Rapid production of T cells co-expressing CAR and membrane-bound IL-15 potentiates antitumor activity and promotes *in vivo* memory

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BACKGROUND

Genetic Modification of T Cells

- Chimeric antigen receptor (CAR) redirects T-cell specificity to CD19 based on fusion of a monoclonal antibody single-chain variable fragment (scFv) coupled to T-cell activation/signaling endodomains
- The *Sleeping Beauty* (SB) non-viral gene transfer system
  - Successfully tested in humans to express a CD19-specific CAR (Kebriaei *et al.* JCI. 2016, PMID: 27482888)
  - Quiescent T cells can be stably genetically modified using SB system DNA plasmids
  - Eliminates the need to propagate cells in tissue culture

Scale-up and Costs of CAR T-cell Therapy

- T cells genetically modified with virus require (i) recombinant retrovirus/lentivirus and (ii) *ex vivo* replication/propagation
- Current manufacture protocols add complexity to produce patient-derived products:
  - Requires time (minimum of 6 days)
  - Expense (viral production & T-cell propagation)

Improving CAR⁺ T Cells with Cytokine Co-signaling

- Interleukin 15 (IL-15)
  - Homeostatic cytokine that supports long-lived memory T cells
  - Inhibits activation-induced cell death
  - Enhances *in vivo* antitumor activity
- Co-expression of a membrane-bound version of IL-15 (mbIL15) significantly enhances *in vivo* persistence and antitumor activity of CAR⁺ T cells (Hurton *et al*. PNAS. 2016, PMID: 27849619)

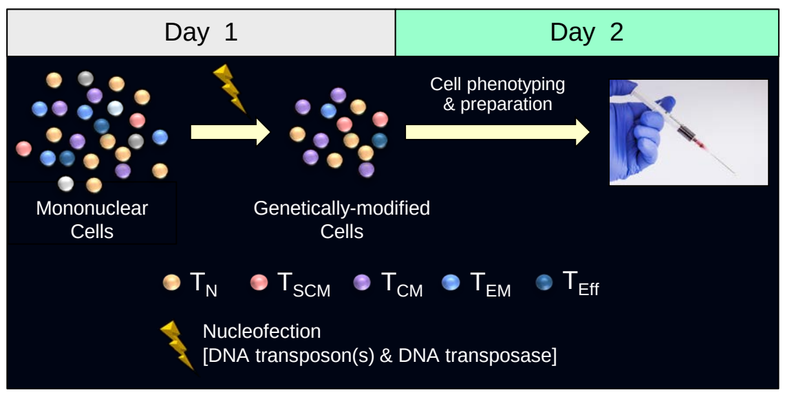

Fig. 1 Very rapid manufacture of T cells under P-O-C. The P-O-C approach can produce genetically-modified T cells in less than 2 days. This manufacturing process does not rely on activating and propagating T cells prior to gene transfer. Thus, following electroporation, the T cells can be "simply" infused. T_N: naïve, T_{SCM}: stem cell memory, T_{CM}: central memory, T_{EM}: effector memory, and T_{Eff}: effector T cells.

Very Rapid Manufacture of T Cells with "Point-of-Care" (P-O-C)

- Current manufacture is based on gene transfer in T cells that undergo *ex vivo* activation and propagation to integrate transgenes from virus to yield clinical numbers of T cells
- Alternatively, T cells can be electroporated with DNA plasmids from SB system to co-express CAR and mbIL15 (**Figure 1**)
- Electroporated T cells manufactured under P-O-C are infused less than two days after gene transfer
  - SB-derived transposition results in stable integration
  - mbIL15 provides preferential survival advantage *in vivo*
- Less manipulation of the product may preserve less differentiated T-cell subsets, thus potentially improving potency
- **We <u>hypothesized</u> that with the co-expression of mbIL15: (i) therapeutically effective CAR⁺ T cells could be generated and very rapidly infused without being activated and propagated *ex vivo* and (ii) there would be enhanced modified-T cell persistence in mice with improved memory subsets.**

<u>Conflict of Interest Disclosures</u>: Hurton: *Intrexon Corporation:* Patent; *Ziopharm Oncology:* Patent, Research Funding; Chan: *Precigen, Inc.:* Employment; Shah: *Precigen, Inc.*: Employment; Cooper: *Targazyme, Inc.:* Equity Ownership; *Intrexon:* Patents, Equity Ownership; *Immatics:* Equity Ownership; *City of Hope:* Patents & Royalties; *Sangamo BioSciences:* Patents & Royalties; *Ziopharm Oncology:* Employment, Equity Ownership, Patents, Research Funding; *MD Anderson Cancer Center:* Visiting Scientist.

METHODS

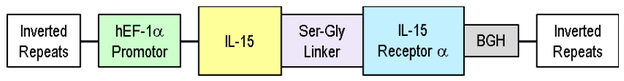

Fig. 2 The mbIL15 construct. The IL-15 cDNA sequence was fused to the full-length IL-15Rα sequence via a flexible serine-glycine linker. The coding sequence was codon optimized and subcloned into a SB-derived DNA plasmid to be used for non-viral gene transfer.

