Memory Phenotype in Allogeneic Anti-BCMA CAR-T Cell Therapy (P-BCMA-ALLO1) Correlates with In Vivo Tumor Control

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ABSTRACT

Background: The emergence of CAR-T cell therapy has transformed the treatment of refractory/relapsed multiple myeloma (MM). Yet, autologous CAR-T cells suffer from many manufacturing challenges including mainly consistency, toxicity, and cost. To address these issues, we engineered a fully allogeneic anti-BCMA CAR-T cell candidate for MM from healthy donors (P-BCMA-ALLO1). Herein, we demonstrate that these cells maintain an early memory phenotype with a high percentage of stem cell memory T cell (T_{SCM}), which correlates with in vivo antitumor efficacy.

Methods: Using Poseida's non-viral piggyBac® (PB) DNA Delivery System in combination with the high-fidelity Cas-CLOVER™ (CC) Site-Specific Gene Editing System and a proprietary "booster molecule", we generated P-BCMA-ALLO1 from healthy donor T cells. We used CC to eliminate surface expression of both the TCR and MHC class I to make fully allogeneic CAR-T cells. In addition to the CAR molecule, PB enables the delivery of a selectable marker allowing the generation of a final cell product that is >95% CAR-positive. The inclusion of the "booster molecule" in the manufacturing process improves the expansion of TCR-negative gene-edited cells without compromising memory phenotype or function. This process can produce up to hundreds of patient doses from a single manufacturing run, which significantly reduces manufacturing cost per dose. We characterized the memory phenotype of P-BCMA-ALLO1 by assessing the mRNA and protein expression profiles of rested and activated CAR-T cells by flow cytometry and Nanostring analysis. We also assessed the antitumor capabilities of these cells using in vitro cytotoxicity assays including a serial in vitro restimulation assay to assess the ability of P-BCMA-ALLO1 to undergo multiple rounds of activation and expansion. We then evaluated the relationship of these characteristics with in vivo efficacy, as defined by control of tumor in an immunodeficient (NSG) RPMI-8226 subcutaneous murine tumor model.

Results: P-BCMA-ALLO1 is comprised of a high percentage of T_{SCM}. It has potent in vivo antitumor activity, which is comparable to non-edited healthy-donor autologous anti-BCMA CAR-T cells. Expression of memory/differentiation markers at both mRNA and protein levels across individual lots significantly correlates with in vivo tumor control. Conversely, suboptimal research products with poor in vivo outcomes expressed an exhausted gene expression profile. Moreover, the best CAR-T products were also more viable, cytotoxic, and proliferative following multiple rounds of restimulation in vitro.

Conclusions: P-BCMA-ALLO1 is a highly potent and safe allogeneic anti-BCMA CAR with a manufacturing process that consistently maintains a T_{SCM} phenotype, which correlates with antitumor efficacy. The P-BCMA-ALLO1 study IND recently received clearance from the FDA and advancing rapidly towards the clinic (NCT04960579).

KEY QUESTIONS

- **What characteristics (in vitro or ex vivo) correlate with preclinical efficacy?**
- **Does the high T_{SCM} percentage in P-BCMA-ALLO1 lend a functional advantage in tumor control?**
- **Can we screen P-BCMA-ALLO1 for favorable profiles that correlate with in vivo tumor efficacy?**

METHODS

Overview of P-BCMA-ALLO1

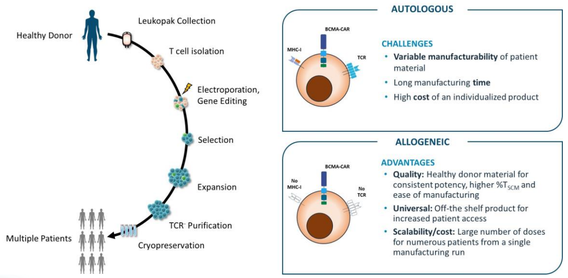

Figure 1: Overview of P-BCMA-ALLO1 manufacturing. Fully resting T cells isolated from healthy donors are edited through piggyBac (PB) and Cas-CLOVER (CC) to express BCMA-CAR and to knockout major histocompatibility complex class I (MHC-I) and T cell receptor (TCR). After T cell activation, CAR$^+$ selection, and expansion, TCR$^-$ CAR-T cells are purified at harvest and cryopreserved in multiple doses ready for infusion. The end result of this process is a CAR-T cell product rendered allogeneic by its lack of TCR and a mix of MHC-I$^+$ and MHC-I$^-$ cells. This manufacturing process carries several advantages over the production of autologous CAR-T cells, which is prone to variability, long manufacturing timelines, and high cost.

