A Novel BCMA-Specific, Centyrin™-Based CAR-T Product for the Treatment of Multiple Myeloma

David Hermanson, Burton E. Barnett, Srinivas Rengarajan, Rebecca Codde, Xinxin Wang, Yening Tan, Christopher E. Martin, Jenessa B. Smith, Jin He, Rohit Mathur, Jing Yan, Satvaa Neelapu, Eric M. Ostertag, Devon J. Shedlock

Poseida Therapeutics, Inc. 4242 Campus Point Court, Suite 700 San Diego, CA, 92121. §MD Anderson Cancer Center, Houston, TX

ABSTRACT

Chimeric-antigen receptor (CAR-T) cell immunotherapies have been remarkably effective in treating acute lymphoblastic leukemia. However, current strategies generally suffer from difficult, inefficient and costly manufacturing processes, significant patient-to-patient variability and poor durability of response in some patients. Here, we report for the first time a CAR-T cell therapeutic, comprising a non-immunoglobulin alternative scaffold Centyrin™ molecule (i.e. “Centyrin™”) manufactured with a novel non-viral piggyBac™ (PB) transposition-based system. Our lead candidate, P-BCMA-101, encodes a Centyrin™ that targets the BCMA receptor, a novel BCMA agonist (BCMA) for the treatment of multiple myeloma (MM) and has several unique aspects that improve upon earlier CAR-T products.

First, P-BCMA-101 is manufactured using only in vitro transcribed mRNA and plasmid DNA without the need for lentivirus or piR-retrovirus, resulting in time and cost savings. Importantly, PB is also less than viral systems due to a less mitogenic insertion profile and is non-oncogenic. Furthermore, PB can efficiently deliver transgenes as large as several hundred kilobases, once transfected, transgenes demonstrate more stable, prolonged and higher expression when compared to those delivered by virus. Second, a selection gene is included to provide a simple and effective method of CAR-T cell enrichment and reduces variability in patient product material. Third, P-BCMA-101 incorporates a safety switch for optional depletion in vivo in case of adverse events. Lastly, the Centyrin™ is comprised of a BCMA-specific Centyrin™, which are based on a human tenascin fibronection type III (FN3) consensus sequence. Centyrin™ have similar binding affinity to the antibodies-derived single chain variable fragments (scFv), but are smaller, more thermostable and predicted to be less immunogenic. Importantly, no signs of toxic signaling leading to T cell exhaustion have been observed with Centyrin™ unlike scFv-based CAR molecules, which can interact with each other on the surface causing non-specific CAR signaling.

The manufacturing process of P-BCMA-101 from primary human T cells is facile, scalable, reproducible, employs no virus, cytokines, or magnetic beads, and easily produces enough CAR-T cells to treat patients. Within 24 hours of electroporation of purified T cells, we demonstrate >95% of the cell product is positive for Cartyrin™ expression and ready to be administered. Notably, our manufacturing process results in >80% of CAR-T cells exhibiting a stem-cell memory phenotype (i.e. CD45RA+ CD27+). P-BCMA-101 cells exhibit specific and robust in vitro activity against MM tumor targets, ranging from high to very low levels of BCMA, as measured by target-cell killing and Cartyrin™ cell proliferation. Importantly, positologging P-BCMA-101 cells was completely protective in vivo to activation of the safety switch. Finally, we have evaluated the anti-tumor efficacy of P-BCMA-101 in 2 separate in vivo experiments as a model of human MM. In the first, NSG™ mice were inoculated with MM cells and treated with PBCMA-101 cells, an aggressive human MM-derived cell line. After the tumors were allowed to grow for two weeks, PBCMA-101 treated animals demonstrated a marked increase in serum M-protein levels, rapid growth of tumor cells demonstrated a reduction of >50% in tumor burden. Tumor cells treated with PBCMA-101 cells exhibited increased levels of Ki67 seen in single cell tumor levels. All untreated control animals demonstrated a marked increase in serum M-protein levels, rapid growth of tumor cells demonstrated a reduction of >50% in tumor burden. Tumor cells treated with PBCMA-101 cells exhibited increased levels of Ki67 seen in single cell tumor levels. In the second experiment, the same tumor model was used to assess the efficacy of PBCMA-101 in 12 aged, non-human primates. Survival data in mice was collected for 12 weeks, serum levels of BCMA were measured. Patients were observed for multiple instances of relapse and then eliminated, suggesting that PBCMA-101 cells persist longer and maintain their anti-tumor efficacy likely due to the stem-cell memory phenotype.

METHODS & RESULTS

Figure 1: Manufacturing Process: Pan T cells are isolated from anapheresis product, and then electroporated with P-BCMA-101 plasmid DNA and in vitro transcribed piggyBac™ transposomal mRNA. The electroporated cells are then activated, expanded, and selected prior to freezing. The process yields a >10^6 cells with >95% Cartyrin™ expression.

Figure 2: P-BCMA-101 Phenotype: P-BCMA-101 cells were evaluated by flow cytometry for typical T cell markers following the manufacturing process. (A) Expression of T cell phenotypic markers on CD3 and CD8 T cells. (B) Expression of commonly associated activation/maintenance markers. (C) Expression of the Cartyrin™ following secondary activation.

Figure 3: In vitro P-BCMA-101 Activity: P-BCMA-101 cells were evaluated for their (A) in vitro cytotoxicity and (B) proliferation in response to HIV-1 (MCF-7) with or without CD138 (BCMA) tumor lines. (A) XTT assay for 10^5 H929 and KG1 cells were plated in the same well of a 96-well plate followed by 1/2generation, 1/3 generation, or 1/6 generation P-BCMA-101 cells. Cells were incubated for the indicated time points, harvested and analyzed by flow cytometry. Cytotoxicity was determined by XTT in triplicate (N=30% no effect/ N=20% no effect). All samples were run in triplicate. (B) CFSE labeled P-BCMA-101 cells were co-cultured in the presence of H929 or KG1 cells for 4 days, harvested and analyzed by flow cytometry. All samples were run in duplicate (N=96% no depletion/ N=92% no depletion). P-BCMA-101 cells were treated with a small molecule for the indicated length of time and concentrations. All data points were collected in triplicate and relative viability determined by dividing the number of live cells in the treatment group by the average number of live cells in the no treatment group per 1,500 bead events collected.

CONCLUSIONS

- P-BCMA-101 is novel CAR T cell product that uses a smaller and less immunogenic Centyrin™ molecule to target BCMA for the treatment of multiple myeloma.
- Manufacturing requires only a single plasmid and in vitro transcribed mRNA using the piggyBac™ system.
- The process does not require virus, cytokines, or magnetic beads, and is easily scalable to generate patient doses yielding >95% Cartyrin™ cells.
- P-BCMA-101 final product cells predominantly exhibit a stem cell memory phenotype and no significant expression of inhibitory/exhaustion markers.
- We have observed no indication of any constitutive signaling.
- In vitro studies demonstrate specific cell lysis and secondary proliferation against BCMA™ targets.
- P-BCMA-101 results in rapid initial eradication of tumor and unprecedented elimination of tumor due to relapse in a standard xenograft model.

Centyrin™ is a registered trademark of Jansen Pharmaceuticals, Inc. Poseida has licensed certain rights to the Centyrin™ technology platform from Jansen Pharmaceuticals, Inc. for use in autologous T cell therapeutics.