*

Keywords: **Chimeric T cell receptor; BiTe; DART; Immunotherapy**

Introduction

T lymphocytes play a critical role in the immune defense against foreign pathogens and cancer. For the past few decades, activating autologous T cells was primarily achieved through vaccination and cytokine stimulation. For native T cells to be activated, T-cell receptors must recognize a peptide that is presented by an antigen-presenting cell through Human Leukocyte Antigen (HLA) classes I and II. In contrast, antibodies, including surface-bound B-cell receptors, can recognize surface antigens independent of HLA restriction. Most antibodies induce anti-tumour response through antibody-dependent cytotoxicity and complement-dependent cytotoxicity. Because most tumour cells are either poor antigen presenters or frequently develop mechanisms to evade immune-cell recognition, investigators developed molecular methods to induce T-cell activation by cell surface proteins, rather than through HLA-restricted peptides. By doing so, a large pool of T cells can be activated by a variety of tumour antigens. One method is to engineer bispecific antibodies that bind to a target antigen on tumour cells, and CD3 on T lymphocytes. Initially, bispecific antibodies consisted of two full antibodies fused at their Fc tails. More recently, smaller molecules were generated to better facilitate tumour site penetration. This review will focus on recent data on the use of engineered chimeric antigen receptor (CAR) T cells and immune checkpoint inhibitors in lymphoma (Figure 1).

Bispecific antibodies

Bispecific T-cell engagers (BiTEs) are 55-KDa fusion proteins consisting of two single-chain variable fragments (scFvs) of two different antibodies from four different genes, on a single polypeptide chain (with 1 N-terminus and 1 C-terminus). Using this platform, one of the scFvs is directed against a CD19, while the second scFv is directed against the CD3ε subunit of the T-cell receptor complex (Figure 1). In contrast, dual-Affinity Re-Targeting (DART) proteins consists of two separate polypeptides that associate and are stabilized by a C-terminal disulfide bridge. Accordingly, DARTs have two N-termini and two C-termini. Both BiTEs and DARTs induce T-cell activation only when bound to target antigens, such as CD19. While *in vitro* experiments suggested that DARTs may have better activity against B cells, there is currently no clinical trial to compare these two platforms. Because of their small molecular size and monovalent binding, both BiTEs and DARTs are rapidly cleared from the circulation and have a fast off-rate and poor tumour retention. Both BiTEs and DARTs activate polyclonal populations of cytotoxic T cells [CD8+, CD4+), which are not dependent on MHC class I for antigen presentation, and may potentially overcome escape mechanisms of tumour cells evading T-cell recognition commonly observed in cancer.

Blinatumomab (Blincyto) was the first BiTE targeting CD3 and CD19 to be approved by the US Food and Drug Administration for the treatment of acute lymphocytic leukaemia (ALL). Blinatumomab was approved in December 2014 to treat relapsed or refractory Philadelphia chromosome–negative precursor B-cell ALL. In a phase II study, 113 patients with relapsed ALL who had detectable residual disease by a Polymerase Chain Reaction (PCR) test were treated with 4 weeks of continual infusion of blinatumomab, followed by a 2-week rest. Responders could receive up to four cycles of treatment or have a stem cell transplant after the first cycle. The treatment eradicated ALL minimal residual disease in 78% of the patients. In a separate study, patients with relapsed diffuse large B-cell lymphoma (DLBCL) were treated with 8 weeks of continuous intravenous infusion of blinatumomab. Patients achieving response after 8 weeks of treatment were allowed to receive a 4-week consolidation cycle after a 4-week treatment-free period. Among the evaluable 21 patients, nine patients responded (four Complete Remissions (CRs), five Partial Remissions (PRs)) resulting in an overall response rate of 43%, and a median duration of response