METHODS

P-BCMA-ALLO1 is Efficiently Edited and Manufactured

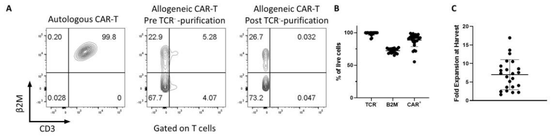

Figure 2: Production of P-BCMA-ALLO1 is highly efficient. CC efficiently targets *B2M* and *TRBC* with few off-target cuts to render resting CAR-T cells allogeneic. A) Representative flow plots show that with CC editing, the majority of P-BCMA-ALLO1 cells at harvest (14d after electroporation) are β2M$^-$TCR$^-$ CAR-T cells. These cells are further enriched after TCR$^-$ purification, with a small β2M$^+$ population left as a hedge against natural killer (NK) cell-mediated attack on CAR-T cells lacking MHC-I. B) Quantitatively, the majority of P-BCMA-ALLO1 are β2M$^-$, TCR$^-$, CAR$^+$ as measured by flow cytometry. C) The yield of P-BCMA-ALLO1 is on average 7X the number of T cells initially electroporated. Error bars represent standard deviation.

RESULTS

P-BCMA-ALLO1 Has a High Percentage of T_{SCM}

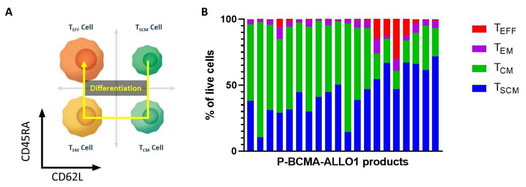

Figure 3: P-BCMA-ALLO1 has a large percentage of T_{SCM}. A) T cells can be categorized into different memory phenotypes based on their expression of CD45RA and CD62L, from naïve/stem cell memory T cells (T_{SCM}) to central memory T cells (T_{CM}) to effector memory T cells (T_{EM}) to their terminal differentiation as effector T cells (T_{EFF}). Memory T cells at the start of this differentiation pathway are quiescent, long-living, and more proliferative, and lose these characteristics as they differentiate. B) P-BCMA-ALLO1 products are majority T_{SCM} and T_{CM}, demonstrating that these cells carry a strong memory phenotype.

P-BCMA-ALLO1 is Effective and Persistent In Vivo

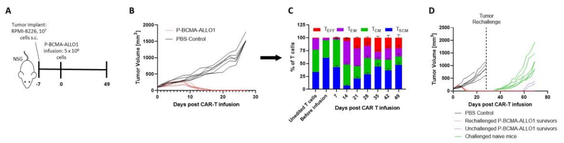

Figure 4: P-BCMA-ALLO1 is effective in vivo with high T_{SCM} persistence. A) We used a subcutaneous RPMI-8226 tumor model in NSG mice to evaluate P-BCMA-ALLO1 efficacy. B) A representative tumor growth curve of one P-BCMA-ALLO1 product showing complete tumor growth by 18 d post infusion. C) In that same experiment, when we look at memory phenotype of peripheral blood CAR-T cells over time, we see the reduction of T_{SCM} in favor of CD62L$^-$ T_{EM} and T_{EFF} to attack the tumor. After complete control, the remaining are not only maintained but enriched over time to near pre-infusion levels. D) This long-term persistence is demonstrated in a rechallenge experiment in the same model, wherein survivors after P-BCMA-ALLO1 treatment with complete tumor control were rechallenged on the opposite flank but remaining engrafted T_{SCM} prevented tumors from growing. Error bars represent standard deviation.

