Rapid point-of-care subcutaneous CAR-T from blood draw to injection in 4 hours with modified LV encoding CARs and synthetic driver elements enables efficient CAR-T expansion and tumor regression

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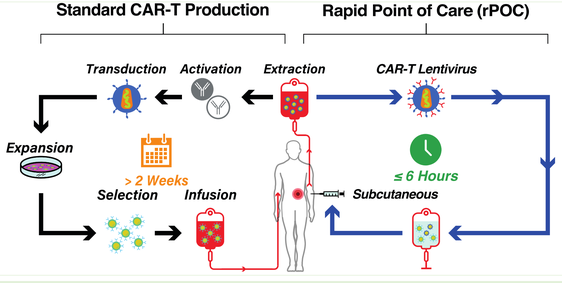
Key Messages

- **We demonstrate that subcutaneous (SC) delivery of human PBMCs modified by a 4-hour exposure to CD3-directed lentiviruses encoding a CD19 or a CD22 Chimeric Antigen Receptor (CAR) resulted in potent anti-tumor efficacy and robust in vivo expansion of CAR-T positive cells.**
- **CAR-T cells driven by a synthetic domain identified from a high-throughput screening strategy exhibited > 10,000-fold expansion in vivo when given SC and demonstrated superior expansion compared to IV dosing.**
- **A single dose of 1 million lentiviral modified PBMC given SC resulted in complete tumor regression in subcutaneous and disseminated Raji tumor models.**
- **The entire process of genetic modification of PBMC and SC dosing can be completed in less than six hours, resulting in targeted genetic modification of T cells w/o prior activation. This represents a significant step forward for advancing rapid Point-of-Care (rPOC) CAR-T therapies.**

Introduction

- Adoptive cellular therapy with chimeric antigen receptor (CAR)-T cells has demonstrated remarkable clinical activity in a number of hematologic malignancies[1], but product chain of custody, individualized manufacturing, preparative chemotherapy, and patient and health care provider complexities present technical and logistical hurdles to broader implementation.
- Despite the clinical success of these products, there are several hurdles that currently limit the widespread deployment of CAR-T[2,3].

- 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
  - Requirement for lymphodepletion



Methods

- Lentiviral constructs for CARs (either CD19- or CD22-directed) co-expressed with a synthetic driver domain identified from a >6x10^5 diversity combinatorial library of proliferative elements, transmembrane domains, leucine zippers, and an EGFR epitope screened for cellular expansion in a lymphoreplete model.

- Modified serum-free-lentiviral manufacturing process was developed to reduce complexity of CAR-T vector production and to introduce CD3-activating elements into the viral envelope allowing activation and transduction of resting lymphocytes from peripheral blood.

Results

Figure 1. CAR-T Expansion and Anti-Tumor Activity

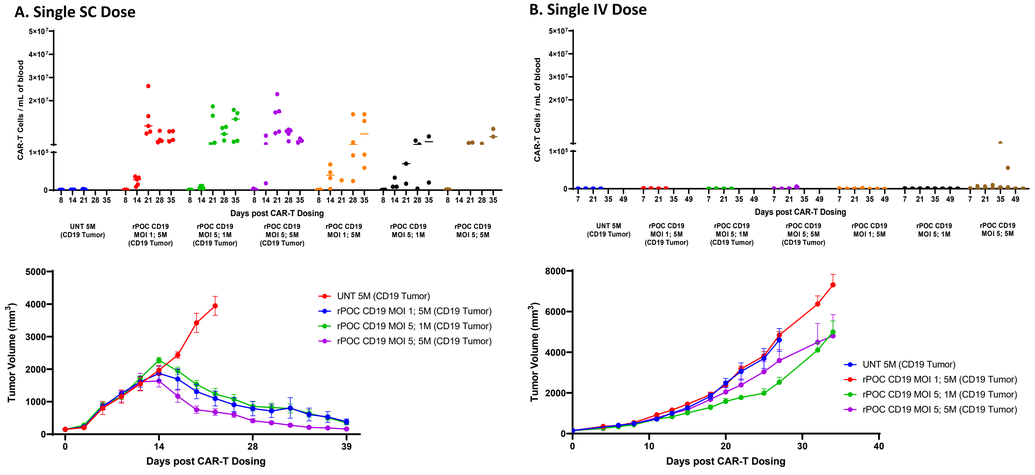


Figure 1: Subcutaneous (SC) administration rPOC gene-modified PBMC (1M or 5M) with a vector co-pseudotyped with VSV-G and a CD3-targeting UCHT1 scFv-Fc-GPI anchor moiety and encoding a CD19 CAR results in (A) superior engraftment and significantly better anti-tumor immunity compared to (B) IV dosing in the B-NDG mice bearing CD19 tumor (Raji). (UNT: 5M untreated PBMC, Dose: 1M or 5M PBMC transduced with LV at MOI: 1 or 5; error bars: SEM)

