T-Cell Rejuvenation: A Novel Approach for Partially Reprogramming T Cells to Improve Their Immunotherapeutic Properties

Poster No. 508

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Introduction

Two key parameters have been shown to affect T-cell function over the lifespan of an organism: T-cell age and identity. In adoptive cell therapies (ACTs), increased age and differentiation of T cells are associated with decreased efficacy and reduced benefit in patients with cancer. The capacity of T cells for self-renewal, proliferation, persistence, and antitumor activity is adversely affected by aging. Methods have been developed to de-differentiate cells into induced pluripotent stem cells (iPSCs) that return to an embryonic developmental stage characterized by epigenetic youth; however, the resulting cells lose their functional identity. The subsequent redifferentiation of iPSCs into the desired functional T-cell phenotype is a process that is complex and time-consuming. Here we report the development of our novel cellular rejuvenation technology with partial reprogramming capable of countering the effects of cellular aging while maintaining T-cell function.

Results

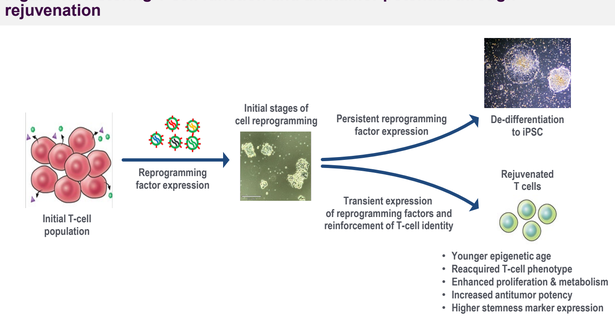
Novel T-cell rejuvenation through partial reprogramming

Via our novel T-cell rejuvenation reprogramming technology, aged T cells transiently express transcription factors associated with iPSC reprogramming in a non-genomic integrative manner. As shown in **Figures 1–4**, these reprogrammed T cells exhibit:

- Younger epigenetic age
- Reacquired conventional T-cell phenotype
- Enhanced cell proliferation and metabolism
- Improved antitumor potency
- Higher expression of stemness markers

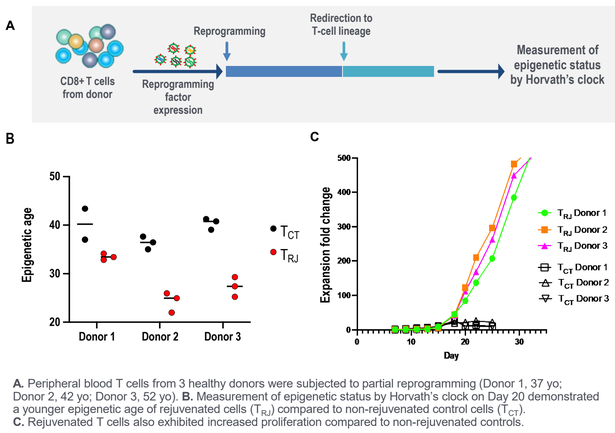
The resulting rejuvenated T cells do not require complex redifferentiation steps, thus reducing the time required for reprogramming and differentiation of conventional T cells. Our data demonstrate the capacity to partially "turn back" the epigenetic clock of a T cell without returning the cell to the pluripotent state associated with complete iPSC reprogramming.

Figure 1. Restoring T-cell function and antitumor potential through rejuvenation



T-cell reprogramming with persistent reprogramming factor expression results in de-differentiated iPSCs. In contrast, partial reprogramming with rejuvenation technology allows for maintenance of T-cell identity and improvement of cellular function without complex iPSC redifferentiation steps.

