Same Day Transduction and *In Vivo E*xpansion of a Chimeric Antigen Receptor with a Synthetic Driver Construct for Adoptive Cellular Therapy

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Key messages

- We demonstrate viral transfer feasibility in a point of care (POC) platform using an engineered lentiviral vector retargeted to CD3, which efficiently transduces freshly isolated resting human PBMCs in four hours
- POC modified cells successfully engraft and expand *in vivo* upon IV administration in mice
- The entire process of PBMCs isolation, genetic modification and dosing can be completed in less than twelve hours, vein to vein
- Altogether, this platform represents a significant step forward in advancing the development of CAR-T therapies with POC potentially expanding patient accessibility and deployment

Introduction

Adoptive cellular therapy (ACT) using *ex vivo* expanded chimeric antigen receptor (CAR) modified T cells to target cancer cells expressing CD19 has been successful in the treatment of hematologic malignancies and the clinical application of this technology for solid tumor malignancies is a major focus of several research and development programs[1].

Despite the clinical success of these products, there are several hurdles that currently limit the widespread deployment of CAR-T:

- Complexity of the process
- Several weeks are required to prepare and release the engineered products
- Centralized manufacturing facilities are required
- Extensive logistical control over the chain of custody of patient specific product is mandatory
- Risks of contamination exist
- Economically toxic

In addition, recent studies suggest that limiting the *ex vivo* expansion time results in less differentiated CAR-T products with enhanced effector function[2].

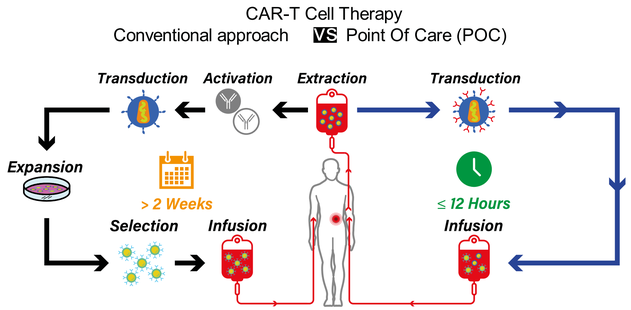
Successful engraftment and persistence for current autologous CAR-T cell products require the depletion of normal lymphocytes in patients with cytotoxic drugs (primarily cyclophosphamide, fludarabine or combinations) prior to administration of CAR-T cells. Although the use of non-myeloablative lymphodepleting regimens prior to CAR-T infusion significantly enhances the successful *in vivo* homeostatic expansion and persistence of administered CAR-T cells[3], non-myeloablative chemotherapy also requires significant supportive care.

The development of a point of care approach to ACT has the potential to reduce the complexity of CAR T-cell immunotherapy and broaden access to a substantially greater number of cancer patients and address many of the limitations discussed above.

The most ideal system would allow for rapid genetic modification of patient's cells next to the patient, thereby eliminating chain of custody risks, combined with successful *in vivo* expansion and engraftment of cells in the patient to achieve therapeutic cellular levels without preconditioning through lymphodepletion.

Here we describe and provide data demonstrating initial proof of concept for a novel point of care approach for CAR-T using engineered CD3-retargeted lentiviral vectors[4] and freshly isolated resting human PBMCs.

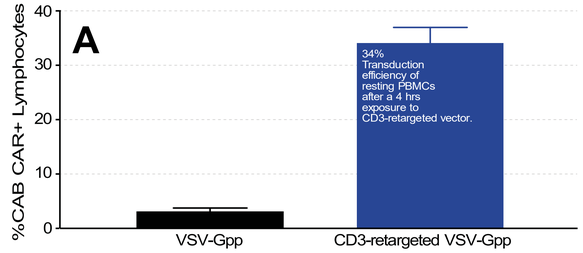
Resting human PBMCs were isolated from fresh blood and successfully transduced within a four hour exposure to CD3-retargeted lentiviral particles. These modified cells successfully engrafted and expanded *in vivo* upon administration in mice

