Very rapid production of CAR+ T cells upon non-viral gene transfer using the *Sleeping Beauty* System

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Our Main Finding

Genetically modified tumor-specific resting (non-activated) T cells were generated and infused in less than 2 days. This was accomplished based on electro-transfer of DNA plasmids from the (SB) coding for a CD19-specific chimeric antigen receptor (CAR) and membrane-bound IL-15 (mbIL15). A single low-dose of these rapidly-generated mbIL15-CAR T cells resulted in sustained persistence that produced potent anti-tumor effect and superior leukemia-free survival. This advancement to T-cell production will reduce manufacturing time within a GMP facility which translates to improved scalability and reduced costs.

BACKGROUND

Chimeric Antigen Receptors (CARs)

- Fusion of a monoclonal antibody single-chain variable fragment with T-cell activation/signaling endodomains.
- Redirects T-cell specificity to a cell surface tumor-associated antigen for an adoptive immunotherapy for cancers, especially hematologic malignancies.
- The Sleeping Beauty (SB) non-viral gene transfer system has been successfully tested in humans to express a CD19-specific CAR (signaling though CD28 and CD3-ζ). (Kebriaei *et al.* JCI. 2016, PMID: 27482888)

Limited T-Cell Persistence after Infusion

- Therapeutic effect is correlated with CAR+ T-cell persistence and engraftment, which depends on the frequency of less differentiated T-cell memory subsets within the infusion product.
- Achieving consistent long-term persistence across a variety of adoptive immunotherapy trials remains a critical issue.

Improving CAR+ T Cells with Cytokine Co-signaling

- Interleukin 15 (IL-15) is a homeostatic cytokine that supports long-lived memory T cells, inhibits activation-induced cell death, and enhances *in vivo* anti-tumor activity.
- Physiologically, IL-15 has tissue restricted activity as it is trans-presented to T cells by IL-15Rα, *e.g.*, by antigen presenting cells.
- We have shown that co-expressing a membrane-bound version of IL-15 (mbIL15) significantly enhances the *in vivo* persistence and anti-tumor activity of CAR+ T cells. (Hurton *et al.* PNAS. 2016, PMID: 27849619)

Rapid Manufacture with "Point-of-Care (POC)" Technology

- Current manufacture is based on gene transfer in activated T cells and *ex vivo* propagation to yield clinical numbers of T cells.
  - Current protocols for viral/non-viral-based platforms take 7 – 28 days.
  - Significant use of resources within a Good Manufacturing Practice (GMP) facility.
  - Activating and propagating T cells *ex vivo* tends to lead to a more differentiated infusion product (Figure 1).
- Reducing the manufacture time of CAR+ T cells is the basis of POC technology and appears essential to: (i) advancing modified cell-based therapies as a manufacturing platform with broad appeal, (ii) shortened time to treatment, and (iii) decreased costs.
- Less manipulation of the product may preserve less differentiated T-cell subsets and therefore improved product potency.

Rationale

- **We <u>hypothesized</u> that, with the co-expression of mbIL15, therapeutically effective CAR+ T cells could be generated and immediately infused without being activated and propagated.**
- We adapted the non-viral-based *Sleeping Beauty* (SB) gene modification system to electroporate and infuse CAR+ T cells in less than 2 days (Figure 1).

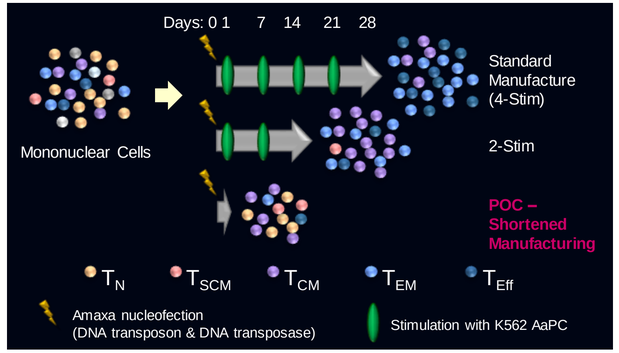

Fig. 1 Reducing manufacture time. Traditional CAR+ T-cell manufacture electroporate mononuclear cells (MNC) followed by several stimulations with K562-derived activating and propagating cells (AaPC). Unlike these methods, the POC approach can generate a genetically-modified T cells in less than 2 days. This manufacture process does not rely on activating and propagating the genetically modified T cells.

