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 endodomain
- The Sleeping Beauty (SB) non-viral gene transfer system
  - Successfully tested in humans to express a CD19-specific CAR (Kebrabiai 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/proagation
- 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)

**METHODS**

**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. TN naive, TSCM stem cell memory, TCM central memory, TEM effector memory, and T Eff effector T cells.

**Fig. 2** The mbIL15 construct. The IL-15 cDNA sequence was fused to the full-length IL-15Ra 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) CAR only, (ii) CAR and mbIL15, or (iii) CAR, mbIL15 & CAR & Co-signaling (IL-15Rα) signals 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. NOG Cg-Prl-Ko/dm1Sg2aRgKrdM2j (NOG) mice were intravenously challenged with 1.1x10⁶ 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 10⁵ CAR+ P-O-C CAR or P-O-C mbIL15-CAR T-cells, 2x10⁵ CAR+ T-cells of P-O-C CAR or 6x10⁵ total cells for P-O-C mbIL15-CAR 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 composite of initial CAR, or 6x10⁶ total cells for P-O-C mbIL15 (CARneg) were intravenously injected for T-cell treatments.

**RESULTS**

**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 mbIL15 CAR+ CD3+ cells for CAR (middle), and mbIL15-CAR (top) T cells.

**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 <3.5x10⁶ pCl/μl. Arrow indicates the day genetically modified cells were injected. Significance determined by log-rank (Mantel-Cox). *P < 0.05, **P < 0.01, ***P < 0.001, one-way ANOVA.

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

**Fig. 8** Persistence and memory composition of CAR+ P-O-C T cells at treatment endpoint. Splenocytes 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 CD4+CD25 and CD69 expression. Memory subset data is shown for mice where a CAR+ population was observed and was gated on CAR+CD3+CD8+CD45RO-CD69+ cells. CD95 expression was used to identify a Teff-like subset (bottom right) from the Tem subset (bottom left). Data were pooled from two independent experiments.

**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 (2x10⁵ CAR+ dose) and P-O-C mbIL15-CAR T cells were primarily Tem at sacrifice of mice, while P-O-C CAR (10⁶ CAR+ dose) T cells had progressed to differentiated Teff
- Low frequency Tem-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:
  - advance genetically modified cell-based therapies as a manufacturing platform with broad appeal
  - shorten time to treatment
  - decrease costs