



## The 65th ASH Annual Meeting Abstracts

## ONLINE PUBLICATION ONLY

## 703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

**Generation of Bi-Specific Chimeric Antigen Receptor (CAR) T Cell Therapy for the Treatment of AIDS-Related B Cell Malignancies from Healthy Donors and HIV Patients**

Saghar Pahlavanneshan<sup>1</sup>, Ryan Urak, PhD<sup>1</sup>, Ryotaro Nakamura, MD<sup>1</sup>, John Zaia, MD<sup>2</sup>, John H. Baird, MD<sup>3</sup>, Mary C Clark, PhD<sup>4</sup>, Stephen J. Forman, MD FACP<sup>1</sup>, Xiuli Wang, PhD<sup>1</sup>

<sup>1</sup>Cellular Immunotherapy Center, Department of Hematology and Hematopoietic Cell Transplantation, Beckman Research Institute of City of Hope, Duarte, CA

<sup>2</sup>Center for Gene Therapy, Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center and Beckman Research Institute, Duarte, CA

<sup>3</sup>Department of Hematology and HCT, City of Hope National Medical Center, Duarte, CA

<sup>4</sup>Department of Clinical and Translational Project Development, City of Hope, Duarte, CA

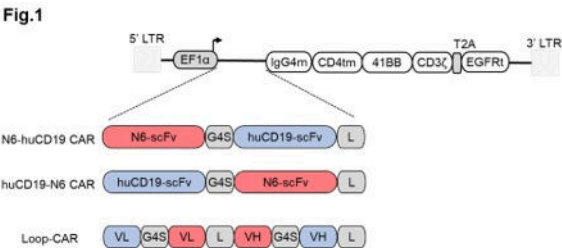
**Introduction:** Individuals with human immunodeficiency virus (HIV) face a substantially greater likelihood of developing cancers compared to the general population. The prevalence of cancer diagnosis among HIV-positive individuals ranges from 25% to 40%. Non-Hodgkin lymphoma (NHL) stands out as the most prevalent cancer type associated with people living with HIV/AIDS (PLWHA). NHL primarily manifests in two forms in PLWHA: Burkitt lymphoma, which poses an 18-fold higher risk, and diffuse large B cell lymphoma (DLBCL), accounting for 75% of cases. The advent of chimeric antigen receptor (CAR) T cell therapies, notably CD19-targeting CAR T cells, has emerged as a groundbreaking treatment for B cell NHL. Unfortunately, PLWHA has been excluded from participating in all of these clinical trials for years due to safety concerns and the challenges associated with manufacturing CAR T cell products from HIV-positive donors. Recent researches explored the feasibility of developing CD19-targeting CAR T cell products utilizing cells from HIV-positive donors with lymphoma. Although these studies have provided promising evidence that alleviates concerns regarding the safety profile and limited effectiveness of CAR T cell therapy in this particular population, they do not address the underlying HIV condition. We previously generated CAR T cells targeting either lymphoma (CD19-CAR) or HIVgp120 (N6-CAR) that show efficacy against their respective diseases in clinical and preclinical testing, respectively. We hypothesized that a dual construct capable of targeting both antigens could simultaneously target lymphoma cells and HIV-infected cells in a same individual. We report our experience developing a bi-specific N6-huCD19 CAR T cell platform that can target both antigens in a single therapeutic product.

**Methods:** We engineered 3 bi-specific CAR candidate designs including 2 tandem and 1 loop constructs. These constructs were developed by integrating a humanized (hu) CD19 single-chain variable fragment (scFv) and an N6 scFv from an anti-HIV broadly neutralizing antibody (bNAb) into a backbone of a 2<sup>nd</sup>-generation CAR construct. The bi-specific tandem CAR constructs 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 CD3-zeta signaling domains, and EGFRt separated by a T2A ribosomal skip sequence (Fig.1). We tested the 3 bi-specific constructs in healthy donor-derived T cells using cytotoxicity co-culture assay against either Raji (CD19+) or 8E5 (gp120+) target cells. We generated N6-huCD19 CAR T cells from HIV-positive patients and confirmed the functionality of these CAR T cells against tumor cells.

