Manufacture of an Allogeneic CAR-T Stem Cell Memory Product Candidate for Multiple Myeloma, P-BCMA-ALLO1, Is Robust, Reproducible and Highly Scalable

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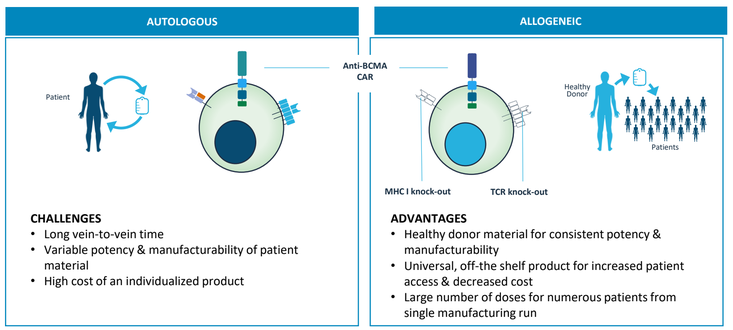
ABSTRACT

Chimeric Antigen Receptor (CAR) T cell therapy has generated unprecedented efficacy in the treatment of multiple hematologic malignancies. For relapsed/refractory Multiple Myeloma (MM), autologous CAR-T products directed against the B cell maturation antigen (BCMA), such as Poseida's P-BCMA-101, have demonstrated significant efficacy. P-BCMA-101 is comprised of a high-percentage of stem cell memory T cells (TSCM), resulting in a product that is much safer and potentially more durable than other anti-BCMA autologous product candidates. However, as individualized products, all autologous CAR-T products are expensive to manufacture and dependent upon patient T-cells of variable quality. We are developing P-BCMA-ALLO1, an off-the-shelf allogeneic (allo) BCMA-specific CAR-T product candidate derived from healthy donor material, which provides numerous advantages over autologous products, increasing patient access by being immediately available and greatly reducing manufacturing cost and variability.

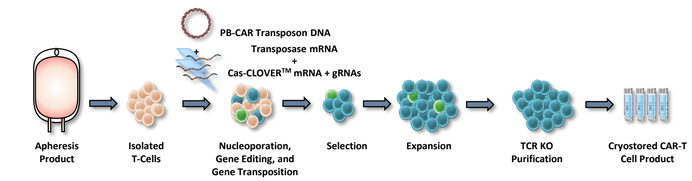
P-BCMA-ALLO1 is produced using two key platform technologies: the nonviral piggyBac® (PB) DNA Modification System and the high-fidelity Cas-CLOVER™ (CC) Site-Specific Gene Editing System. The mRNA coding for hyperactive, or "Super PB" transposase (SPB), and CC enzymes are codelivered with the P-BCMA-ALLO1 PB-based DNA transgene via electroporation to healthy donor T cells to stably integrate the transgene, as well as to knockout (KO) several mediators of allo graft-versus-host and host-versus-graft responses to maximize patient safety and durability of response. The P-BCMA-ALLO1 transgene encodes three genes, a BCMA-specific single-domain variable heavy chain (VH)-CAR (VCAR) gene, a drug selection gene to generate a ~100% CAR+ product, as well as a caspase-based safety switch gene to reduce or eliminate the product in vivo, if desired. The CC System is used to KO the endogenous T Cell Receptor (TCR) and beta-2 microglobulin, thereby decreasing Major Histocompatibility Complex (MHC) class I expression. KO of these key targets is aimed to prevent graft-versus-host disease, as well as reduce host-versus-graft rejection of the product. The CC System can efficiently edit resting T cells, thereby maintaining a high-percentage of TSCM cells, and does not create unwanted off-target mutations, another important consideration when creating an allo product candidate. To maximize the number of doses produced from a single manufacturing run, we have developed a proprietary "booster molecule" that allows for significant expansion of TCR-KO CAR-TSCM cells to potentially produce hundreds of doses.

To date, large-scale manufacturing of significant doses of potent allo CAR-T products has been challenging for the field. P-BCMA-ALLO1 manufacturing uses a potentially unlimited number of individual serial donors. We have currently produced P-BCMA-ALLO1 at both research and near-commercial scale from >35 donors with >97% manufacturing success. While a range of TCR-KO efficiencies was observed (~50-90%), the final product was always >99% homozygous TCR-KO after a purification step. Overall expansion of TCR-KO cells ranged from ~2-20 fold, and after removal of unedited TCR+ cells ~0.42-7.04x10e9 TCR-KO cells were recovered from 0.75x10e9 starting cells. However, working at clinical production scale (starting with ~3x10e9 cells), up to 250 doses of P-BCMA-ALLO1 could be manufactured per run, at a dose of 150x10e6 cells/patient. Importantly, with this level of donor and manufacturing robustness, no significant prior screening of donor material, other than to meet standard FDA requirements, would be needed.
P-BCMA-ALLO1 made from multiple donors were comprised of an exceptionally high-percentage of the desirable TSCM cells (CD45RA+CD62L+CD45RO-) and had minimal to no expression of exhaustion markers, such as PD-1 or Lag3. Furthermore, P-BCMA-ALLO1 demonstrated potent efficacy in the RPMI-8226 xenograft model in NSG mice across multiple products generated from separate individual healthy donors. Altogether, these data demonstrate a robust, reproducible and highly scalable manufacturing process. Moreover, this manufacturing process can easily be expanded for use with additional CAR targets for treatment of other hematologic or solid tumor malignancies.

