P-CD19CD20-ALLO1: Potent Fully Allogeneic CAR-T Therapy Targeting CD19 and CD20 with Superior Efficacy over Single-Target Products

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ABSTRACT

Autologous Chimeric Antigen Receptor (CAR) T cells targeting CD19 have revolutionized the treatment of relapsed or chemotherapy-refractory B cell malignancies. However, many patients experience disease progression due to the loss or reduced expression of CD19. Autologous therapies also pose challenges for access, as each CAR-T dose requires patient apheresis and individual manufacture, leading to associated wait times and the need for bridging therapy. Here, we describe P-CD19CD20-ALLO1, a fully allogeneic CAR-T product expressing two full-length CARs targeting CD19 and CD20, respectively. P-CD19CD20-ALLO1 is currently being investigated in an open-label, multicenter Phase 1 study in subjects with relapsed/refractory B cell malignancies (NCT06014762) and is the most advanced allogeneic dual-targeting CAR-T in clinical development.

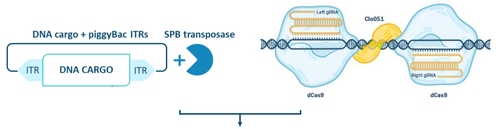
P-CD19CD20-ALLO1 is a T stem cell (T_{SCM})-rich product derived from healthy donor pan-T cells using a nonviral transposon-based system for transgene delivery, with full knockout of TRBC1/2 and partial KO of B2M. Clinical data from Poseida and others have demonstrated safety and efficacy advantages associated with T_{SCM} CAR-T, including for P-BCMA-ALLO1, our BCMA-targeting allogeneic CAR-T for Relapsed/Refractory Multiple Myeloma (NCT04960579), which is built upon the same platform as P-CD19CD20-ALLO1. Both the CD19 and CD20-targeting CARs employ novel fully human single-domain VH binders.

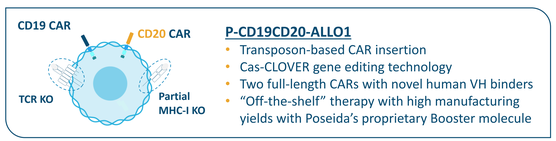
In nonclinical studies, P-CD19CD20-ALLO1 demonstrated potent antigen-specific anti-tumor activity against multiple tumor models in vivo, across multiple healthy donors and dose levels. Beyond expected advantages of targeting two antigens to prevent antigen escape, we also observed potency advantages for P-CD19CD20-ALLO1 compared to its CD19- and CD20-single targeting counterparts. In vitro potency was evaluated using serial restimulation against the CD19- and CD20-positive RAJI cell line, as well as RAJI cells engineered to express only CD19 or CD20. In these assays, P-CD19CD20-ALLO1 CAR-T cells showed superior potency against WT (CD19+CD20+) RAJI cells compared to the CD19- or CD20-single targeting products. Interestingly, this superiority was also observed against Raji cells expressing only one of the two target antigens, CD19 or CD20, suggesting that the increased potency of P-CD19CD20-ALLO1 is not solely due to the dual binding of CAR molecules to more antigens on the same target cell. Mechanistically, we observed that over three restimulations with WT (CD19+CD20+) RAJI cells, P-CD19CD20-ALLO1 CAR-T cells expressed and/or sustained higher expression of effector cytokines such as IFNγ, FasL, Granzyme A, and Granulysin than either CD19 or CD20 single-antigen targeting CAR-T cells.

In addition to improved in vitro potency, we assessed the in vivo efficacy of the dual-targeting product in comparison with the single-targeting products in a stress xenograft model of WT (CD19+CD20+) RAJI. P-CD19CD20-ALLO1 CAR-T cells were superior to the CD19-targeting CAR-T product.

P-CD19CD20-ALLO1, a dual-targeting, fully allogeneic T_{SCM}-rich CAR-T product for CD19 and CD20-positive B-cell malignancies, demonstrates robust antigen-specific activity against DLBCL and CLL models and outperforms its single-targeting counterparts even in the presence of only a single antigen on target cells.

