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

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ABSTRACT

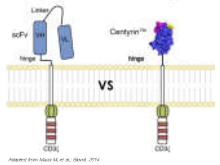
Chimeric-antigen receptor (CAR)-T cell immunotherapies have been remarkably effective in treating acute lymphoblastic leukemia. However, current strategies generally suffer from difficult, inefficient and costly manufacturing processes, significant patient side effects and poor durability of response in some patients. Here, we report for the first time a CAR-T cell therapeutic comprising a non-immunoglobulin alternative scaffold Centyrin™ molecule (a "CARTyrin") manufactured with a novel non-viral piggyBac™ (PB) transposon-based system. Our lead candidate, P-BCMA-101, encodes a CARTyrin that targets the B cell maturation antigen (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 γ–retrovirus, resulting in time and cost savings. Importantly, PB is also safer than viral systems due to a less mutagenic insertional profile and is non-oncogenic. Furthermore, PB can efficiently deliver transgenes as large as several hundred kilobases, and, once inserted, 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 CARTyrin+ 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 CARTyrin is comprised of a BCMA-specific Centyrin™, which are based on a human tenascin fibronectin type III (FN3) consensus sequence. Centyrins™ have similar binding affinities to the antibody-derived single chain variable fragments (scFv), but are smaller, more thermostable and predicted to be less immunogenic. Importantly, no signs of tonic signaling leading to T cell exhaustion have been observed with CARTyrins unlike scFv-based CAR molecules, which can interact with each other on the surface causing non-specific CAR signaling.

The manufacture 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 CARTyrin+ cells to treat patients. Within 18 days 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 > 60% of CARTyrin+ T cells exhibiting a stem-cell memory phenotype (i.e. CD45RA+ CD62L+). P-BCMA-101 cells exhibit specific and robust in vitro activity against BCMA+ tumor targets, ranging from high to very low levels of BCMA, as measured by target-cell killing and CARTyrin-T cell proliferation. Importantly, proliferating P-BCMA-101 cells were highly sensitive in vitro to activation of the safety switch. Finally, we have evaluated the anti-tumor efficacy of P-BCMA-101 in 2 separate *in vivo* experiments in a model of human MM. In the first, NSG™ mice were injected IV with 1.5x10^6 luciferase+ MM.1S cells, an aggressive human MM-derived cell line. After the tumor cells were allowed to grow for 21 days, animals received a single IV administration of 5x10^6 P-BCMA-101 cells. All untreated control animals demonstrated a marked increase in serum M-protein levels, rapid growth of tumor cells demonstrated by bioluminescent imaging (BLI), and death within four weeks. In stark contrast, 100% of animals that received P-BCMA-101 rapidly eliminated tumors within 3-7 days as measured by BLI and serum M-protein levels and improved survival out to at least 60 days post-treatment. In a second larger experiment, the same tumor model was used, but animals received either 4x10^6 or 12x10^6 P-BMCA-101 cells. Strikingly, we observed multiple instances of relapse and then elimination, suggesting that P-BCMA-101 cells persist longer and maintain their anti-tumor efficacy likely due the stem-cell memory phenotype.

P-BCMA-101 is the first-in-class of Centyrin™-based CAR therapeutics. The CARTyrin, combined with our advanced manufacturing processes, represents a significant improvement over first generation, immunoglobulin-based and virally-transduced CAR-T products. P-BCMA-101 exhibited an advantageous stem-cell memory phenotype and demonstrated specific and potent anti-tumor efficacy against BCMA+ myeloma cells both in vitro and in vivo. Based on these results, we plan to initiate a phase I clinical trial of P-BCMA-101 for the treatment of patients with relapsed and/or refractory MM.

INTRODUCTION



	scFv	Centyrin™
Size:	~250 aa	~90 aa
Components:	Heavy & Light Chain Variable Regions, Flexible Linker	Fibronectin type III domain
Derived:	Mouse/Rodent Abs	Human
Stability:	Not as stable as original IgG format	Stable

I. PiggyBac™-Produced CAR-T Cells Exhibit Stem-Cell Memory Phenotype Session 703 Poster I
II. Development of Novel Non-Immunoglobulin Centryin™-Based CARs (CARTyrins) Targeting Human BCMA Session 703 Poster III

METHODS & RESULTS

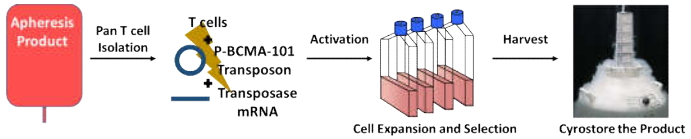


Figure 1: Manufacturing Process: Pan T cells are isolated from an apheresis product, and then electroporated with P-BCMA-101 plasmid DNA and in vitro transcribed piggyBac™ transposase mRNA. The electroporated cells are then activated, expanded, and selected prior to freezing. The process yields >1 x 10^9 cells with >95% CARTyrin expression.

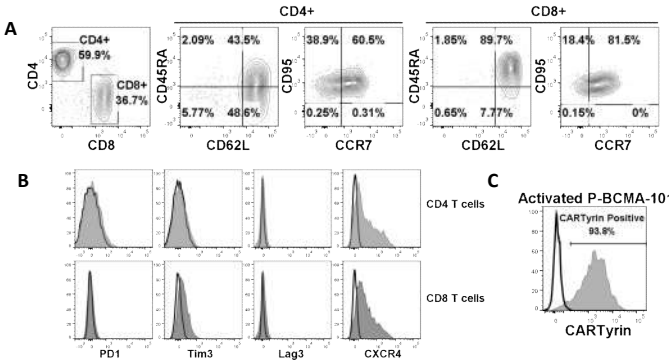


Figure 2: P-BCMA-101 Phenotype: P-BCMA-101 cells were evaluated by flow cytometry for typical T cell markers following the manufacturing process. (A) Expression of T cell phenotypic markers on CD4+ and CD8+ T cells. (B) Expression of commonly associated exhaustion/activation markers. (C) Expression of the CARTyrin following secondary activation.