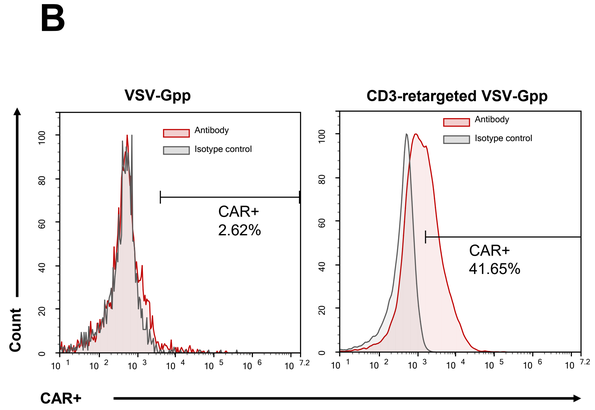


Retargeting lentiviral vectors to the CD3 antigen can increase the transduction efficiency and allow efficient T cell modification and CAR expression in a point of care (POC) setting, in less than 12h. In an animal model, we show that 4h-transduced PBMCs engraft and expand efficiently.

Results

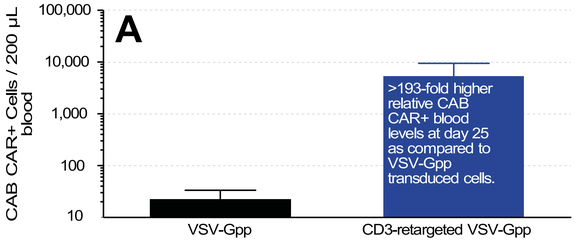
Figure 1. *In vitro* analysis of four hours transduced PBMCs with a CD3-retargeted CAB CAR lentiviral vector containing a lymphoproliferative element demonstrates efficient transduction of freshly isolated human T cells

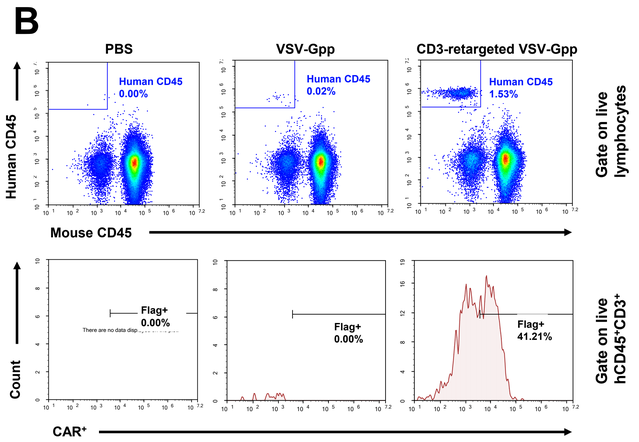




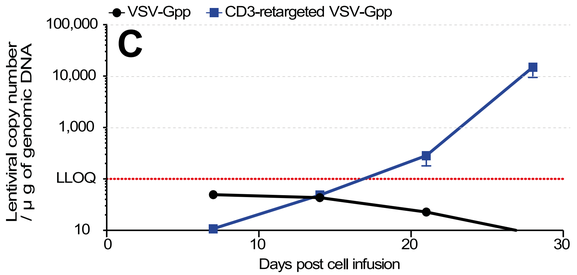
158 mL of fresh human donor blood isolated by venipuncture was processed to PBMCs using a Sepax2 instrument in 2 x 1h 20 min. These purified PBMCs were immediately transduced in complete CTS medium (OpTimizer CTS completed with OpTimizer CTS T cell expansion supplement, CTS immune cells serum replacement and GlutaMax (Gibco) with a control lentiviral vector pseudotyped with VSV-G alone (black) or with VSV-G and a UCHT1 scFv-Fc GPI-anchored CD3-retargeting moiety (blue), at an MOI of 1, for four hours. The lentiviral vector is third generation and encodes a FLAG-tagged CAB CAR[4] and a lymphoproliferative element[2]. The transduced cells were subsequently washed three times with PBS-2% HSA. To evaluate transduction efficiency duplicate aliquots of cells were seeded in 6 well plates in 2 mL of complete CTS medium at a concentration of 1e6 PBMCs/mL. Six days post-transduction, expression of the FLAG-tagged CAB CAR on the CD3+ transduced cells cultivated *in vitro* was quantified by flow cytometry. (A) Mean +/- SD of the duplicate *in vitro* cultures. (B) Representative flow cytometry gating strategy.