P-CD19CD20-ALLO1 TECHNOLOGY

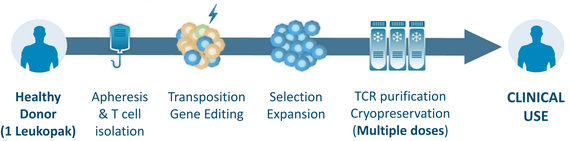




P-CD19CD20-ALLO1

- Transposon-based CAR insertion
- Cas-CLOVER gene editing technology
- Two full-length CARs with novel human VH binders
- "Off-the-shelf" therapy with high manufacturing yields with Poseida's proprietary Booster molecule

P-CD19CD20-ALLO1 MANUFACTURING PROCESS



P-CD19CD20-ALLO1 is produced using the same platform and manufacturing process as our BCMA-targeting allogeneic product P-BCMA-ALLO1 (Poster #4828)

METHODS AND RESULTS

P-CD19CD20-ALLO1 is Rich in T Stem Cell Memory (T_{SCM}) Cells

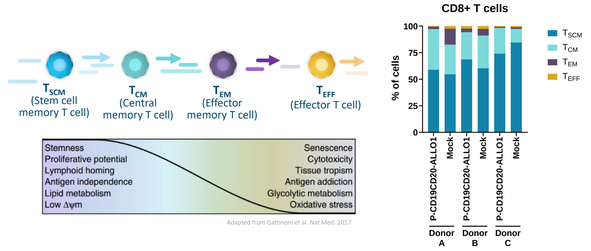


Figure 1. P-CD19CD20-ALLO1 CAR-T cells are rich in T Stem Cell Memory (T_{SCM}) cells. Poseida's non-viral transposon-based CAR insertion technology preferentially modifies naïve and T_{SCM} cells and creates P-CD19CD20-ALLO1 CAR-T cells products rich in long-lived and self-renewing T_{SCM} cells that can differentiate into highly potent effector cells upon antigen recognition while providing durable anti-tumor protection.

P-CD19CD20-ALLO1 is a Potent CAR-T Product Against CD19- and CD20-Dual Positive B-Cell Malignancies

Figure 2. P-CD19CD20-ALLO1 CAR-T cells produced from three healthy donors exhibit robust antitumor efficacy against CD19- and CD20-dual positive Raji Burkitt's lymphoma cell line in vitro and in vivo. A. Luciferase-expressing WT Raji cells (CD19+CD20+) were co-cultured for 48 hours with P-CD19CD20-ALLO1 CAR-T cells at the indicated E:T ratios and viable cells were quantified using a luminescence-based assay. Cytotoxicity was calculated by normalizing to samples containing only Raji cells. B. NSG mice were intravenously implanted with WT Raji (CD19+CD20+) cells and received 4 days later the indicated doses of P-CD19CD20-ALLO1 CAR-T cells. Tumor burden was monitored by bioluminescence imaging. Data is presented as mean ± SEM (n=5 mice/group).

P-CD19CD20-ALLO1 Can Target CD19 or CD20 Single-Positive Tumor Cells and Could Prevent Antigen Escape

Figure 3. Dual-targeting P-CD19CD20-ALLO1 CAR-T cells can target CD19 or CD20 single-positive Raji cells. P-CD19CD20-ALLO1 show strong in vivo antitumor efficacy against Raji cells genetically engineered to express only CD19 (CD20 KO) or CD20 (CD19 KO) in a dose-dependent manner. NSG mice were intravenously implanted with CD19 or CD20-expressing Raji cells and 4 days later received the indicated doses of P-CD19CD20-ALLO1 CAR-T cells. Tumor burden was monitored by bioluminescence imaging. Data is presented as mean ± SEM (n=5 mice/group).