Figure 2. Rejuvenated healthy donor T cells acquire epigenetic youth (Horvath's clock) and enhanced proliferation



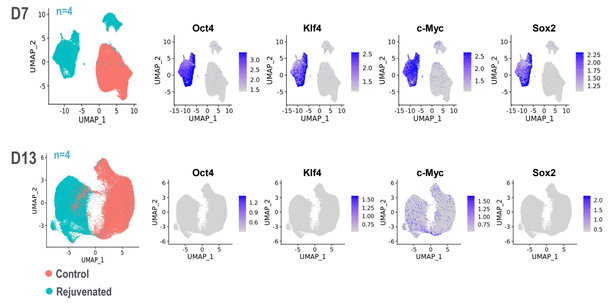
A. Peripheral blood T cells from 3 healthy donors were subjected to partial reprogramming (Donor 1, 37 yo; Donor 2, 42 yo; Donor 3, 52 yo). **B.** Measurement of epigenetic status by Horvath's clock on Day 20 demonstrated a younger epigenetic age of rejuvenated cells (T_{RJ}) compared to non-rejuvenated control cells (T_{CT}). **C.** Rejuvenated T cells also exhibited increased proliferation compared to non-rejuvenated controls.

Results (cont.)

Expression of reprogramming factors in rejuvenated T cells is transient

Single-cell RNA-Seq analysis of control and rejuvenated T cells from 4 healthy donors aged 50 to 55 years showed that rejuvenated T cells expressed OSKM factors (Oct4, Sox2, Klf4, and c-Myc) on Day 7, but expression disappeared prior to Day 13. Critically, this non-integrative, transient expression of reprogramming factors mitigates the risk of generating immortalized cells.

Figure 3. RNA-Seq analysis of transient expression of OSKM factors in rejuvenated T cells



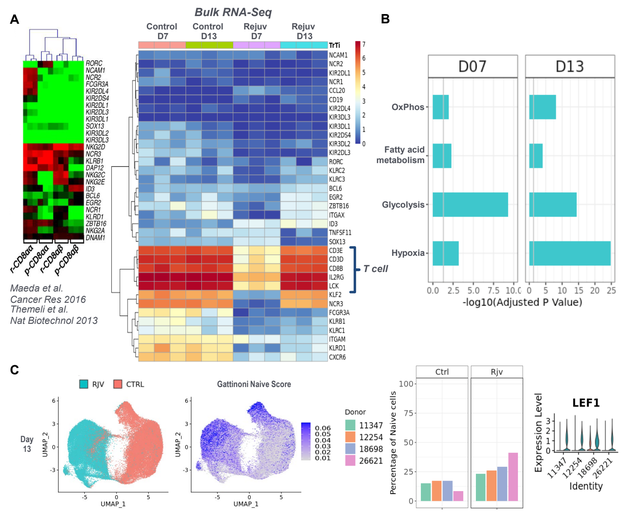
RNA-Seq data from Days 7 and 13 demonstrated transient expression of OSKM factors (Oct4, Sox2, Klf4, and c-Myc) in rejuvenated T cells. Note: Expression of c-Myc on Day 13 was endogenous.

Rejuvenation produces a conventional T-cell phenotype with improved stemness and metabolism

After the redirection phase, RNA-Seq analysis of control and rejuvenated T cells from healthy donors aged 50 to 55 years showed that the rejuvenated T cells re-express T-cell–associated genes and compared to controls:

- Did not exhibit abnormal expression of unconventional T-, NK-, or B-cell markers typically expressed by human iPSC-derived T cells **(Figure 4A)**
- Were enriched for expression of genes associated with glycolysis and oxidative phosphorylation at Day 7 and Day 13 compared to control cells, indicating enhanced metabolism **(Figure 4B)**
- Exhibited higher expression of naive-associated markers characteristic of more stem-like T-cell populations **(Figure 4C)**

Figure 4. Rejuvenated T cells demonstrate a conventional T-cell phenotype with improved metabolism and stemness based on transcriptomic analyses



