Rejuvenation: Improving T-cell Antitumor Properties Through Partial Reprogramming

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Introduction

Cellular identity and age determine T-cell function and fitness over an organism's lifespan.[1,2] Increased T-cell age and differentiation are associated with reduced T-cell function and efficacy of solid tumor cell therapy.[3,4] As such, methods to de-differentiate T cells into iPSCs and re-differentiate them into T cells have been explored under complex and time-consuming processes characterized by the production of innate and unconventional T-cell phenotypes.[5–7] Here, we report the development of a novel cellular rejuvenation technology with partial reprogramming capable of countering the effects of cellular aging while maintaining T-cell function. Rejuvenation is a rapid way to improve cell fitness. T cells under these processes are characterized by a reduced epigenetic age, better stemness phenotype, improved cell expansion potential, and enhanced antitumor efficacy.

Results

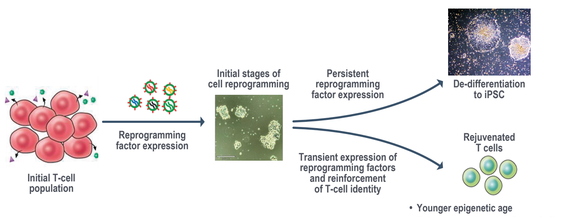
Novel T-cell rejuvenation through partial reprogramming

With Lyell's 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 2–4**, these reprogrammed T cells exhibit:

- Younger epigenetic age
- Enhanced cell proliferation and metabolism
- Improved antitumor potency
- Preservation of stemness markers and TCR repertoire

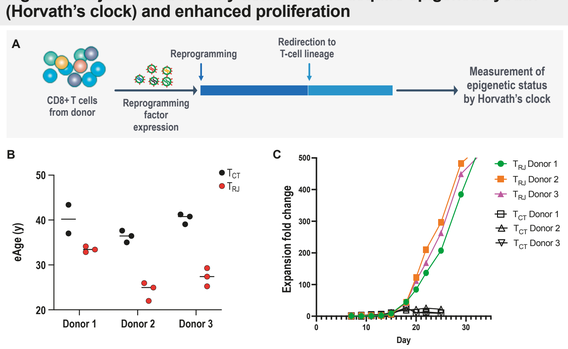
The resulting rejuvenated T cells do not require complex redifferentiation steps, thus reducing the time required for reprogramming and differentiation of conventional T cells. Lyell's data demonstrate the capacity to partially "turn back" the epigenetic clock of a T cell without returning the cell to 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



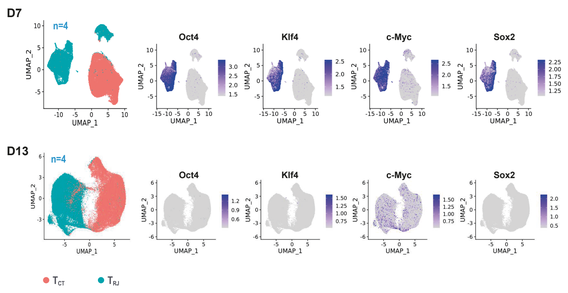
(A) Peripheral blood T cells from 3 healthy donors were subjected to partial reprogramming (Donor 1, 37 yo; Donor 2, 42 yo; Donor 3, 52 yo). **(B)** Measurement of epigenetic status by Horvath's clock on Day 20 demonstrated a younger epigenetic age of rejuvenated cells (T_{RJ}) compared with non-rejuvenated control cells (T_{CT}). **(C)** Rejuvenated T cells also exhibited increased proliferation compared with non-rejuvenated controls. For reference: Average fold difference between T_{RJ} and T_{CT} at Day 25 was 255.6 vs 14.0, respectively.

Results (continued)

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 50 to 55 years of age 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



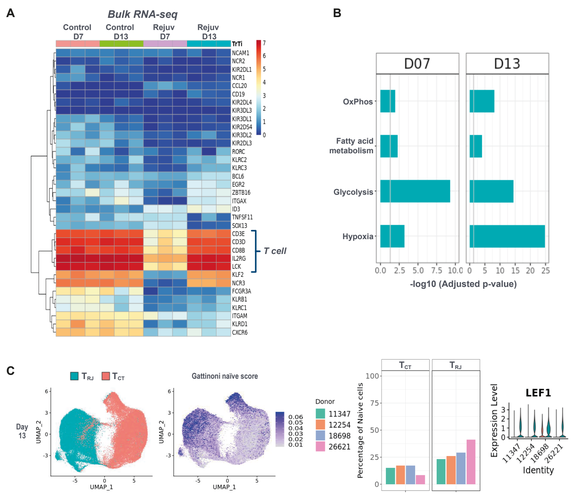
RNA-seq data from Days 7 and 13 demonstrated transient expression of OSKM factors (Oct4, Sox2, Klf4, and c-Myc) in rejuvenated T cells (T_{RJ}). Note: Expression of c-Myc on Day 13 was endogenous.