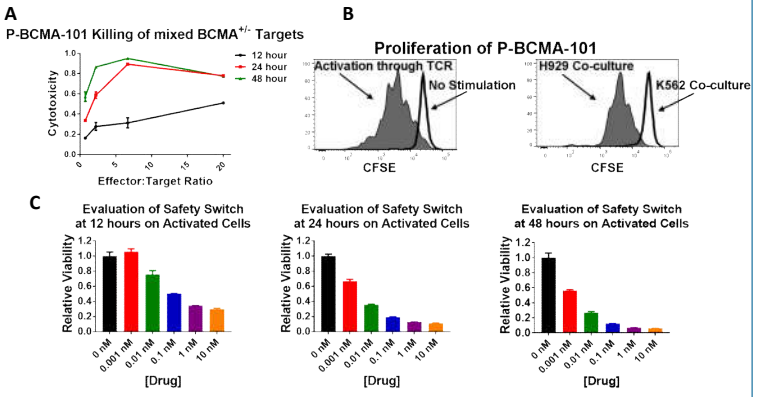


Figure 3: *In vitro* P-BCMA-101 Activity: P-BCMA-101 cells were evaluated for their (A) *in vitro* cytotoxicity and (B) proliferation in response to H929 (BCMA+) and K562/GFP (BCMA-) tumor lines. (A) 5x10^4 H929 and K562 cells were plated in the same well of a 96-well plate followed by 1x10^6, 3.33x10^5, 1.11x10^5, or 3.7x10^4 P-BCMA-101 cells. Cells were incubated for the indicated time points, harvested, and evaluated by flow cytometry. Cytotoxicity was determined by 1-((%H929 in sample/%K562 in sample)/(%H929 no effectors/%K562 no effectors)). All samples were run in triplicate. (B) CFSE labeled P-BCMA-101 cells were co-cultured in the presence of H929 or K562 cells for 4 days, harvested and evaluated by flow. All samples were run in duplicate. (C) Activated 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.

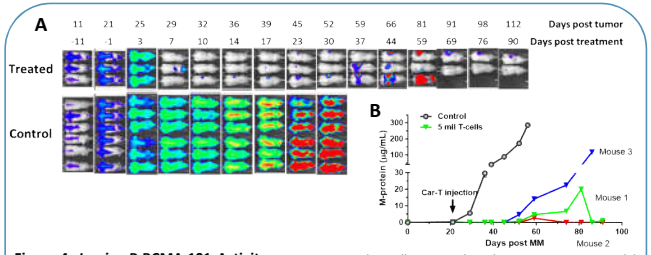


Figure 4: *In vivo* P-BCMA-101 Activity: P-BCMA-101 product cells were evaluated in an NSG™ mouse model. Male mice were injected with 1.5x10^6 MM.1S/Luciferase+ Cells that were allowed to implant over a 21 day period. Following tumor implant, mice were dosed with 5x10^6 P-BCMA-101 cells via i.v. administration. Tumor burden was monitored using (A) Bioluminescent imaging as well as through (B) M-Protein measurements in blood.

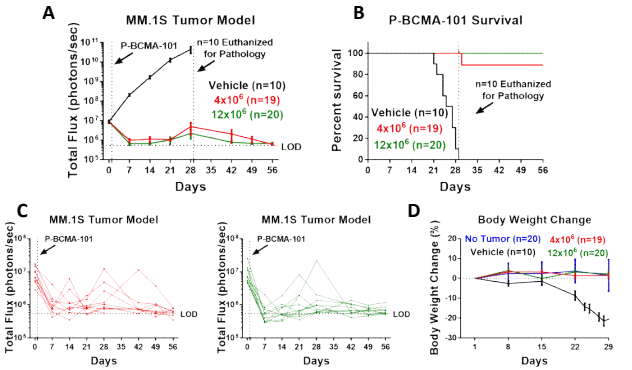


Figure 5: Expanded *in vivo* study using P-BCMA-101: P-BCMA-101 cells were again evaluated in a NSG™ mouse models using MM.1S/luciferase+ cells. Female mice aged 6-8 weeks were injected with 7.5x10^5 MM.1S/luciferase+ cells that were allowed to grow for 21 days. Once tumor burden had been confirmed, mice were injected i.v with vehicle, 4x10^6, or 12x10^6 P-BCMA-101 cells. Tumor burden was monitored using Bioluminescent imaging. (A) Graph represents the Mean ± SEM for each treatment group. (B) Survival graph. Both BLI and necropsy revealed no sign of tumor in the single death in the 4x10^6 dose. (C) Individual mice are plotted as a single line. (D) Body weight change. At Day 29, 10 mice from each treatment group were euthanized and submitted for pathology. The study is still on-going.

CONCLUSIONS

- P-BCMA-101 is novel CAR T cell product that uses a smaller and less immunogenic Centryin™ 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+ target cells
- P-BCMA-101 results in rapid initial eradication of tumor and unprecedented elimination of tumor due to relapse in a standard xenograft model