METHODS

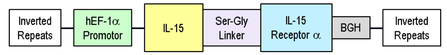

Fig. 2 The mbIL15 construct. The IL-15 cDNA sequence was fused to the full-length IL-15Rα sequence via a flexible serine-glycine linker. The coding sequence was codon optimized and subcloned into a SB-derived DNA plasmid to be used for non-viral gene transfer.

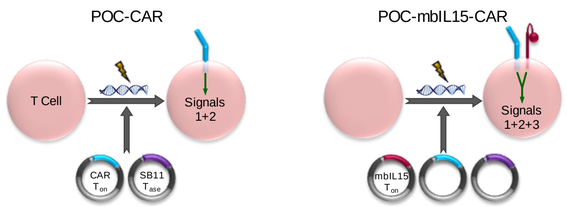

Fig. 3 POC-CAR T-cell generation. MNC were genetically modified, using nucleofection (electroporation), with mbIL15 and/or 2nd generation CD19-specific CAR (signaling through CD28 and CD3-ζ) coded from individual SB DNA transposon plasmids and SB11 transposase DNA plasmid. CAR engagement provides T-cell activation and co-stimulation signals while mbIL15 provides a 3rd stimulatory signal. Cells were then briefly placed in culture (with no exogenous cytokines) prior to injection into mice.

Established disseminated leukemia mouse model. NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice were intravenously injected with 1.5x10^4 CD19+ NALM-6 leukemia cells that expressed firefly luciferase (ffluc). Six days later when leukemia was established, CAR or mbIL15-CAR T cells were intravenously injected with 7.5x10^5 CAR+ T cells per mouse (*n*=5/group). POC-CAR^{neg} T cells (mock electroporated) were injected at an equivalent total T-cell dose. Tumor burden was serially monitored by bioluminescence imaging (BLI).

RESULTS

POC T-cell infusion product phenotype

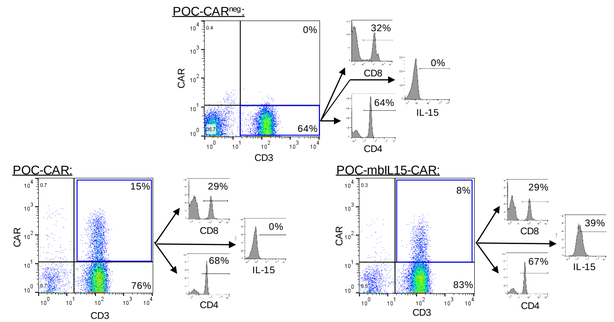

Fig. 4 Phenotype of genetically modified T cells at infusion. Less than 2 days after genetic modification, the POC T cells were harvested for infusion. The T cells were assessed for CAR and mbIL15 expression, as well as CD4 and CD8 ratio.

POC-mbIL15-CAR T cells mitigate systemic leukemia & increase survival

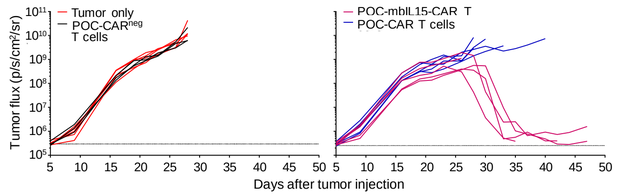

Fig. 5 POC-mbIL15-CAR T cells exhibit potent anti-tumor activity. NSG mice bearing established disseminated CD19+ NALM-6 leukemia were treated on day 6 with one 7.5x10^5 CAR+ T-cell dose of POC-CAR or POC-mbIL15-CAR T cells. Control POC-CAR^{neg} T cells were given at an equivalent total T cell dose. Quantified tumor burden (*ffLuc* activity) was measured by BLI. Each line represents an individual animal.