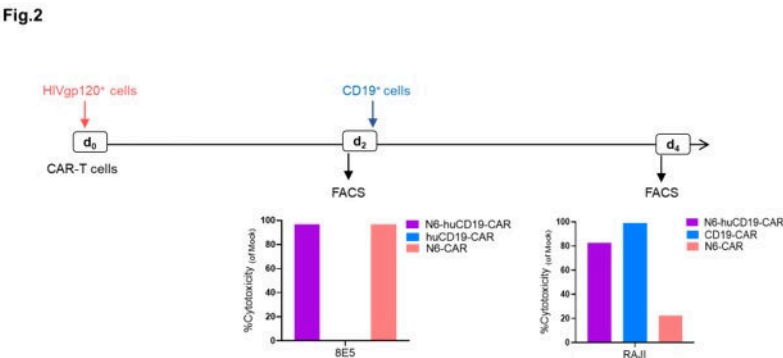
**Results:** The functional assessment of all 3 bi-specific CAR constructs revealed their efficacy against CD19 and HIVgp120 antigens. However, the N6-huCD19 tandem CAR T cells demonstrated comparable and better efficacy against both antigens. Notably, the N6-huCD19 tandem CAR T cells exhibited the capability to sequentially target either a single antigen or alternate between the 2 antigens (Fig.2). We successfully generated N6-huCD19 tandem CAR T cells using HIV-positive donors, signifying the practicality of this approach within the context of PLWHA. Remarkably these HIV donor-derived N6-huCD19 CAR T cells effectively killed tumor cells expressing CD19 or HIVgp120 antigens, thus confirming their dual functionality against these 2 antigens.

**Conclusion:** Here we demonstrate the development of the novel N6-huCD19 bi-specific CAR T cells with functionality against both CD19 and HIVgp120 antigens. We also showed the feasibility of generating these CAR T cells from HIV-donors. This strategy could potentially provide life-saving approach tailored for treatment of HIV-patients who develop CD19+ lymphomas.

**Disclosures Nakamura:** Jazz Pharmaceuticals: Consultancy, Other: research collaboration; NCCN: Other: guideline panel for HCT; Leukemia & Lymphoma Society: Other: grant reviewer; International Consortium: Other: consortium chair; Mt. Sinai: Other: Acute GVHD; Blue Bird: Consultancy; Napajen: Consultancy; Miyarisan: Research Funding; Omeros: Consultancy; NCTN Lymphoma Steering Committee: Membership on an entity's Board of Directors or advisory committees; BMT CTN Steering Committee: Membership on an entity's Board of Directors or advisory committees; Sanofi: Consultancy. **Baird:** Kite Pharma-Gilead: Research Funding, Speakers Bureau; Genentech-Roche: Research Funding; Regeneron Pharmaceuticals: Research Funding; Cellular Biomedicine Group: Research Funding.



**Figure 1. Bi-specific CAR construct designs.** Schema of 3 tandem CAR designs: N6-huCD19, huCD19-N6, and huCD19-N6 loop bi-specific CAR constructs in a 2<sup>nd</sup>-generation CAR construct backbone.



**Figure 2. Serial killing assay using healthy donor-derived N6-huCD19 CAR T cells.** On day0, healthy donor-derived N6-huCD19 CAR T cells were co-cultured with HIVgp120-expressing cells (8E5). After a 48-hour incubation period, the cells were examined to determine the survival of HIVgp120-expressing cells. Simultaneously, CD19-expressing cells (RAJI) were introduced into the co-culture. Following an additional 48-hour incubation, the survival of CD19-expressing cells was quantified. huCD19 and N6 monospecific CAR T cells were included as control groups.

**Figure 1**

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