Rejuvenation of Tumor-Infiltrating Lymphocytes (TIL) Through Partial Reprogramming

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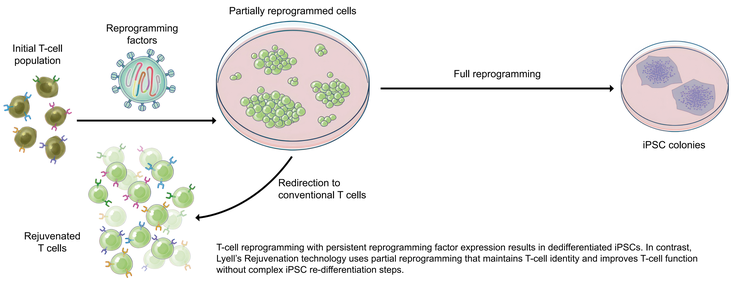
Abstract

- TIL therapy is a promising approach for the treatment of advanced solid tumors; however, efficacy is limited by T-cell exhaustion and terminal differentiation[1,2]
- Recent studies have highlighted the detrimental effects of aging in T cells, including reduced T-cell function, and the efficacy of solid tumor cell therapy[3,4]
- Reprogramming T cells to iPSCs and re-differentiating back to a T-cell lineage has proven complex and time-consuming,[5–7] as reprogramming via iPSCs requires each TCR in the final product to be derived from an individual iPSC clone
- To overcome these barriers, Lyell has developed a novel technology called Rejuvenation, which does not require full reprogramming to iPSCs (**Figure 1**)
- Here, we report that rejuvenated TIL (TIL_{RJ}) retain polyclonality, have reduced epigenetic age, and improved cellular function

Figure 1: Restoring T-cell function and antitumor potential through Rejuvenation



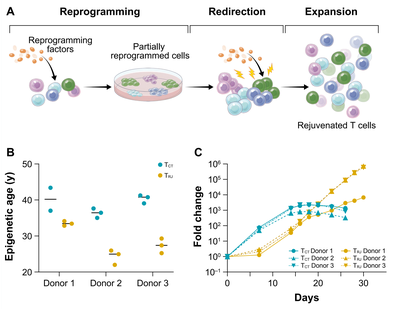
T-cell reprogramming with persistent reprogramming factor expression results in dedifferentiated iPSCs. In contrast, Lyell's Rejuvenation technology uses partial reprogramming that maintains T-cell identity and improves T-cell function without complex iPSC re-differentiation steps.

Results

Novel T-cell Rejuvenation through partial reprogramming

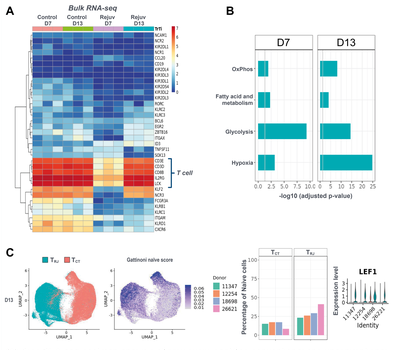
- With Lyell's novel T-cell Rejuvenation technology, aged peripheral blood (PB) T cells transiently express transcription factors associated with iPSC reprogramming in a non-genomic integrative manner
- Once successfully redirected to T cells, rejuvenated T cells (T_{RJ}) acquire the following favorable features compared to control T cells (T_{CT}): (**Figures 2 and 3**)
  - Reduced epigenetic age
  - Enhanced proliferation and metabolism
  - Preservation of stemness markers
- The T_{RJ} do not require complex redifferentiation steps; thus reducing the time required for reprogramming to iPSC and re-differentiation to conventional T cells
- 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 2: Rejuvenated peripheral blood T cells from healthy donors showed reduced epigenetic age and increased proliferation



(A) Schematic representation of T-cell Rejuvenation process. (B) PB T cells from three healthy donors were subjected to Rejuvenation (Donor 1, 37 yo; Donor 2, 42 yo; Donor 3, 52 yo). Measurement of epigenetic status by Horvath's clock on D20 demonstrated a reduced epigenetic age of T_{RJ} compared with T_{CT}. (C) Fold change expansion curve of T_{RJ} compared with T_{CT}. T_{RJ} exhibited increased proliferation. For reference: Average fold expansion of T_{RJ} and T_{CT} at D26 was 61,250 and 868, respectively.

Figure 3: Rejuvenated peripheral blood 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 conventional and unconventional genes related to T-cell identity. (B) Bulk RNA-seq showed enrichment of DEGs associated with key metabolic features in T_{RJ} at D7 and D13 compared with T_{CT}. (C) Single-cell RNA-seq showed elevated expression of naïve-associated markers in T_{RJ} compared with T_{CT}.