RESULTS

Memory Phenotype Correlates with Antitumor Efficacy

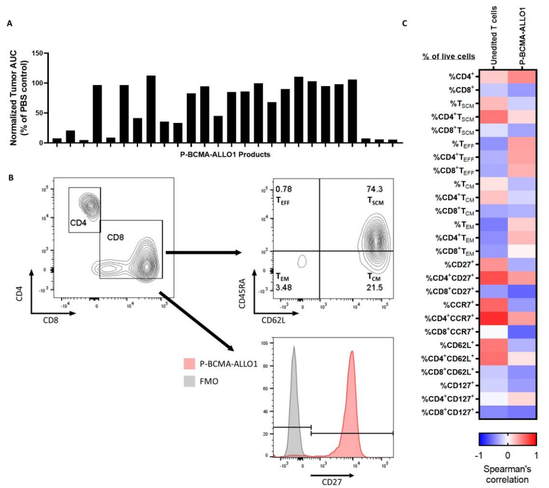

Figure 5: The memory phenotype of P-BCMA-ALLO1 correlates with in vivo tumor control. A) Using tumor area under the curve (AUC) normalized to PBS control, we get a range of efficacy that allows us to understand what phenotypes and characteristics correlate with in vivo tumor control. B) Gating strategy to measure the different memory phenotypes (T_{SCM}, T_{CM}, T_{EM}, T_{EFF}) and the percentage of positive expression of other markers of memory like CD27, CCR7, CD62L, and CD127 using a fluorescence minus one (FMO) control. C) In our correlative analysis against tumor AUC, we found strong significant correlations with lower tumor growth (or better tumor control) between the number of CD8$^+$ T cells expressing CD27, CCR7, and CD127 in P-BCMA-ALLO1 products. We also found strong positive correlations with memory CD4$^+$ T cells expressing CD27 and CCR7 prior to editing, which we hypothesize is a reflection that memory CD8$^+$ T cells are preferable to memory CD4$^+$ T cells.

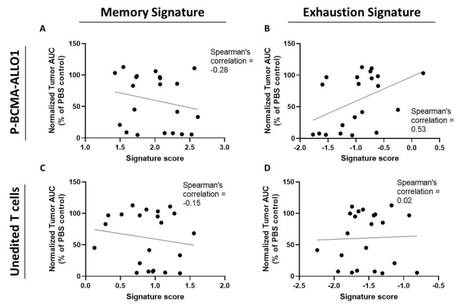

Figure 6. Memory and exhaustion signatures correlate with in vivo tumor efficacy. Correlations of signature scores derived from geneset analysis (GSA) with Nanostring mRNA counts for (A,C) memory over exhaustion or (B,D) exhaustion or memory in (A,B) P-BCMA-ALLO1 and (C,D) Unedited T cells. A memory expression profile is negatively correlated with tumor AUC, showing its importance in tumor control, while an exhaustion signature is positively correlated with tumor control.

RESULTS

In Vitro Functionality Correlates with In Vivo Tumor Control

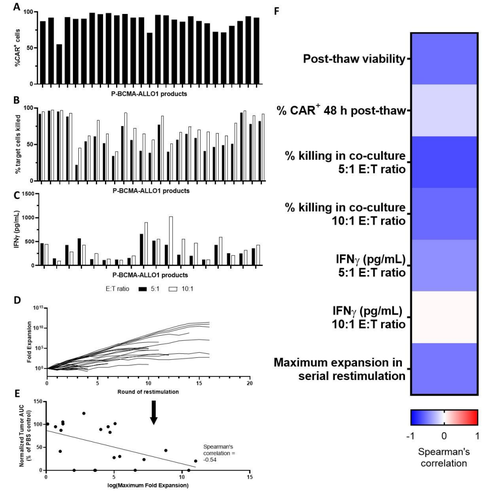

Figure 7: Effective P-BCMA-ALLO1 products are viable, proliferative, and cytotoxic in vitro. We assayed P-BCMA-ALLO1 for other characteristics in vitro to assess their correlations with in vivo efficacy. A) The percentage of CAR$^+$ after 48 h of rest post-thaw was measured by flow in P-BCMA-ALLO1. B) We also measured their cytotoxicity against K562-BCMAs in a 48h co-culture assay at different effector:target (E:T) ratios. C) From those same co-cultures, we measured IFNγ concentration. We also assayed the proliferative capacity of P-BCMA-ALLO1 using a serial restimulation assay against irradiated K562 cells expressing BCMA. D) Growth as a function of restimulation round shows the range of proliferation in P-BCMA-ALLO1 products. E) When maximum expansion is plotted against tumor AUC, we find a significant negative correlation. F) Our heatmap of correlations between in vitro assays and tumor AUC shows that viable, cytotoxic, and proliferative cells are strongly correlated with tumor control.

CONCLUSIONS

- **P-BCMA-ALLO1 is manufactured from healthy donors using piggyBac and Cas-CLOVER to produce potentially hundreds of allogeneic CAR-T doses**
- **In vivo, P-BCMA-ALLO1 is potent and exhibits the ability to persist**
- **P-BCMA-ALLO1 is comprised of a high percentage of T_{SCM} cells, whose phenotype correlates strongly with in vivo tumor control**
- **In vitro, viability and cytotoxicity of P-BCMA-ALLO1 are also strong correlates with in vivo tumor control**
- **These results support better screening methods for healthy donors and P-BCMA-ALLO1 products to assess cell health and fitness and possibly predict clinical efficacy**

REFERENCES

1. Wherry, EJ et al. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* **27**, (2007)