P-CD19CD20-ALLO1 Outperforms Single CD19- and CD20-Targeting CAR-T Counterparts in an In Vitro Tumor Serial Rechallenge Assay

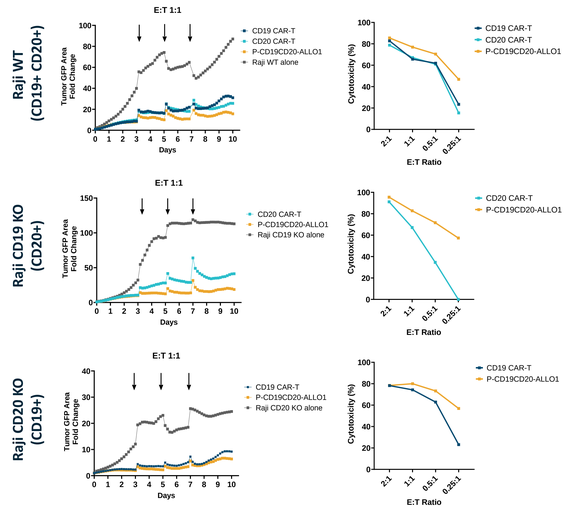


Figure 4. P-CD19CD20-ALLO1 CAR-T cells outperform single CD19- and CD20-targeting CAR-T products in an in vitro serial rechallenge assay. Dual-targeting P-CD19CD20-ALLO1 CAR-T cells show higher and more durable killing over three rechallenges (indicated by the black arrows) with Raji cells than their single CD19- and CD20-targeting CAR-T cell counterparts, even in the presence of only one tumor antigen (Raji CD19 KO or Raji CD20 KO). For each cell line, cytotoxicity for various E:T ratios is indicated in the right panels. P-CD19CD20-ALLO1 exhibits higher cytotoxicity than single-targeting CAR-T cells at all E:T ratios and as low as 0.25:1.

P-CD19CD20-ALLO1 CAR-T Cells Produce Higher and More Sustained Levels of Effector Cytokines

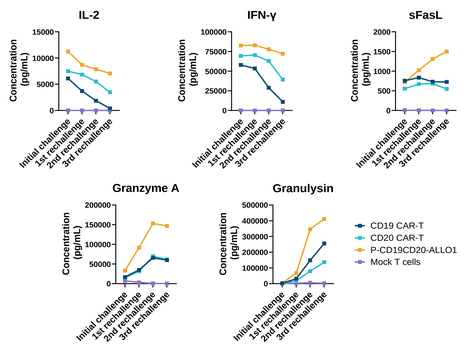


Figure 5. P-CD19CD20-ALLO1 CAR-T cells produce higher levels of effector cytokines than single CD19- or CD20-targeting CAR-T cells. P-CD19CD20-ALLO1 CAR-T cells secrete higher levels of IL-2, IFN-γ, soluble FasL (sFasL), Granzyme A and Granulysin compared to the single targeting CAR-T cells, after an initial challenge and three serial rechallenges with Raji WT (CD19- and CD20-positive) cells at a 1:1 E:T ratio.

P-CD19CD20-ALLO1 CAR-T Cells Show Higher In Vivo Antitumor Efficacy Than CD19-Single Targeting CAR-T Cells

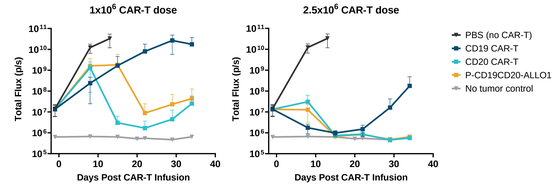
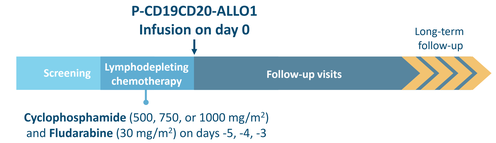


