Preclinical Development of LYL797, a ROR1-Targeted CAR T-Cell Therapy Enhanced With Genetic and Epigenetic Reprogramming for Solid Tumors

Spencer Park, Xiao Wang, Courtney Simianer, Sydney Spadinger, Neeraj Sharma, Jia Lu, Byoung Ryu, Lisa Song, Brian Weitzner, Howell Moffett, Marc Lajoie, Scott Boyken, Queenie Vong, Purnima Sundar, Suman Vodnala, Hajime Hiraragi, Bijan Boldajipour, Rachel Lynn, Shobha Potluri, and Blythe Sather

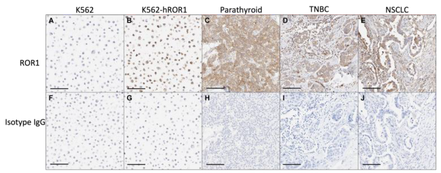
Lyell Immunopharma, Inc., South San Francisco, CA, and Seattle, WA

Abstract No. 661

Introduction

- Chimeric antigen receptor (CAR) T-cell therapy has produced profound results in certain hematologic malignancies but has been less successful for the treatment of solid tumors
  - Studies suggest that T-cell exhaustion plays a role in limiting the ability of CAR T cells to eradicate solid tumors[1]
  - Conventional CAR T-cell products consist of large proportions of differentiated T cells; however, products with a higher proportion of more stem-like, or less-differentiated, T cells are associated with improved anti-tumor efficacy[2]
  - Therefore, maintaining stem-like qualities and overcoming T-cell exhaustion may be key to improving the clinical efficacy of CAR T-cell products in solid tumors
- LYL797 is a novel, ROR1-targeted CAR T-cell product that incorporates epigenetic and genetic reprogramming technologies to overcome these barriers to CAR T-cell therapy in solid tumors
  - Epi-R™ is a proprietary ex vivo epigenetic reprogramming technology designed to create T cells with durable stemness
  - Gen-R™ is an ex vivo genetic reprogramming technology designed to overcome T-cell exhaustion by overexpression of c-Jun
  - ROR1 is a cell-surface antigen expressed in several tumor types (**Fig. 1**), including:
    - Triple-negative breast cancer (57%)[3]
    - Lung adenocarcinoma (42%) and squamous cell carcinoma (12%)[3]
- ROR1 is an attractive therapeutic target for these cancers

Figure 1: Immunohistochemistry analysis of ROR1 cell-surface expression in normal tissues and solid tumors



Anti-ROR1 IHC assay and ROR1 staining in TNBC and in NSCLC samples. Representative images of IHC staining are shown for K562 cells (ROR1 negative control) (A), K562 cells transduced to express human ROR1 (hROR1) (B), and normal parathyroid gland (C) that endogenously expresses ROR1, as assay controls. Representative images of ROR1-positive TNBC sample (D) (BR1301; U.S. Biomax, Inc.) and NSCLC sample (E) (LC2081; U.S. Biomax Inc.) are shown, (F–J): IHC staining with isotype-matched control. Scale bar: 100 µm.

Abbreviations

AP-1, activator protein 1; ATACseq, assay for transposase-accessible chromatin with sequencing; CAR, chimeric antigen receptor; CCR7, C-C motif chemokine receptor 7; CD, cluster of differentiation; CD45RA, CD45 200–245 kDa isoform; CD62L, L-selectin; E, effector; EGFR, epidermal growth factor receptor; IFN-γ, interferon gamma; IgG, immunoglobulin G; IHC, immunohistochemistry; IL-2, interleukin-2; NSCLC, non-small cell lung cancer; ROR1, receptor tyrosine kinase-like orphan receptor-1; scRNAseq, single-cell transcriptome sequencing; T, target; TIL, tumor-infiltrating lymphocyte; TNBC, triple-negative breast cancer; TTE, T-cell terminal exhaustion.

References

1. Fraietta JA, et al. *Nat Med*. 2018;24(5):563-571. 2. Gattinoni L, et al. *Nat Med*. 2017;23(1): 18-27. 3. Balakrishnan A, et al. *Clin Cancer Res*. 2017;23(12):3061-3071. 4. Lynn RC, et al. *Nature*. 2019;576(7786):293-300. 5. Caushi JX, et al. *Nature*. 2021;596:126-132. 6. Oliveira G, et al. *Nature*. 2021;596:119-125.

Acknowledgments

Medical writing and editorial support were funded by Lyell Immunopharma, Inc. and provided by Melanie Styers, PhD, of Verascity Science. Animal studies were performed by Lyell's in vivo pharmacology core. Transcriptome profiling was performed by Lyell's next-gen sequencing core and flow core.

