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 immature and unconventional T-cell phenotypes.5,6 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 cellular fitness. T cells under these processes are characterized by a reduced epithenic 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

Rejuvenation produced a conventional T-cell phenotype with improved stemness and metabolism
After the reprogramming 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 increased expression of non-conventional 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 naive-associated markers characteristic of more stem-like T-cell populations (Figure 4C)

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 T, but expression disappeared prior to Day 13. Critically, this non-transient, transient expression of reprogramming factors mitigates the risk of generating immortalized cells.

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

• Increased eAge
• Enhanced proliferation
• Increased stem-like features
• Retained T-cell function and antigen specificity

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

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

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

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

Figure 5. Rejuvenation enhanced the functional properties of CD4+ and CD48- NY-ESO-1 TCR T cells

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

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

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

Rejuvenation enhanced properties associated with T-cell functionality of tumor-infiltrating lymphocytes (TIL)
Application of rejuvenation to TIL derived from metastatic melanoma demonstrated enhanced proliferative capacity and stem-like properties 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 epithenic age while maintaining the CD4+CD8+ population and TCR repertoire in metastatic melanoma TIL

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

Conclusion
1. A novel rejuvenation technology, utilizing a simple reprogramming procedure to produce T cells that exhibit (1) rejuvenated epigenetic age, (2) improved metabolic and antigen specificity, and (3) enhanced antitumor potency, is associated with reduced tumor burden and improved survival.

2. Application of this technology in preclinical settings has demonstrated improved efficacy in solid tumors, including severe subsets such as melanoma and pancreatic cancer.

3. Application of this technology provides a novel platform for in vivo potentiation of conventional T-cell-based immunotherapies, resulting in enhanced antitumor efficacy.

4. Future studies to assess the clinical utility of rejuvenation in the context of a phase I clinical trial (Poster No. 263)

References

Abbreviations
APC, antigen-presenting cell; CAR, chimeric antigen receptor; eAge, epigenetic age; HLA, human leukocyte antigen; iPSC, induced pluripotent stem cell; MHC, major histocompatibility complex; NK, natural killer; OSKM,Oct4, Sox2, Klf4, and c-Myc; PBS, phosphate-buffered saline; PBS, phosphate-buffered saline; TCR, T-cell receptor; TCT, control T cell; TRJ, rejuvenated T cell; y, years; yo, year old.

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