Rejuvenation produced 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 50 to 55 years of age showed that the rejuvenated T cells re-express T-cell–associated genes and compared with 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 with control cells, indicating enhanced metabolism (**Figure 4B**)
- Exhibited higher expression of naïve-associated markers characteristic of more stem-like T-cell populations (**Figure 4C**)

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



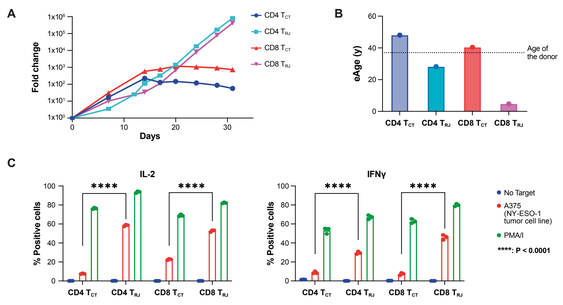
(A) Bulk RNA-seq heatmap analysis of previously identified[6] conventional and unconventional genes related to T-cell identity. **(B)** Bulk RNA-seq showed enrichment of DEGs associated with key metabolic features in rejuvenated cells (T_{RJ}) at Day 7 and Day 13 compared with control cells (T_{CT}). **(C)** Single-cell RNA-seq showed elevated expression of naïve-associated markers in rejuvenated cells (T_{RJ}) vs control cells (T_{CT}).

Rejuvenation enhanced the properties associated with T-cell functionality of engineered T-cell therapies

Rejuvenation was evaluated in two models of engineered T-cell therapies: CD4+ and CD8+ NY-ESO-1 TCR T cells (**Figure 5**) and CD19-targeted CAR T cells (**Figure 6**). Rejuvenation of these models resulted in:

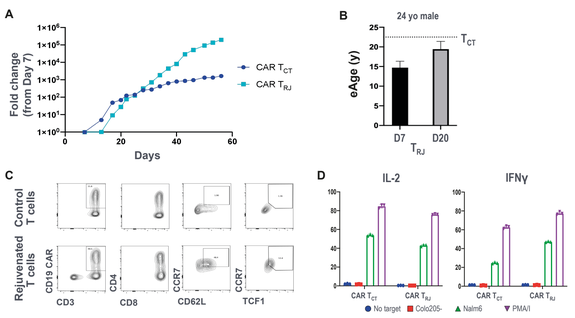
- Decreased epigenetic age
- Enhanced proliferation
- Increased stem-like features
- Retained T-cell function and antigen specificity

Figure 5. Rejuvenation enhanced the functional properties of CD4+ and CD8+ NY-ESO-1 TCR T cells



Rejuvenated (T_{RJ}) and control (T_{CT}) CD4+ and CD8+ NY-ESO-1 TCR T cells of a 24 yo healthy donor were evaluated to compare **(A)** cell proliferation, **(B)** epigenetic age, and **(C)** T-cell function and antigen specificity.

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



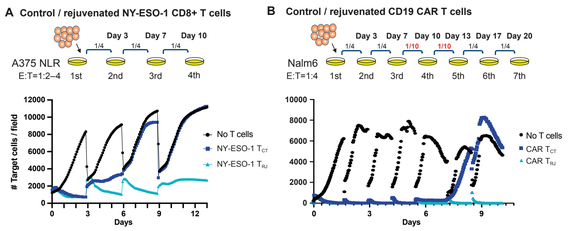
Rejuvenated (T_{RJ}) and control (T_{CT}) CD19 CAR T cells from a 24 yo healthy donor were evaluated to compare **(A)** cell proliferation, **(B)** epigenetic age, **(C)** stemness phenotype, and **(D)** T-cell function and antigen specificity.

Rejuvenation improved the 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 with conventional NY-ESO-1 TCR T cells (**Figure 7A**) or CD19 CAR T cells (**Figure 7B**).