Figure 6. P-CD19CD20-ALLO1 CAR-T cells outperform CD19-single targeting CAR-T cells in a stress xenograft model of the CD19- and CD20-dual positive Raji cell line. NSG mice were intravenously implanted with WT Raji (CD19- and CD20-dual positive) cells and received 4 days later a stress dose of $2.5x10^6$ or a "super" stress dose of $1x10^6$ P-CD19CD20-ALLO1, CD19-single targeting or CD20-single targeting CAR-T cells. Tumor burden was monitored by bioluminescence imaging. Data is presented as mean ± SEM (n=5 mice/group). CAR-T cell expansion was minimal in that disseminated model, especially at the stress CAR-T doses used here.

P-CD19CD20-ALLO1 is Currently Under Investigation in a Phase 1 Study (NCT06014762) in Partnership with Roche



KEY INCLUSION CRITERIA

- Relapsed/refractory DLBCL NOS, HGBL, PMBCL, and tFL or FL Grade 3B
- Must have received prior systemic chemoimmunotherapy that includes an anti-CD20 antibody, an anthracycline, and either no response or refractory/relapsed to first-line therapy, progressive disease following two or more lines of therapy, or refractory to ASCT
- ECOG 0 or 1

PRIMARY OBJECTIVE

- Assess safety and MTD based on DLT

SECONDARY OBJECTIVES

- Evaluate anti-B cell malignancy effect of P-CD19CD20-ALLO1
- Study effect of cell dose and LD regimen to guide dose/LD regimen selection for pivotal studies

For information about enrolling patients, please email clinicaltrials@poseida.com

CONCLUSIONS

- P-CD19CD20-ALLO1 is a potent **allogeneic non-viral "off-the-shelf" CAR-T product, rich in self-renewing and long-lived T_{SCM} cells**
- P-CD19CD20-ALLO1 expresses **two full-length CARs against CD19 and CD20** and could **prevent disease relapse due to antigen escape**
- Dual-targeting P-CD19CD20-ALLO1 CAR-T cells **outperform single CD19- or CD20-targeting CAR-T counterparts** and produce higher and sustained **higher levels of effector cytokines** in vitro
- Dual-targeting P-CD19CD20-ALLO1 CAR-T cells **outperform CD19-single targeting CAR-T** cells in vivo
- **P-CD19CD20-ALLO1 is currently under investigation in a Phase 1 clinical study (NCT06014762) for CD19- and CD20-positive B-cell malignancies**

PLEASE SEE OUR OTHER POSEIDA PRESENTATIONS

- A Phase 1 Study of P-BCMA-ALLO1, a Non-Viral, Allogeneic BCMA Directed CAR-T in Relapsed/Refractory Multiple Myeloma (RRMM): Results from Optimized Lymphodepletion Cohort; **Poster #4828**
- Late Polyclonal P-BCMA-101 CAR-T Cell Re-Expansion and Rapid Complete Response in a Patient with Relapsed Multiple Myeloma Treated with One Cycle of Talquetamab, More Than 3 Years after CAR-T Infusion; **Poster #2083**

ABBREVIATIONS: ASCT = Autologous Stem Cell Transplant; DLBCL NOS = Diffuse Large B Cell Lymphoma, Not Otherwise Specified; DLT = Dose Limiting Toxicities; ECOG = Eastern Cooperative Oncology Group; E:T = Effector-to-Target; FL = Follicular Lymphoma; HGBL = High-Grade B cell Lymphoma; KO = Knockout; LD = Lymphodepletion; MHC-I = Major histocompatibility complex Class I; MTD = Maximum Tolerated Dose; PMBCL = Primary Mediastinal Large B-cell Lymphoma; T_{SCM} = Stem Cell Memory T cells; T_{CM} = Central Memory T cells; TCR = T Cell Receptor; T_{EFF} = Effector T cells; T_{EM} = Effector Memory T cells; tFL = Transformed Follicular Lymphoma; SEM = Standard Error of the Mean; WT = Wild Type