Supplementary Materials

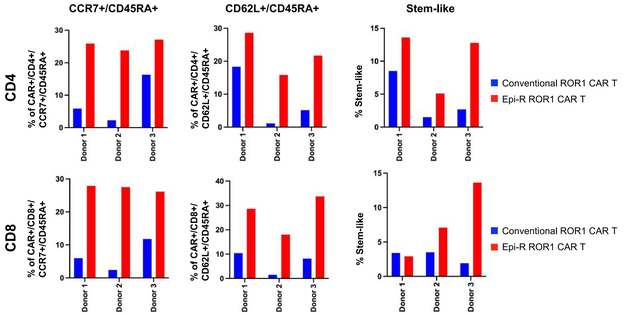
Please scan the Quick Response (QR) code to download a copy of the poster. Copies of content obtained through the QR code are for personal use only and may not be reproduced without permission from the author of this poster: Spencer Park (spark@lyell.com).

Results

Epi-R improves the product phenotype and long-term function of ROR1 CAR T cells

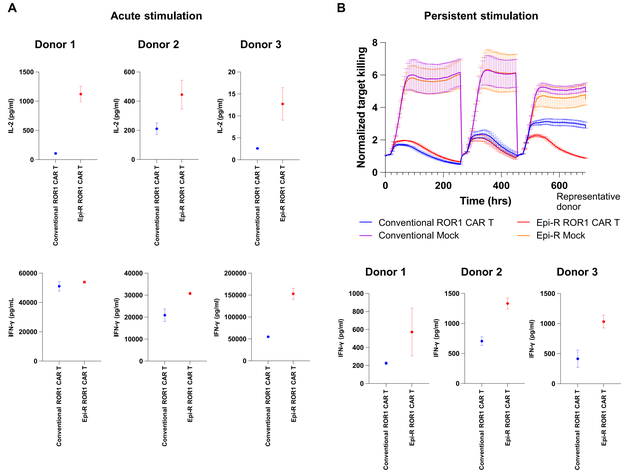
- Epi-R technology is designed to intentionally and reproducibly generate T-cell populations with durable stemness that can proliferate and persist, providing sustained anti-tumor functionality

Figure 2. Composition of conventional versus Epi-R ROR1 CAR T-cell preparations



Epi-R preparation of ROR1 CAR T cells resulted in higher proportions of naive (CCR7+/CD45RA+ or CD62L+/CD45RA+) and stem-like (CD45RO-/CCR7+/CD45RA+/CD62L+/CD27+/CD28+/TCF1/7+) populations compared to conventional ROR1 CAR T cells.

Figure 3. Cytokine production after acute (A) or persistent (B) antigen stimulation of conventional versus Epi-R ROR1 CAR T-cell preparations

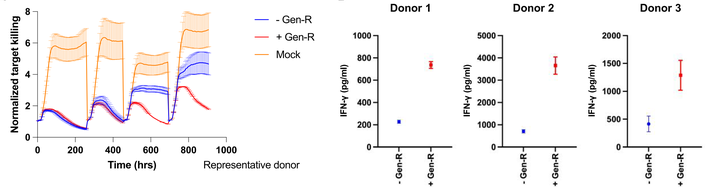


(A) Epi-R improved early cytokine production during short-term co-culture with ROR1+ A549 lung adenocarcinoma cells. (B) Epi-R generated durable anti-tumor function during persistent stimulation with A549 cells and improved the capacity to secrete IFN-γ following 3 consecutive rounds of co-culture.

Gen-R prolongs effector function and delays T-cell exhaustion during serial antigen stimulation

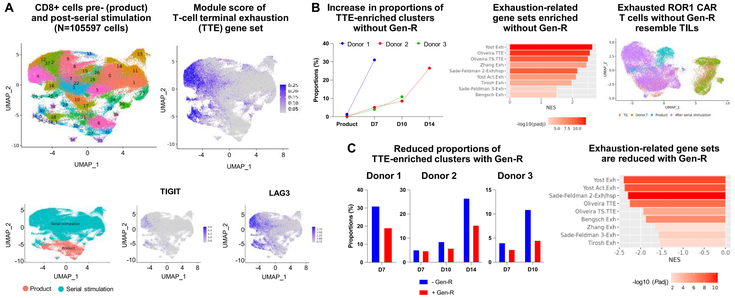
- Exhausted T cells exhibit dysregulation of AP-1 complexes that can be countered by overexpression of the AP-1 family transcription factor c-Jun[4]
- Gen-R technology, the overexpression of c-Jun, rebalances AP-1 complexes in T cells in favor of activation rather than exhaustion

Figure 4. Cytotoxicity (A) and cytokine secretion (B) by ROR1 CAR T cells with Gen-R (+ Gen-R) or without Gen-R (- Gen-R) during serial antigen stimulation