Rejuvenation enhanced the properties associated with the T-cell functionality of TIL

- Rejuvenated TIL (TIL_{RJ}) derived from metastatic melanoma (**Figure 4**) showed improved cellular proliferation and stem-like properties as well as a maintained TCR repertoire compared with control TIL (TIL_{CT}) (**Figures 5 and 6**)
- TIL_{RJ} derived from NSCLC demonstrated enhanced proliferative capacity and stem-like properties compared with TIL_{CT} (**Figure 7**)

Figure 4: Restoring TIL function and antitumor potential through Rejuvenation

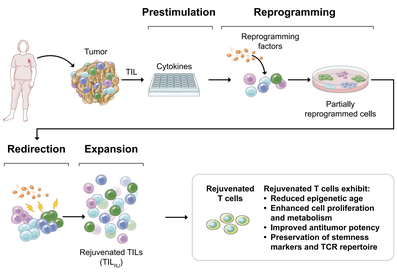
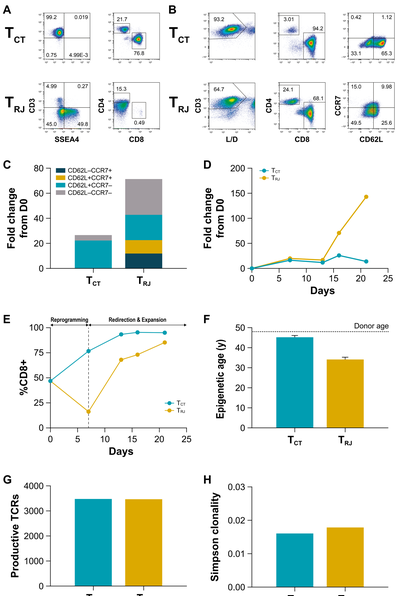
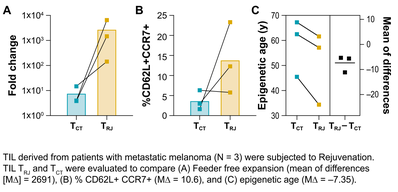


Figure 5: Rejuvenation improved cellular proliferation capacity, stemness phenotype, and epigenetic age while maintaining the CD4+/CD8+ population and TCR repertoire in metastatic melanoma TIL



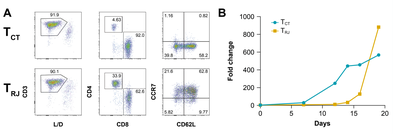
TIL derived from a patient with metastatic melanoma (48 yo) were subjected to Rejuvenation. (A) Partially reprogrammed TIL showed downregulation of the T-cell markers CD3, CD4, and CD8 and temporal expression of a reprogramming marker (SSEA4). (B) Partially reprogrammed T cells redirected to CD4+ T cells and CD8+ T cells and preserved T_{CM} phenotype (CD62L+ CCR7+). TIL T_{RJ} and T_{CT} were evaluated to compare (C) phenotype, (D) cellular proliferation, (E) %CD8+, (F) epigenetic age, and (G, H) TCR repertoire.

Figure 6: Rejuvenation improved cellular proliferation capacity, stemness phenotype, and epigenetic age in metastatic melanoma TIL



TIL derived from patients with metastatic melanoma (N = 3) were subjected to Rejuvenation. TIL T_{RJ} and T_{CT} were evaluated to compare (A) Feeder free expansion (mean of differences [MΔ] = 2691), (B) % CD62L+ CCR7+ (MΔ = 10.6), and (C) epigenetic age (MΔ = –7.35).

Figure 7: Rejuvenation improved stemness phenotype and cellular proliferation capacity in NSCLC TIL

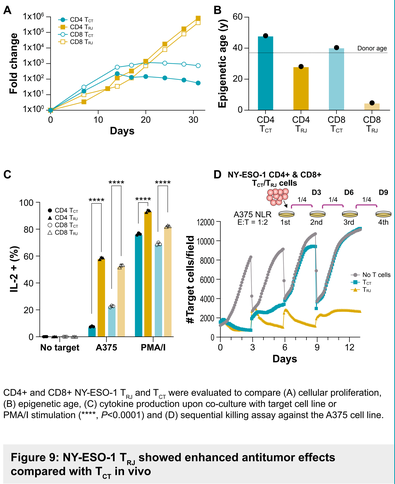


TIL derived from a patient with NSCLC were subjected to Rejuvenation. (A) T_{RJ} demonstrated a better-preserved T_{CM} phenotype (CD62L+ CCR7+) compared with T_{CT}. (B) T_{RJ} demonstrated greater cellular proliferation compared with T_{CT}.