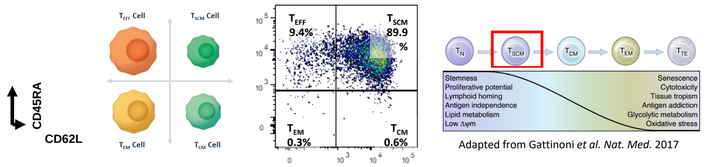
INTRODUCTION



Overview of P-BCMA-ALLO1 Manufacturing Process



Phenotype of P-BCMA-ALLO1 Product



Adapted from Gattinoni *et al. Nat. Med.* 2017

METHODS & RESULTS

Cas-CLOVER™ Gene Editing System - The Best of Both Worlds

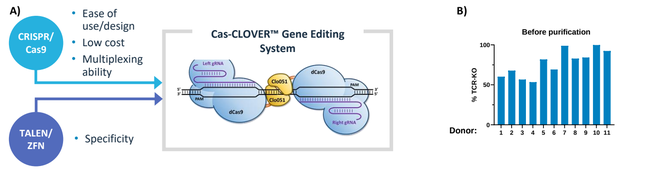


Fig. 1 Cas-CLOVER™ Gene Editing System combines the strengths of CRISPR/Cas9 and TALEN/ZFN editing approaches, and efficiently edits in resting T cells. A) The site-specific Cas-CLOVER™ Gene Editing System consists of a dimeric gRNA-guided nuclease. Editing activity is guided by a pair of gRNAs allowing for ease of design as well as multiplexing ability found in CRISPR/Cas9-based systems. At the same time editing activity is contingent on dimerization of two half domains, similar to TALENs or ZFNs, leading to exquisite editing specificity. B) Cas-CLOVER™ efficiently edits resting T cells. Upon editing, efficient disruption of surface expression of TCRα/β and CD3 was detected via flow cytometry and graphed.

Cas-CLOVER™ Shows no Off-Target Activity

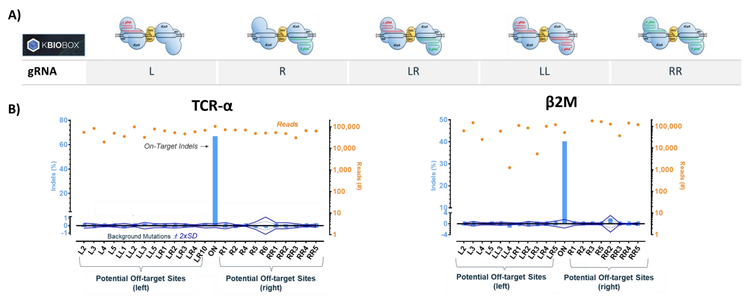


Fig. 2 Cas-CLOVER™ editing in the TRAC and B2M loci shows no off-target editing activity by NGS. A) Cas-CLOVER™ was used to edit resting T cells using gRNA pairs targeting TRAC or B2M and potential off-target sites for different combinations of gRNAs were surveyed via NGS. B) NGS revealed efficient on-target editing for both TRAC and B2M loci while no above-background off-target editing events were detectable. Non-edited resting T cells from 3 donors were used to assess the level of non-editing related background mutations.

Booster Molecule Increases Yield of P-BCMA-ALLO1

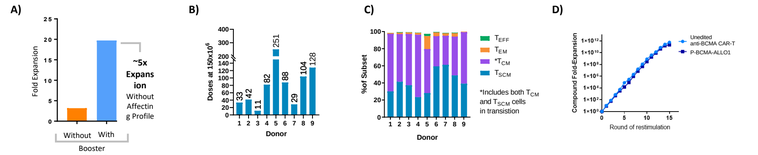


Fig. 3 A Booster Molecule increases the yield of P-BCMA-ALLO1 cells while maintaining proliferative potential and the desirable early memory phenotype. P-BCMA-ALLO1 CAR-T cells were produced in presence or absence of the Booster Molecule. Addition of the Booster Molecule led to significant increases in cell expansion during production A) and increased the percentage of TCR KO cells at the end of production B) leading to increased product yield of P-BCMA-ALLO1. C) At the same time, P-BCMA-ALLO1 grown in the presence of the Booster Molecule maintained a high fraction of desirable T_{SCM} cells and D) in an *in vitro* serial restimulation assay, P-BCMA-ALLO1 had a similar proliferative potential to TCR-replete (unedited) CAR-T.

