A novel microfluidic device for rapidly generating potent CAR T cells

Saba Ghassemi
Center for Cellular Immunotherapies,
Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Adoptive cellular immunotherapy (ACT) using T cells that are genetically modified to express a chimeric antigen receptor (CAR) yield durable clinical responses in patients with cancer. The effectiveness of ACT led to the regulatory approval of several CD19-specific CAR T cell therapies, including tisagenlecleucel and axicabtagene ciloleucel for patients with B cell acute lymphoblastic leukemia (ALL)[1]. While initial response rates are high, relapse can occur as CAR T cells undergo senescence and engraftment is lost[2].

CAR T cell therapies almost universally involve the isolation of mononuclear cells containing T cells from peripheral blood of a patient, followed by activation of the T cells, their genetic modification using a viral vector, and the expansion of the T cells ex vivo before reinfusion back into the patient (**Fig. 1**). Beads coated with agonist antibodies to the CD3 complex and the CD28 costimulatory receptor, are commonly used to activate T cells. We have shown that the activation step and propagation phase, triggers differentiation toward effector cells with a loss of anti-leukemic potency[3]. This complements other studies showing that the ability of T cells to engraft is related to their state of differentiation with less differentiated naïve-like and central memory cells showing the greatest potency[4, 5].

We developed an innovative strategy to express CAR transgenes in non-activated T cells, generating superior CAR T cells in as little as 24 hrs[6]. While promising, there are several mechanisms that restrict the ability of lentivirus to infect non-activated T cells. The limited diffusion of lentivirus in large culture vessels is a major barrier to gene transfer. By adjusting the geometric conditions, we enhanced the colocalization of vector particles with T cells. By increasing the surface area to volume ratio of the culture vessel, while keeping the volume constant, we increased the transduction of non-activated T cell by 4 fold (**Fig. 2**).

There is a critical unmet need to design and develop a microfluidic device that optimizes the interaction of lentiviral particles and T cells- thereby enhancing CAR gene transfer into quiescent T cells. Our vision of the ultimate device is a fully automated and closed manufacturing system for genetically-modified nonactivated T cells. As this approach involves the use of non-activated T cells within 24h, it offers the most rapid, efficient process to generate functionally competent CAR T cells described to date.

Reference:

1. Melenhorst, J.J. et al. Decade-long leukaemia remissions with persistence of CD4(+) CAR T cells. *Nature* **602**, 503-509 (2022).
2. Maude, S.L. et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *The New England journal of medicine* **371**, 1507-1517 (2014).
3. Ghassemi, S. et al. Reducing Ex Vivo Culture Improves the Antileukemic Activity of Chimeric Antigen Receptor (CAR) T Cells. *Cancer Immunol Res* **6**, 1100-1109 (2018).
4. Graef, P. et al. Serial transfer of single-cell-derived immunocompetence reveals stemness of CD8(+) central memory T cells. *Immunity* **41**, 116-126 (2014).
5. Hinrichs, C.S. et al. Human effector CD8+ T cells derived from naive rather than memory subsets possess superior traits for adoptive immunotherapy. *Blood* **117**, 808-814 (2011).
6. Ghassemi, S. et al. Rapid manufacturing of non-activated potent CAR T cells. *Nat Biomed Eng* **6**, 118-128 (2022).

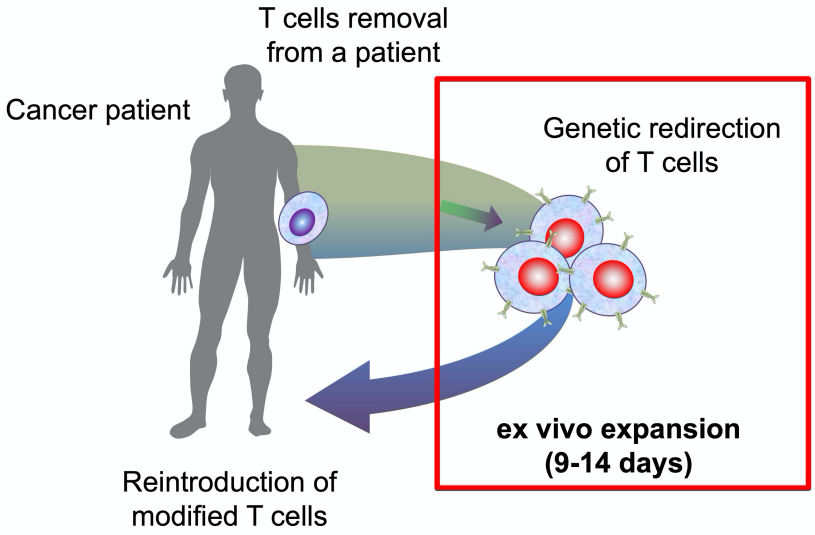

Figure 1. A general schematic of generating of CAR T cells for adoptive immunotherapy.

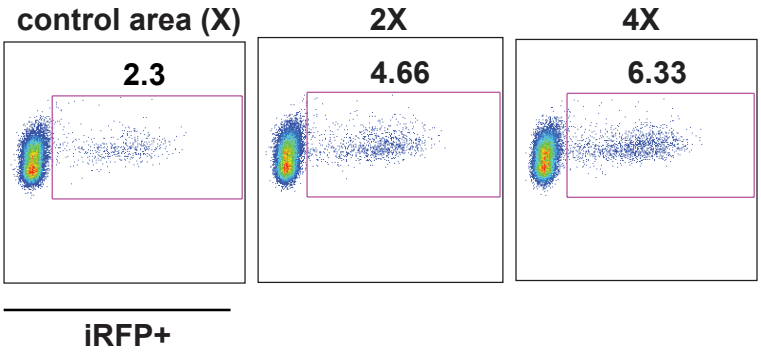

Figure 2. Transducing conditions can enhance lentiviral gene transfer in non-activated T cells. T cells were transduced with lentiviral vector iRFP cultured in either one well, or two wells, or four wells or eight wells with total culture volume held constant. iRFP+ cell frequency was determined by flow cytometry.