Rejuvenation improved the antitumor efficacy of engineered T-cell therapy in vitro and in vivo

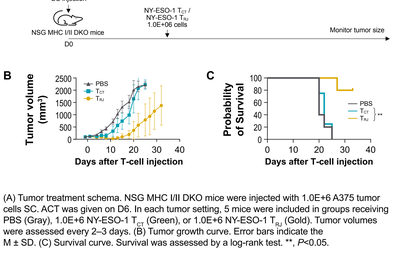
- The NY-ESO-1 TCR transduced system was used as a surrogate model of TIL Rejuvenation
- NY-ESO-1 TCR transduced T cells were rejuvenated, and their function was assessed by cytokine production assays and sequential killing assays
- NY-ESO-1 T_{RJ} showed better proliferation, reduced epigenetic age, higher cytokine production, and better persistence upon repeated encounter with target cells (**Figure 8**)
- In an in vivo tumor model, NY-ESO-1 T_{RJ} showed suppression of tumor growth and improved probability of survival compared with NY-ESO-1 T_{CT} (**Figure 9**)

Figure 8: Rejuvenation improved cellular proliferation capacity and epigenetic age, and enhanced the functional properties of CD4+ and CD8+NY-ESO-1 TCR T cells in vitro



CD4+ and CD8+ NY-ESO-1 T_{RJ} and T_{CT} were evaluated to compare (A) cellular proliferation, (B) epigenetic age, (C) cytokine production upon co-culture with target cell line or PMA/I stimulation (****, $P<0.0001$) and (D) sequential killing assay against the A375 cell line.

Figure 9: NY-ESO-1 T_{RJ} showed enhanced antitumor effects compared with T_{CT} in vivo



(A) Tumor treatment schema. NSG MHC I/II DKO mice were injected with 1.0E+6 A375 tumor cells SC. ACT was given on D6. In each tumor setting, 5 mice were included in groups receiving PBS (Gray), 1.0E+6 NY-ESO-1 T_{CT} (Green), or 1.0E+6 NY-ESO-1 T_{RJ} (Gold). Tumor volumes were assessed every 2–3 days. (B) Tumor growth curve. Error bars indicate the M ± SD. (C) Survival curve. Survival was assessed by a log-rank test. **, $P<0.05$.

Conclusions

- Lyell's T-cell Rejuvenation technology utilizes a partial reprogramming process to produce T cells that are characterized by reduced epigenetic age, enhanced cellular proliferation, improved metabolism, and higher expression of stemness biomarkers. Additional research may further characterize T_{RJ} in terms of their capacity for tumor antigen–specific polyclonality, long-term engraftment, and solid tumor eradication in vivo
- Metastatic melanoma and NSCLC TIL were successfully rejuvenated and acquired improved functionality while retaining a broad TCR repertoire
- 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 TCR T cells exhibited improved antitumor properties compared with control T cells
  - Rejuvenated NY-ESO-1 TCR T cells also showed improved tumor suppression and survival compared with control T cells in an in vivo setting
- Through partial reprogramming, Lyell's T-cell Rejuvenation technology has the potential to be developed as the first rejuvenated autologous polyclonal TIL therapy

Abbreviations

ACT, adoptive cell therapy; CCR7, C-C motif chemokine receptor 7; CD, cluster of differentiation; D, days; DEG, differentially expressed gene; DKO, double knock out; E:T, effector:target; LEF1, lymphoid enhancer binding factor 1; IL-2, interleukin-2; iPSC, induced pluripotent stem cells; L/D, live/dead; M, mean; MΔ, mean of differences; MHC, major histocompatibility complex; N, number of patients; NLR, NucLightRed; NSCLC, non-small cell lung cancer; NSG, NOD scid gamma; NY-ESO-1, New York esophageal squamous cell carcinoma 1; OxPhos, oxidative phosphorylation; PB, peripheral blood; PBS, phosphate-buffered saline; PMA/I, phorbol 12-myristate 13-acetate/ionomycin; RNA-seq, RNA sequencing; SC, subcutaneous; SD, standard deviation; SSEA4, stage-specific embryonic antigen-4; TCR, T-cell receptor; T_{CM}, central memory T cell; T_{CT}, control T cell(s); TIL, tumor-infiltrating lymphocyte(s); TIL_{CT}, control tumor-infiltrating lymphocyte(s); TIL_{RJ}, rejuvenated tumor-infiltrating lymphocyte(s); T_{RJ}, rejuvenated T cell(s); y, years; yo, years old.

References

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Acknowledgments

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