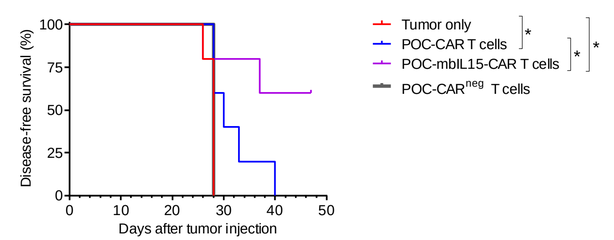

Fig. 6 POC-mbIL15-CAR T cells significantly improves survival. Kaplan Meier survival curves show disease-free survival whereby mice were considered disease-free when tumor flux was less than 10^7 p/s/cm^2/sr. Significance determined by log-rank (Mantel-Cox). *P < 0.05.

POC-mbIL15-CAR T cells persist at high frequencies

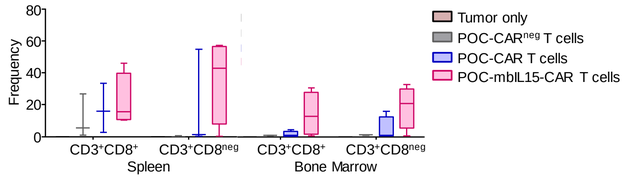

Fig. 7 Persistence of CD4 and CD8 subsets of POC-T cells. Spleen and bone marrow of mice at necropsy were analyzed 41 days after T-cell infusion to assess the level of human T-cell persistence, as well as the CD4 and CD8 ratio. Data is plotted as box and whiskers showing the median (*horizontal bar*).

SB11 is not detected in recovered POC-T cells

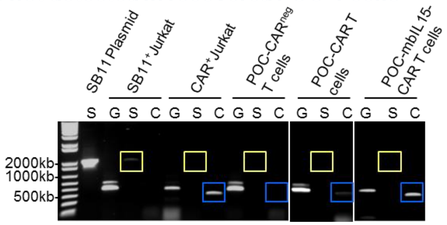

Fig. 8 SB11 transposase is not detected in cells isolated from mice. PCR of bone marrow harvested from mice at day 47 assessed the presence of SB11 transposase DNA and CAR transgene. S=SB11 primers, G= human GAPDH primers, and C=CAR primers.

CONCLUSIONS

- Therapeutically effective POC-CAR T cells can be rapidly generated without the need for *ex vivo* activation and extended culture time.
- Infusions of bulk POC cell products yielded *in vivo* engraftment of both CD4+ and CD8+ T cells, with skewing to engraftment of CD8+ T cells for mice treated with POC-CAR T cells and a more balanced *in vivo* CD4:CD8 ratio observed with the POC-mbIL15-CAR T-cell treatment.
- POC-mbIL15-CAR T cells sustained persistence and eradicated tumor with one low-dose of genetically modified cells (7.5 x 10^5 CAR+ T cells).
- Residual SB11 transposase from the genetic modification was not detected in cells isolated from mice.
- These data support a clinical trial to rapidly manufacture genetically modified T cells based on non-viral gene transfer using SB system to co-express CAR and mbIL15.

Conflict of Interest Disclosures: Hurton: *Intrexon:* Equity Ownership, Patents & Royalties; *Ziopharm Oncology:* Equity Ownership, Patents & Royalties. Singh: *Immatics:* Equity Ownership, Patents & Royalties; *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership, Patents & Royalties. Switzer: *Intrexon:* Equity Ownership, Patents & Royalties; *Ziopharm Oncology:* Equity Ownership, Patents & Royalties. Mi: *Intrexon:* Equity Ownership, Patents & Royalties; *Ziopharm Oncology:* Equity Ownership, Patents & Royalties. Maiti: *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership, Patents & Royalties. Su: *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership, Patents & Royalties. Huls: *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Employment, Equity Ownership, Patents & Royalties. Champlin: *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership, Patents & Royalties. Cooper: *Ziopharm Oncology:* Employment, Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership; *City of Hope:* Patents & Royalties; *Targazyme, Inc.:* Equity Ownership; *Immatics:* Equity Ownership; *Sangamo BioSciences:* Patents & Royalties; *Miltenyi Biotec:* Honoraria; *MD Anderson Cancer Center:* Visiting Scientist.