P-BCMA-ALLO1 - a Highly Pure Allogeneic CAR-T Product

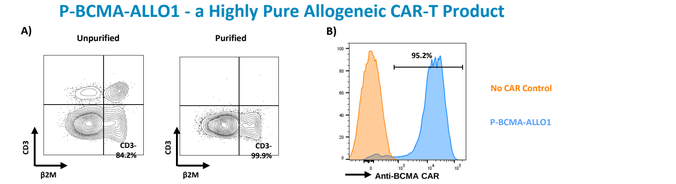


Fig. 4 The final P-BCMA-ALLO1 product is >99% TCR KO and >95% CAR-positive. A) At the end of P-BCMA-ALLO1 production, a clinically-scalable purification step is added to deplete remaining TCR-positive CAR-T cells and yield a highly pure allogeneic CAR-T cell product comprised of more than >99.9% TCR KO cells. B) The presence of a selectable marker in the P-BCMA-ALLO1 transposon allows for the generation of a P-BCMA-ALLO1 cell product that is >95% CAR-positive.

P-BCMA-ALLO1 Exhibits Potent Anti-tumor Effects *In Vivo*

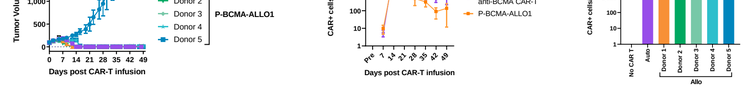


Fig. 5 P-BCMA-ALLO1 exhibits potent anti-tumor effects in an *in vivo* mouse model of MM. P-BCMA-ALLO1 (ALLO) was tested in alongside with healthy donor non-edited cells (HDNE, CAR-positive/TCR-positive) in an *in vivo* model of MM tumor control. A) NGS mice were implanted sub-cutaneously with $1x10^7$ RPMI-8226 BCMA+ tumor cells and tumors were established for 7 days before injection with $1x10^7$ CAR-T cells. P-BCMA-ALLO1 exhibited potent and sustained anti-tumor efficacy that was comparable to non-edited control CAR-T cells. B-C) P-BCMA-ALLO1 also demonstrated robust *in vivo* proliferation that could be detected in the blood of treated animals by day 14 after T cell administration. C) The peak of expansion (Day 14) correlated with the timing of tumor control observed in A) and was similar to expansion levels observed for TCR-replete (unedited) CAR-T.

P-BCMA-ALLO1 Does not Mediate an Allogeneic Response

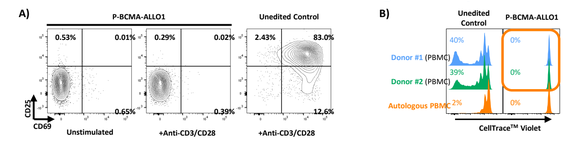


Fig. 6 P-BCMA-ALLO1 is not capable of mediating allogeneic responses. A) To show TCR KO in the P-BCMA-ALLO1 final product on a functional level, cells were exposed to an activation regiment comprised of anti-CD3 and anti-CD28 antibodies for 24 h. P-BCMA-ALLO1 did not show any upregulation of the activation markers CD25 and CD69 by flow cytometry. In contrast, unedited control CAR-T cells showed a strong induction of CD25 and CD69 in response to activation. B) P-BCMA-ALLO1 cells or donor-matched, unedited control CAR-T cells were exposed to PBMCs from mismatched donors as well as autologous PBMCs. While the unedited control CAR-T cells showed robust induction of CAR-T cell proliferation in response to the allogeneic PBMCs. In contrast, P-BCMA-ALLO1 cells did not proliferate, showing that P-BCMA-ALLO1 cannot mediate an allogeneic response.

CONCLUSIONS

- Highly desirable T_{SCM}-rich product phenotype and potent proliferative potential
- Allogeneic CAR-T equivalent or better than unedited healthy donor CAR-T *in vitro* & *in vivo*
- Robust non-viral manufacturing process compatible with majority of healthy donors & ability to generate hundreds of doses per manufacturing run
- Superior safety due to T_{SCM} phenotype, no/low off-target gene editing, and safety switch
- Results support rapid advancement of P-BCMA-ALLO1 into the clinic for treatment of MM