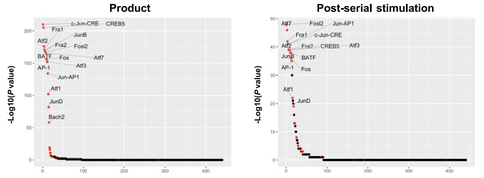
In a serial antigen stimulation model developed to characterize the effects of Gen-R on CAR T-cell exhaustion, ROR1 CAR T cells lacking Gen-R displayed functional characteristics of exhaustion, such as expedited loss of cytolytic capabilities (A) and cytokine secretion (B) following serial antigen stimulation. In contrast, ROR1 CAR T cells modified with Gen-R exhibited prolonged effector function.

Figure 5. Transcriptome profiling of CD8+ ROR1 CAR T cells with Gen-R (+ Gen-R) or without Gen-R (- Gen-R) following serial antigen stimulation



Transcriptome profiling was performed on CD8+ CAR+ T cells sorted at different timepoints of the serial stimulation assay. (A) T-cell clusters with enrichment of T-cell terminal exhaustion gene set (TTE)[5] were identified from analyzing all CD8+ T cells in the single-cell RNA-seq data (black outline). The identification of TTE clusters indicates presence of exhausted CD8+ T cells. (B) in vitro serial stimulation exhausts ROR1 CAR T cells lacking Gen-R. (C) In contrast, ROR1 CAR T cells reprogrammed with Gen-R exhibit reduced exhaustion.

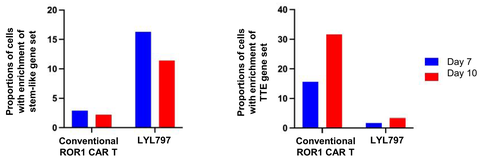
Figure 6. Transcription factor motifs enriched with Gen-R pre- (product) and post-serial antigen stimulation compared to ROR1 CAR T cells without Gen-R



ATACseq analysis revealed c-Jun motif was active and remained active in Gen-R–modified cell products (left) and after serial stimulation (right), suggesting the impact of Gen-R on T cells is durable.

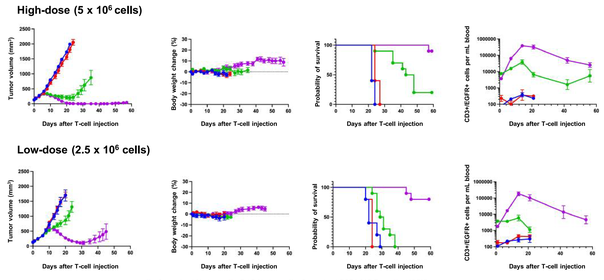
Combined use of Epi-R and Gen-R (LYL797) increases the stem-like phenotype, decreases exhaustion, and enhances in vivo functions of ROR1 CAR T cells

Figure 7. Transcriptome profiling of CD8+ conventional ROR1 CAR T cells and LYL797 following serial antigen stimulation



The proportions of clusters enriched in the stem-like gene set[5] increased (left) while the proportion of clusters enriched in the exhaustion-related gene set[6] decreased (right) in LYL797 compared to conventional ROR1 CAR T cells following serial antigen stimulation.

Figure 8. Improved functional activity of LYL797 (Gen-R + Epi-R) compared to conventional ROR1 CAR T cells



In vivo efficacy of ROR1 CAR T cells in an established human ROR1+ H1975 NSCLC mouse xenograft model. When the mean tumor volume reached ~125 mm³, mice were infused with the indicated dose. Pharmacokinetic profile of T cells in the peripheral blood was monitored over the entirety of the study using EGFR staining. ROR1 CAR T cells with combined Epi-R and Gen-R (LYL797) demonstrated improved survival and expansion in the peripheral blood of tumor-bearing animals, which correlated with improved anti-tumor activity.

Conclusions

- **Collectively, these preclinical results demonstrate that:**
  - **Epi-R produces higher proportions of naive and stem-like T-cell populations, improves cytokine production, and generates durable anti-tumor function in ROR1 CAR T cells**
  - **Gen-R prolongs effector function and delays exhaustion of ROR1 CAR T cells**
  - **Combined use of Epi-R and Gen-R increases the stem-like phenotype, decreases exhaustion, and enhances in vivo function of LYL797, a novel ROR1 CAR T cell therapy**
- **Based on these promising preclinical data, LYL797 is being evaluated for safety and efficacy in a first-in-human phase 1, single-arm, dose-escalation and -expansion study in advanced ROR1+ TNBC and NSCLC (NCT05274451)**

Presented at ASGCT Annual Meeting 2022; May 16–18; Washington, DC, USA