Maeda et al. *Cancer Res* 2016
Themeli et al. *Nat Biotechnol* 2013

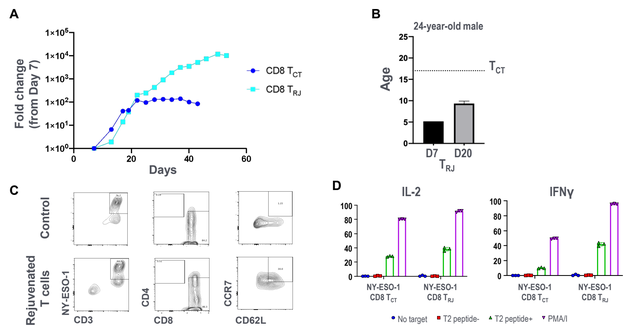
A. Bulk RNA-Seq heatmap analysis of conventional and unconventional genes related to T-cell identity. **B.** Bulk RNA-Seq showed enrichment of DEGs associated with key metabolic features in rejuvenated cells at Day 7 and Day 13 compared to control cells. **C.** Single-cell RNA-Seq showed elevated expression of naive-associated markers in rejuvenated cells vs control cells.

Rejuvenation enhances properties associated with T-cell functionality of engineered T-cell therapies

Rejuvenation was evaluated in two models of engineered T-cell therapies: CD8+ NY-ESO-1 TCR T cells **(Figure 5)** and CD19-targeted CAR T cells **(Figure 6)**. Rejuvenation of these models resulted in:

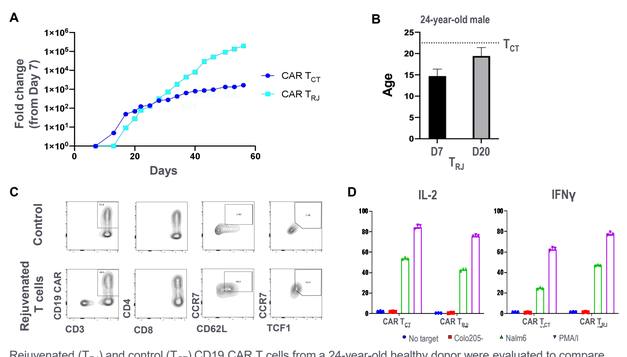
- Decreased epigenetic age
- Enhanced proliferation
- Increased stem-like features
- Retained T-cell function and antigen specificity

Figure 5. Rejuvenation enhanced functional properties of CD8+ NY-ESO-1 TCR T cells



Rejuvenated (T_{RJ}) and control (T_{CT}) CD8+ NY-ESO-1 TCR T cells of a 24-year-old healthy donor were evaluated to compare **(A)** cell proliferation, **(B)** epigenetic age, **(C)** stemness phenotype, and **(D)** T-cell function and antigen specificity.

Figure 6. Rejuvenation enhanced the functional properties of CD19 CAR T cells

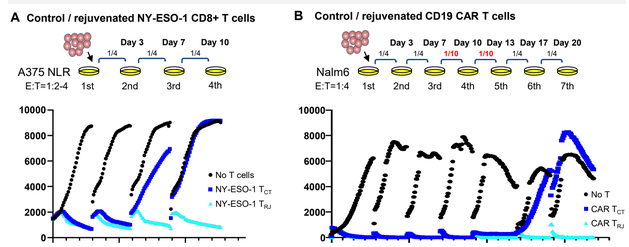


Rejuvenated (T_{RJ}) and control (T_{CT}) CD19 CAR T cells from a 24-year-old healthy donor were evaluated to compare **(A)** cell proliferation, **(B)** epigenetic age, **(C)** stemness phenotype, and **(D)** T-cell function and antigen specificity.

Rejuvenation improves antitumor efficacy of engineered T-cell therapies

Sequential killing assays were used to evaluate the cytotoxic function of two rejuvenated ACTs, demonstrating greater persistent antitumor efficacy compared to conventional NY-ESO-1 TCR T cells **(Figure 7A)** or CD19 CAR T cells **(Figure 7B)**.