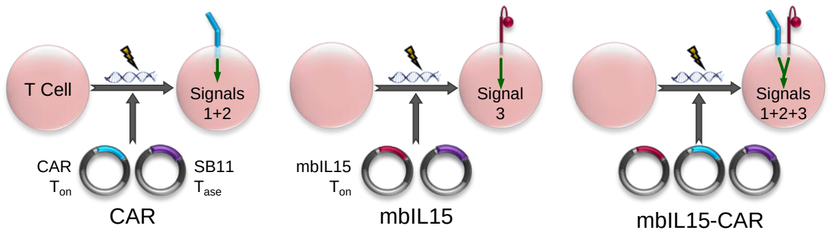

Fig. 3 Generation of T cells under P-O-C that express CAR (*left*), mbIL15 (*center*), and CAR with mbIL15 (*right*). Mononuclear cells were genetically modified, using nucleofection (electroporation), with SB11 transposase DNA plasmid and: (i) 2nd generation CD19-specific CAR (signaling through CD28 and CD3-ζ), (ii) mbIL15, or (iii) mbIL15 & CAR coded from separate SB DNA transposon plasmids. CAR engagement provides T-cell activation and co-stimulation signals while mbIL15 provides a 3rd stimulatory signal to enhance survival. Electroporated T cells were then placed overnight in culture (with no exogenous cytokines) prior to injection into mice.

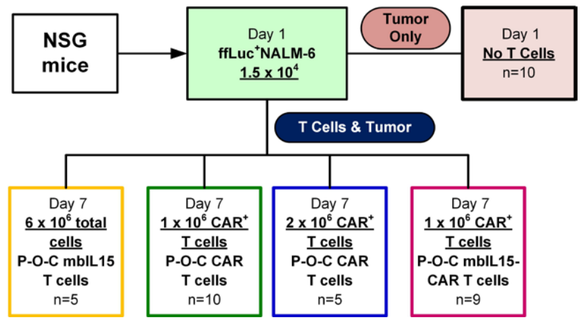

Fig. 4 Mouse model with established and disseminated leukemia. NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice were intravenously injected with 1.5x10⁴ CD19⁺ NALM-6 leukemia cells that expressed firefly luciferase (ffLuc). Six days later when leukemia was established via bioluminescent imaging (BLI), a single T-cell infusion of 10^6 CAR⁺ P-O-C CAR or P-O-C mbIL15-CAR T cells, 2x10^6 CAR⁺ T cells of P-O-C CAR, or 6x10^6 total cells for P-O-C mbIL15 (CAR^{neg}) were intravenously injected for T-cell treatments. To calculate T-cell dosing, CAR on the cell surface was measured the day after electroporation which is a sum of integrated and episomal expression. Tumor burden was serially monitored by BLI.

RESULTS

P-O-C T cell infusion product phenotype

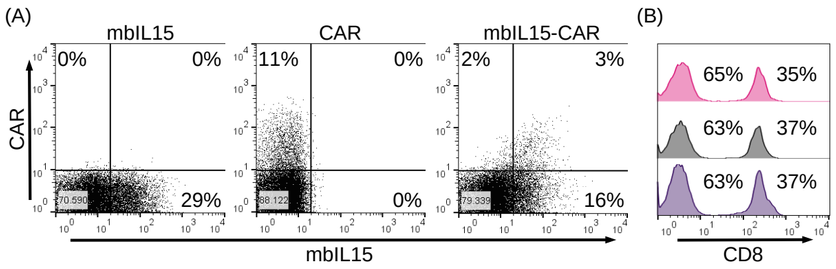

Fig. 5 Phenotype of genetically modified T cells at infusion. Less than 2 days after genetic modification, the P-O-C T cells were harvested for infusion. The T cells were assessed for (**A**) CAR and mbIL15 expression of gated CD3⁺ cells (a sum of integrated and episomal expression), as well as (**B**) CD4 and CD8 ratio of gated CD3⁺ cells for mbIL15 *(bottom)* and gated CAR⁺CD3⁺ cells for CAR *(middle)*, and mbIL15-CAR (*top*) T cells.

P-O-C mbIL15-CAR T cells increase survival

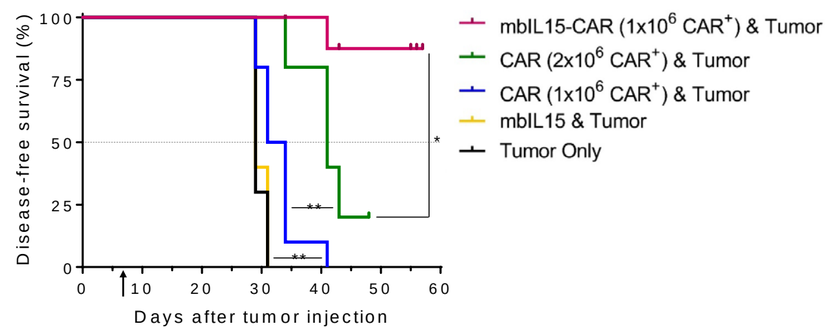

Fig. 6 P-O-C mbIL15-CAR T cells significantly improve survival. Kaplan Meier survival curves show disease-free survival whereby mice were considered disease-free when tumor flux was <3.5x10^7 p/s/cm²/sr. Arrow indicates the day genetically modified cells were injected. Significance determined by log-rank (Mantel-Cox). *P < 0.05, ** P < 0.01.

