DEVELOPMENT OF BI-SPECIFIC CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY FOR THE TREATMENT OF AIDS-RELATED B CELL MALIGNANCIES


Background People living with human immunodeficiency virus (HIV) are at significantly higher risk than the general population of developing cancer, with a lifetime prevalence of cancer diagnosis of 25–40%. Non-Hodgkin lymphomas (NHL) are the most common type of AIDS-defining malignancy and predominantly manifests as Burkitt lymphoma (25%) or diffuse large B cell lymphoma (75%). Although CD19-directed (CD19) CAR T cell therapies are promising in treating B cell NHL, clinical trials have excluded HIV-positive patients due to concerns about safety (infectious complications), viral control, and limited CAR efficacy.

Recent reports have demonstrated the safety and feasibility of using CD19CAR T cell therapy to treat NHL in patients with HIV, indicating a move toward addressing the unmet need of this patient population. We previously designed mono-specific CAR constructs targeting either lymphoma (CD19) or HIVgp120 (N6) that show efficacy against their respective diseases in clinical and preclinical testing, respectively. We hypothesized that a dual construct targeting both antigens could simultaneously target both lymphoma cells and HIV-infected cells in the same individual. Thus, we developed a bi-specific CD19/N6 CAR T cell platform that can target both antigens in a single therapeutic product.

Methods We generated 3 bi-specific CAR constructs (2 tandem and 1 loop) (figure 1A) that incorporate a humanized (hu) CD19 single-chain variable fragment (scFv) and an N6 scFv from an anti-HIV broadly neutralizing antibody (bNAb) into a 2nd-generation CAR backbone. The tandem CARs consisted of N6 and huCD19 scFvs fused with a G4S linker in either huCD19:N6 or N6:huCD19 orientation. The loop CAR was generated by fusing huCD19(VL):N6(VH):N6(VL):huCD19 (VH) with a Whitlow linker. All constructs included the CD4 transmembrane domain, a double-mutated IgG4 Fc spacer, 4–1BB co-stimulatory and CD3ζ signaling domains, and EGFRt separated by a T2A ribosomal skip sequence. We tested the 3 bi-specific constructs in healthy-donor T cells using cytotoxicity co-culture assay (figure 1B) against either Raji (CD19+) or 8E5 (gp120+) target cells and confirmed functionality in HIV-positive donors.

Results Although all 3 bi-specific CAR constructs were functional against both CD19 and HIVgp120 antigens, the N6:huCD19CAR tandem CAR demonstrated equivalent or better efficacy against both antigens and could serially target single or alternating antigens (figure 1C,D). Moreover, we successfully generated N6:huCD19CAR tandem CAR T cells using HIV-positive donors and confirmed functionality.

Conclusions The development of the N6:huCD19CAR bi-specific CAR represents a novel CAR T cell therapy that could potentially provide life-saving treatment for patients with HIV-associated NHL through simultaneous targeting of tumor and viral suppression.

Ethics Approval This study was approved by the City of Hope IRB 09025.

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Abstract 314 Figure 1  N6:huCD19 bi-specific CAR-mediated elimination of HIVgp120- and CD19-expressing cells. (A) Schema of three tandem designs: N6-huCD19 scFv, huCD19-N6 scFv, huCD19-N6 loop design. (B) Serial killing assay scheme. On day 0, healthy donor N6-huCD19 CAR bi-specific T cells were co-cultured with gp120-expressing cells (8E5). After a 48-hour incubation period, the cells were examined to determine the survival of gp120-expressing cells. Simultaneously, CD19-expressing cells were introduced into the co-culture. Following an additional 48-hour incubation, the survival of CD19-expressing cells was quantified. (C) Cytotoxic analysis was performed on Day 2 to evaluate the targeting of HIV gp120. (D) On Day 4, the cytotoxic analysis was conducted to assess CD19 targeting. huCD19 and N6 monospecific CARs were included as control groups.