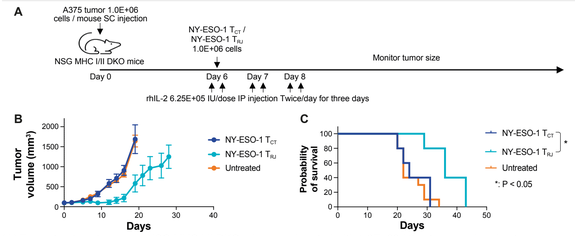
Additionally, in an in vivo tumor model, rejuvenated NY-ESO-1 TCR T cells showed improved tumor volume and probability of survival compared with control NY-ESO-1 TCR T cells (**Figure 8**).

Figure 7. Rejuvenation improved the 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)** rejuvenated CD19 CAR T cells (T_{RJ}) compared with controls (T_{CT}).

Figure 8. In vivo NY-ESO-1 TCR T cells showed enhanced antitumor effects

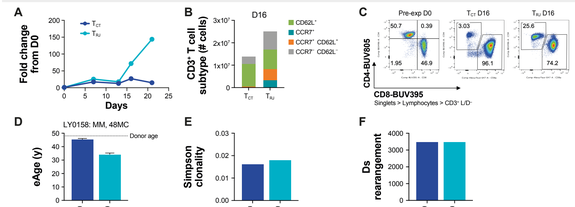


(A) Tumor treatment schema. NSG MHC I/II DKO mice were injected with 1.0E+06 A375 tumor cells subcutaneously. ACT was given on Day 6. In each tumor setting, 5–10 mice were included in groups receiving PBS (orange), 1.0E+06 NY-ESO-1 T_{CT} (dark blue), or 1.0E+06 NY-ESO-1 T_{RJ} (light blue). All mice were given 6.25E+05 IU of rhIL-2 after ACT twice daily for 3 days. Tumor volumes were assessed every two to three days. **(B)** Tumor growth curve. Error bars indicate the mean ± SEM. **(C)** Survival curve. Survival was assessed by a log-rank test.

Rejuvenation enhanced properties associated with T-cell functionality of tumor-infiltrating lymphocytes (TIL)

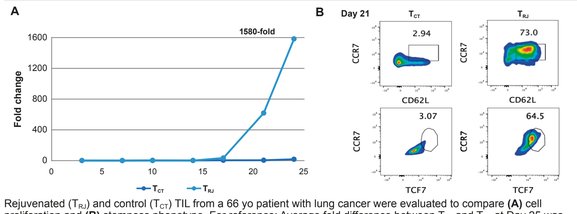
Application of rejuvenation to TIL derived from metastatic melanoma showed improved cell proliferation and stem-like properties as well as a maintained Simpson clonality and TCR repertoire compared with control TIL (**Figure 9**). Similarly, rejuvenated TIL derived from epithelial cancer demonstrated enhanced proliferative capacity and stem-like properties compared with control TIL (**Figure 10**).

Figure 9. Rejuvenation improved cell proliferation capacity, stemness phenotype, and epigenetic age while maintaining the CD4+/CD8+ population and TCR repertoire in metastatic melanoma TIL



Rejuvenated (T_{RJ}) and control (T_{CT}) TIL from a 48 yo patient with metastatic melanoma were evaluated to compare **(A)** cell proliferation, **(B)** stemness phenotype, **(C)** preservation of CD4+ and CD8+ populations, **(D)** epigenetic age, **(E)** Simpson clonality and **(F)** number of TCR rearrangements.

Figure 10. Phenotypic and proliferative analysis of rejuvenated TIL from an epithelial cancer



Rejuvenated (T_{RJ}) and control (T_{CT}) TIL from a 66 yo patient with lung cancer were evaluated to compare **(A)** cell proliferation and **(B)** stemness phenotype. For reference: Average fold difference between T_{RJ} and T_{CT} at Day 25 was 1580.0 vs 17.2, respectively.

Conclusion

- 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 with non-rejuvenated T-cell controls. Rejuvenated NY-ESO-1 TCR T cells also show better tumor suppression and survival than control in an in vivo setting. 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, Lyell's 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; Ds, diversity gene segment; eAge, epigenetic age; E:T, effector to target ratio; IFNγ, interferon gamma; IL-2, interleukin-2; IP, intraperitoneal; iPSC, induced pluripotent stem cells; IU, international units; Klf4, Krüppel-like factor 4; MM, multiple myeloma; 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; PBS, phosphate-buffered saline; RNA-seq, RNA sequencing; SC, subcutaneous; SEM, standard error of the mean; 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; y, years; yo, year old.

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