P-O-C mbIL15-CAR T cells demonstrate the most potent antitumor response

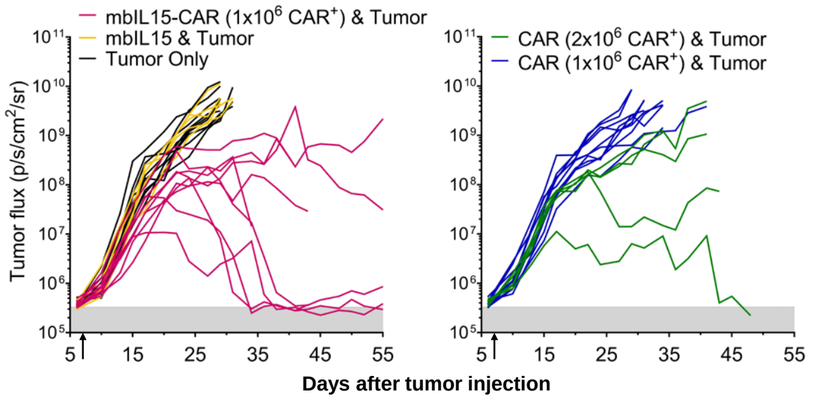

Fig. 7 P-O-C mbIL15-CAR T cells exhibit potent antitumor activity after one low T-cell dose. Quantified tumor burden (ffLuc activity) was measured by BLI. Each line represents an individual animal. Arrows indicated the day genetically modified cells were injected.

P-O-C mbIL15-CAR T cells show improved persistence & memory composition than P-O-C CAR T cells

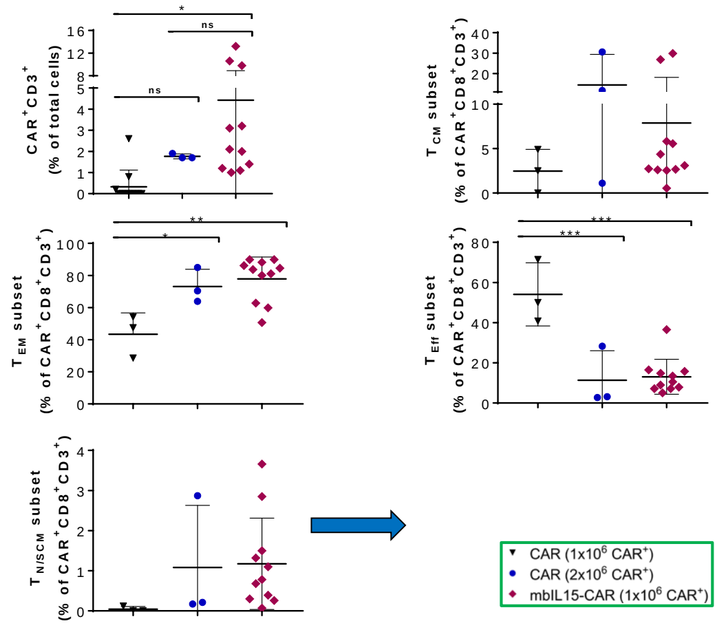

Fig. 8 Persistence and memory composition of CAR⁺ P-O-C T cells at treatment endpoint. Spleens of moribund mice were analyzed to assess the persistence of genetically modified human T cells *(top left)*, as well as the frequencies of memory subsets delineated by CD45RO and CCR7 expression. Memory subset data is shown for mice where a CAR⁺ population was observed and was gated on CAR⁺CD3⁺CD8⁺CD45⁺eGFP^{neg}CD45.1^{neg} cells. CD95 expression was used to identify a T_{SCM}-like subset (*bottom right*) from the $T_{N/SCM}$ subset (*bottom left*). Data were pooled from two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, one-way ANOVA.

CONCLUSIONS

- P-O-C mbIL15-CAR T cells can be rapidly generated without *ex vivo* activation or propagation
- With one low-dose, P-O-C mbIL15-CAR T cells exhibited enhanced survival and more potent antitumor activity than other treatments
- Doubling the dose of P-O-C CAR improved survival and antitumor activity, but did not achieve the treatment effects of P-O-C mbIL15-CAR T-cells
- Recovered P-O-C CAR (2x10^6 CAR⁺ dose) and P-O-C mbIL15-CAR T cells were primarily T_{EM} at sacrifice of mice, while P-O-C CAR (10^6 CAR⁺ dose) T cells had progressed to differentiated T_{eff}
- Low frequency T_{SCM}-like cells were observed in the P-O-C mbIL15-CAR T-cell treated mice
- These data support a clinical trial to very rapidly manufacture genetically modified T cells under P-O-C
- Reducing the manufacture time of CAR⁺ T cells under P-O-C can:
  i. advance genetically modified cell-based therapies as a manufacturing platform with broad appeal
  ii. shorten time to treatment
  iii. decrease costs