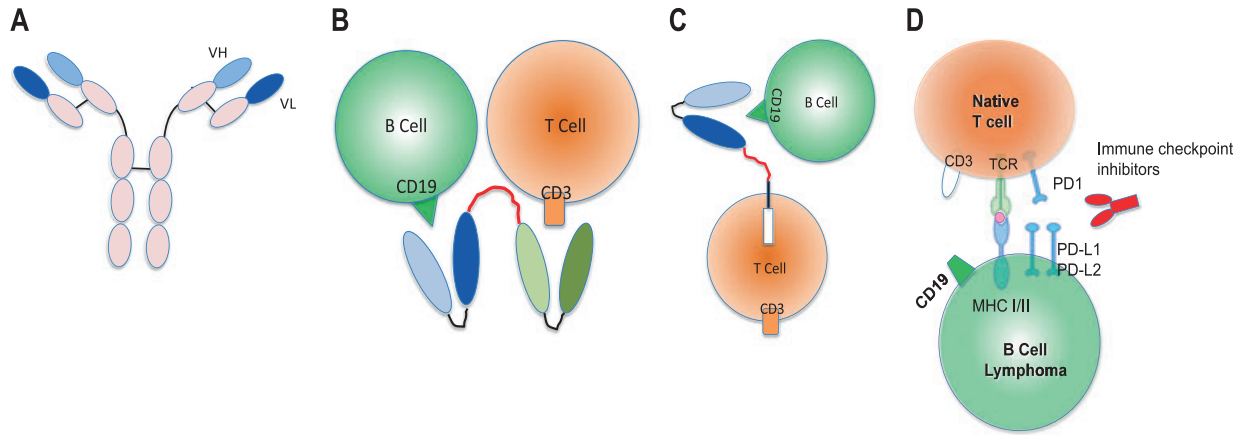

Figure 1. Platforms for inducing anti-tumour immune response. (A) Classic monoclonal antibodies, such as rituximab. (B) BiTE, (C) CAR T cells and (D) targeting PD-1/PD-L1/2 axis

was 11.6 months. All patients who responded did so within the first 8-week cycle. The most common adverse events were tremor (52%), pyrexia (44%), diarrhoea (24%), fatigue (24%), edema (24%) and pneumonia (24%).

Engineered chimeric antigen receptor T cells

First generation CARs are composed of an extracellular scFv of a desired monoclonal antibody, fused through a transmembrane domain to an intracellular T-cell receptor signalling motif (CD3ζ). Gene transfer is typically achieved through viral infection (retrovirus or lentivirus) or through non-viral methods (sleeping beauty). Second generation CARs added one costimulatory domains (such as CD28 or 4-1BB/CD137) to CD3ζ signalling domain, whereas third generation CARs added two costimulatory domains. Incorporating tandem intracellular costimulatory domains resulted in an enhanced T-cell proliferation and improved antitumor efficacy, but also was associated with increased risk of excessive cytokine release-related toxicity. To produce CD19-directed CAR T cells, peripheral blood mononuclear cells are collected, T cells are then transduced with a vector encoding the CAR genes, followed by *ex vivo* T-cell expansion. This process typically takes 3 to 4 weeks, allowing adequate time for the administration of lymphocyte depleting chemotherapy, before CAR T cells are adoptively transferred back to the patient.

Clinical responses in patients with CD19-expressing lymphoid malignancies were first reported using second generation CARs. Infusion of anti-CD19 scFv-CD137-CD3ζ CAR T cells resulted in durable responses, including complete remissions in patients with relapsed/refractory follicular lymphoma and chronic lymphocytic leukemia. Treatment-related adverse events included tumour lysis syndrome, lymphopenia and prolonged hypogammaglobulinemia. In a separate study, 15 patients with advanced B-cell malignancies (nine patients had DLBCL, two had indolent lymphomas and four had chronic lymphocytic leukemia) were treated with conditioning chemotherapy regimen of cyclophosphamide and fludarabine followed by a single infusion of anti-CD19 scFv-CD28-CD3ζ CAR T cells. Of the 15 patients, eight achieved complete remissions, and four achieved partial remissions. Remarkably, four of the seven evaluable patients with chemotherapy-refractory DLBCL achieved complete remissions. Acute toxicities including fever, hypotension, delirium and other neurologic toxicities were observed, which resolved within 3 weeks after cell infusion. Finally, Anti-CD19 scFv-CD28-CD3ζ CAR T cells also demonstrated major activity in patients with relapsed ALL. In a phase I study, all five adult patients with relapsed B-cell ALL who were treated with the autologous CAR T cells responded, including eradication of detectable circulating DNA. Similar to the other studies, cytokine storm-related symptoms were observed, which were more severe in patients with higher disease burden at the time of treatment.