Table 1. Blood Immunophenotype after Single SC Dose

Group	Treatment	Tumor	CD4+CAR-T+ Cells/mL blood (SD)			CD8+CAR-T+ Cells/mL blood (SD)		
			Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
1	UNT 5M	None	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1,000 (548)
2	rPOC CD19 MOI = 1; 5M	CD19 Tumor	0 (0)	3,000 (894)	212,000 (139,836)	0 (447)	15,000 (8,735)	10,425,000 (6,875,662)
3	rPOC CD19 MOI = 5; 1M	CD19 Tumor	0 (0)	0 (547)	86,000 (91,243)	0 (0)	4,000 (2,280)	6,003,000 (6,896,402)
4	rPOC CD19 MOI = 5; 5M	CD19 Tumor	0 (447)	169,000 (289,005)	470,000 (373,226)	1,000 (894)	890,000 (1,343,976)	10,794,000 (5,725,248)
5	rPOC CD19 MOI = 1; 5M	None	0 (0)	4,000 (2,944)	12,000 (2,986)	0 (0)	25,000 (17,970)	117,000 (83,974)
6	rPOC CD19 MOI = 5; 1M	None	0 (0)	2,000 (1,732)	12,000 (17,926)	0 (0)	11,000 (9,539)	196,000 (281,703)
7	rPOC CD19 MOI = 5; 5M	None	0 (0)	42,000 (17,039)	184,000 (75,963)	0 (0)	156,000 (59,282)	1,037,000 (397,583)

Table 1: Total numbers of CD4+ and CD8+ CAR-T+ cells in blood collected from B-NDG mice w/ CD19 tumor 1-3 weeks after a single SC dose of transduced PBMC show vast majority of CD8+CAR-T+ cells. (UNT: 5M untreated PBMC, Dose: 1M or 5M PBMC transduced with LV at MOI: 1 or 5; SD: standard deviation)

Table 2. CAR Product Integration after Single SC Dose

Group	Treatment	Tumor	LV Copies/Cell (SD)		
			Week 1	Week 2	Week 3
1	UNT 5M	None	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
2	rPOC CD19 MOI = 1; 5M	CD19 Tumor	0.37 (0)	0.63 (0.43)	1.1 (0.18)
3	rPOC CD19 MOI = 5; 1M	CD19 Tumor	0.0 (0.0)	0.52 (0.11)	0.93 (0.20)
4	rPOC CD19 MOI = 5; 5M	CD19 Tumor	0.60 (0.08)	1.0 (0.28)	1.2 (0.16)
5	rPOC CD19 MOI = 1; 5M	None	0.21 (0.15)	0.81 (0.34)	0.96 (0.29)
6	rPOC CD19 MOI = 5; 1M	None	0.0 (0.0)	0.44 (0.28)	0.72 (0.50)
7	rPOC CD19 MOI = 5; 5M	None	0.40 (0.01)	1.2 (0.09)	0.96 (0.34)

Table 2: Day 21 qPCR analysis of integrated CAR vector copies per genome of blood cells collected from B-NDG mice bearing CD19 tumor shows less than 3 copies per genome. (UNT: 5M untreated PBMC, Dose: 1M or 5M PBMC transduced with LV at MOI: 1 or 5; SD: standard deviation)

Figure 2. Anti-Tumor Activity

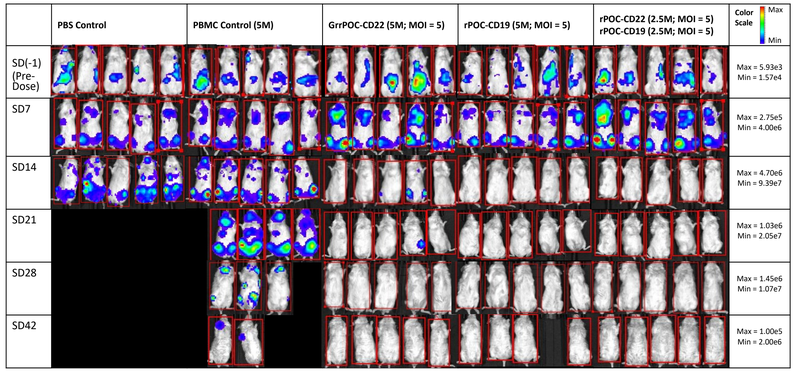


Figure 2: Disseminated Raji-Luciferase tumor model in NSG MHC-I/II KO mice:
Subcutaneous administration of 1 million rPOC 4hr-gene modified PBMC with a vector co-pseudotyped with VSV-G and a CD3-targeting UCHT1 scFv-Fc-GPI anchor moiety encoding a CD19 or a CD22 CAR results in robust anti-tumor immunity. (Dose=5M PBMC transduced with LV (rPOC CD19 and/or rPOC CD22) at MOI=5; visualized on Study Day (SD from -1 to 42))

Conclusion

- CAR-T cells driven by a synthetic domain identified from a high-throughput screening strategy exhibited **> 10,000-fold expansion in vivo** when given subcutaneously (SC) and demonstrated superior expansion compared to IV dosing.
- A single dose of 1 million lentiviral modified PBMC generated **in 4 hours and given subcutaneously (SC)** resulted in complete tumor regression in subcutaneous and disseminated Raji tumor models.
- The entire process of genetic modification of PBMC and subcutaneous dosing can be completed in less than six hours, representing a significant step forward for advancing **rapid Point-of-Care (rPOC) CAR-T therapies.**

References

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Acknowledgments

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