Figure 2. Infusion of cells immediately following four hour exposure to a CD3-retargeted CAB CAR vector with a lymphoproliferative element allows engraftment of CD3+ CAB CAR+ human lymphocytes *in vivo* in mice without preliminary *in vitro* expansion or lymphodepletion





Freshly isolated, four hour transduced and 3x washed human PBMCs as described in Figure 1 were concentrated to 100e6 PBMCs/mL in PBS before being injected IV (100 µL/mouse) in NSG mice (n=6). To assess for engraftment and expansion *in vivo*, ~ 100 µL of peripheral blood samples were collected from each mouse by retro-orbital puncture at different intervals post-transduction and IV injection. Expression of the FLAG-tagged CAB CAR in the human hCD45+ CD3+ T cells population was quantified by flow cytometry. The number of lentiviral copies per µg of extracted genomic DNA from the mice peripheral blood was quantified by TaqMan qPCR targeting a short region of the LTR of the integrated third generation lentiviral genome. (A) FACS based CAR T cell detection data, Mean +/- SEM. (B) Representative flow cytometry in A. Gate on live lymphocyte population (top) or live human CD45+CD3+ cell population (bottom). (C) qPCR data representing the CAR T copies / µg genomic DNA, Mean +/-SEM.



Conclusions

Here we show that a point of care (POC) approach via the retargeting of lentiviral vectors through co-pseudotyping of VSV-G with a CD3-targeting UCHT1 scFv-Fc-GPI anchor moiety:

1. Overcame the well-known low transduction efficiency of human resting T cells[5] and increased the transduction efficiency *in vitro* more than 10 fold, compared to VSV-G alone, after only a 4h transduction time.
2. Resulted in successful engraftment of modified CAR-T cells in peripheral blood in a mouse model.

Of importance, the entire process of PBMC isolation, transduction and dosing was completed within ***twelve hours vein to vein*** and represents a significant step forward in advancing the development of potential POC CAR-T therapies by:

1. Reducing the chain of custody
2. Favoring the engraftment by use of autologous cells
3. Expanding upon patient accessibility and deployment

References

1 – Li J. *et al.* Chimeric antigen receptor T cell (CAR-T) immunotherapy for solid tumors: lessons learned and strategies for moving forward. J Hematol Oncol, 2018; PMCID: PMC5809840

2 – Ghassemi S. *et al.* Reducing *Ex Vivo* Culture Improves the Antileukemic Activity of Chimeric Antigen Receptor (CAR) T Cells. Cancer Immunol Res, 2018; PMID: 30030295

3 – Nguyen LT. *et al.* Phase II clinical trial of adoptive cell therapy for patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and low-dose interleukin-2. Cancer Immunol Immunother, 2019; PMID: 30747243

4 – Frank AM. and Buchholz CJ. Surface-Engineered Lentiviral Vectors for Selective Gene Transfer into Subtypes of Lymphocytes. Mol Ther Methods Clin Dev, 2018; PMCID: PMC6216101

5 – Amirache F. *et al.* Mystery solved: VSV-G-LVs do not allow efficient gene transfer into unstimulated T cells, B cells, and HSCs because they lack the LDL receptor. Blood, 2014; PMID: 24578496

6 – Jiandfang Hu *et al.* A novel conditionally active biologics (CAB) approach to minimize on-target off-tumor effects in adoptive immunotherapy. F1 Oncology poster #3189

7 – Laurence Jadin *et al.* A high-throughput screening strategy for the identification of novel lymphoproliferative elements, F1 Oncology poster #3523

Acknowledgements

F1 Oncology would like to thank Wendy Zhang, Feng Zhang, Xiaoli Zhang, Ling Jiang, Wenjie Yin, Tao Wang, Qing Yang, Jianhong Ye, Qiuying Ding and Xun Zhun for their precious contribution to advancing this work.