Figure 7. Rejuvenation improves antitumor efficacy of NY-ESO-1 TCR and CD19 CAR T cells

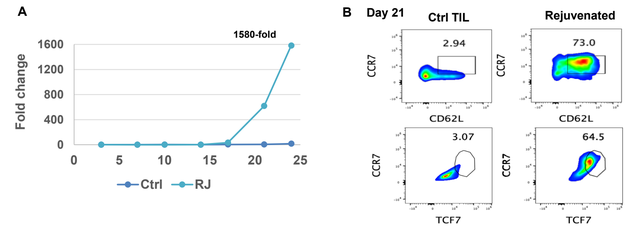


Sequential killing assays of **(A)** rejuvenated NY-ESO-1 TCR T cells (T_{RJ}) and **(B)** CD19 CAR T cells (T_{RJ}) compared to controls (T_{CT}).

Rejuvenation enhances properties associated with T-cell functionality of tumor-infiltrating lymphocytes (TILs)

Application of rejuvenation to TILs derived from a patient with lung cancer showed enhanced proliferative capacity and expression of CCR7, CD62L, and TCF7, markers of T-cell populations with stem-like properties **(Figure 8)**.

Figure 8. Phenotypic and proliferative analysis of rejuvenated lung TILs from single donor compared to controls



Rejuvenated (RJ) and control (Ctrl) TILs from a 66-year-old patient with lung cancer were evaluated to compare **(A)** cell proliferation and **(B)** stemness phenotype.

Conclusions

Lyell's T-cell rejuvenation technology utilizes a partial reprogramming process to produce T cells that are characterized by reduced epigenetic age, enhanced cell proliferation, improved metabolism, and higher expression of stemness biomarkers. Additional research could further characterize rejuvenated T cells in terms of their capacity for tumor antigen–specific polyclonality, long-term engraftment, and solid tumor eradication in vivo.

Application of this technology has demonstrated improvements in engineered adoptive T-cell products; in vitro sequential cell killing assays revealed that rejuvenated NY-ESO-1–targeted TCR and CD19-targeted CAR T cells exhibit improved antitumor properties compared to non-rejuvenated T-cell controls. Early application with TIL products yielded similar results, indicating potential utility across several T-cell therapy modalities.

Lyell's T-cell rejuvenation technology is being advanced for applications in cancer cellular therapy. Through partial reprogramming, our T-cell rejuvenation technology has the potential to transform conventional T-cell immunotherapies and improve outcomes for patients with solid tumors.

Abbreviations

ACT, adoptive cell therapy; CAR, chimeric antigen receptor; CCR7, C-C motif chemokine receptor 7; CD, cluster of differentiation; c-Myc, MYC proto-oncogene; DEG, differentially expressed gene; IFNγ, interferon gamma; IL-2, interleukin-2; iPSC, induced pluripotent stem cell; Klf4, Krüppel-like factor 4; NK, natural killer; NY-ESO-1, New York esophageal squamous cell carcinoma 1; Oct4, octamer-binding transcription factor 4; OSKM factors, Oct4, Sox2, Klf4, and c-Myc; OxPhos, oxidative phosphorylation; RNA-Seq, RNA sequencing; Sox2, SRY-box transcription factor 2; TCF1, T-cell factor 1 (encoded by TCF7); TCF7, transcription factor 7; TCR, T-cell receptor; T_{CT}, control T cell; TIL, tumor-infiltrating lymphocyte; T_{RJ}, rejuvenated T cell.

Acknowledgments

This project was fully supported by Lyell Immunopharma, Inc. We would like to thank Lyell's T-cell rejuvenation and BATT teams for their technical support and Luca Gattinoni for critical feedback. We would also like to thank the Clock Foundation for epigenetic clock analyses, Maria Romanova (Molecular House, Inc.) for graphical design support, and Melanie Styers (Verascity Science) for medical writing support.

Supplementary Materials

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Presented at ISSCR Annual Meeting 2022; June 15–18; San Francisco, CA, USA