Collectively, these data suggest that CAR T-cell therapy is a promising treatment modality for lymphoma. However, it is important to know that all these trials enrolled a small number of highly selected patients. At the present time, different CAR platforms have been licensed to pharmaceutical companies, creating a challenge for future standardization of these platforms. However, industry sponsorship of these platforms will likely speed up the conduct of future clinical trials using CAR T cells, as more data will need to be generated to determine the safety and efficacy of this novel treatment strategy. Furthermore, it is imperative to develop management and preventive strategies for cytokine release-related adverse events, as some of these toxicities can be severe. Current strategies are focusing on expressing antigens, such as CD20 and EGFR, on CAR T cells. By doing so, one can eliminate or downsize CAR T cells using ant-CD20 or anti-EGFR antibodies, if severe adverse events are caused by uncontrolled T-cell expansion and activation.

DOI: 10.1002/hon

Programmed cell death-1/Program Death Ligand(PDL)1

Programmed cell death-1 (PD-1) is an immune checkpoint receptor that is typically expressed on T cells. PD-1 inhibits T-cell activation upon interaction with its ligands *PD-L1* or *PD-L2*. Several lymphoid malignancies have been shown to over express *PD-L1* and/or PDL-2 providing a mechanism for evading the host anti-tumour immunity. Therapeutic inhibition of the interaction between PD-1 and its ligands using blocking monoclonal antibodies may therefore reactivate T cells to generate an anti-tumour immune response. Within the lymphoid malignancies, both Hodgkin lymphoma (HL) and primary mediastinal large cell lymphoma have been reported to overexpress PD-L1 and PD-L2 proteins. Studies suggested that genetic alterations in chromosome 9p24.1 and Ebstein Barr Virus (EBV) infection may be responsible for the observed over expression of PD-L1 and PD-L2 in the malignant Hodgkin and Reed–Sternberg cells of HL.

Early results from two separate phase I studies using anti-PD-1 blocking antibodies demonstrated high response rates in patients with relapsed HL. In the first study, nivolumab, a fully human IgG4 monoclonal blocking anti-PD-1 antibody demonstrated 87% response rate in 23 patients with relapsed and refractory HL. Most responses (70%) were partial and were achieved within the first 8 weeks of therapy. Surprisingly, tumour infiltrating T cells expressed only low levels of PD-1, but this low level of expression may be related to the immunohistochemistry detection methods. In a parallel study, pembrolizumab, also an IgG4 anti-PD-1 antibody, produced a 53% response rate in 15 patients with relapsed HL (Table 1). Phase II studies are ongoing to confirm these favourable responses, and to determine the duration of responses. Responses were also reported in patients with relapsed B-cell and T-cell lymphomas, although not as impressive as HL (Table 1). PD-1 blocking antibody therapy is associated with infusion-related adverse events, and in rare cases autoimmune pneumonitis, enteritis, hepatitis and endocrinopathy. Antibodies targeting PD-L1 are currently being evaluated. Furthermore, ongoing trials are evaluating the safety and efficacy of combining two immune checkpoint antibodies, in addition to combining PD-1/PD-L1 antibodies with anti-CD20 antibodies. Future trials will evaluate the contribution of immune checkpoint inhibitors with small molecules and with chemotherapy regimens.

Table 1. Reported response rates in patients with HL and NHL using PD-1 antibodies

Drug	Target	DLBCL (%)	FL (%)	T cell (%)	HL (%)
Nivolumab	PD-1	36	40	40	87
Pambrolizumab	PD-1	—	—	—	53

FL, Follicular Lymphoma; HL, Hodgkin lymphoma; NHL, Non Hodgkin Lymphoma; PD-1, programmed cell death-1; DLBCL, diffuse large B-cell lymphoma.

Conflict of interest

Received research funding from Genentech, Roche, Janssen. Received honoraria from Janssen and Roche.

DOI: 10.1002/hon