# LYL797, a ROR1 CAR T-Cell Therapy With Genetic and Epigenetic Reprogramming for Solid Tumors

Abstract 2754

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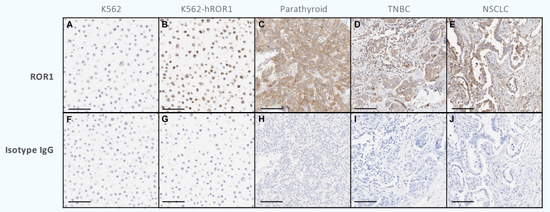
## Background

Chimeric antigen receptor (CAR) T-cell therapy has been shown to produce profound results in the treatment of certain hematologic malignancies, but treatment of solid tumors has not been as successful.[1] Studies suggest that T-cell exhaustion plays a role in limiting the ability of CAR T cells to eradicate solid tumors.[2] Additionally, higher degrees of differentiation in T-cell products have been associated with reduced efficacy, with more stem-like products correlating with improved outcomes.[3] Therefore, a key to improving clinical efficacy of CAR T cells in patients with solid tumors lies in overcoming T-cell exhaustion and increasing stem-like product qualities.

### Targeting ROR1 in Solid Tumors

- Patients with relapsed, refractory, or advanced triple-negative breast cancer (TNBC) and non-small cell lung cancer (NSCLC) have limited effective treatment options
- ROR1 is a cell-surface antigen expressed in chronic lymphocytic leukemia (CLL) and several solid tumor types; it is expressed in approximately 60% of TNBC and 40% of the adenocarcinoma subtypes of NSCLC,[4] findings confirmed in our research (**Figure 1**)
- Thus, ROR1 is an attractive target for novel therapies, including adoptive cell therapy (ACT)

**Figure 1. ROR1 Cell Surface Expression by Immunohistochemistry on Normal Tissues and Solid Tumors**

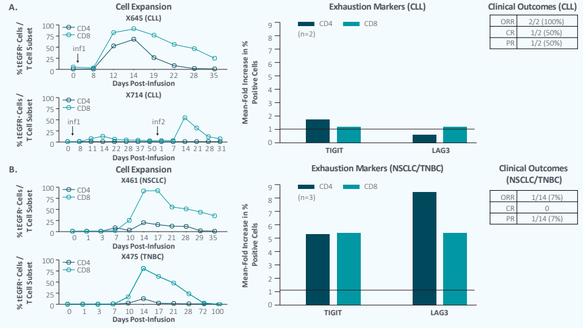



Representative images of IHC staining are shown for K562 cells (ROR1 negative control) (A) K562 cells transduced to express human ROR1 (hROR1) (B) and normal parathyroid gland (C) that endogenously expresses ROR1, as assay controls. Representative images of ROR1-positive TNBC sample (D) (BR1301; U.S. Biomax Inc.) and NSCLC sample (E) (LC2081; U.S. Biomax Inc.) are shown. (F) through (J): IHC staining with isotype-matched control antibody (Clone DA1E; Cell Signaling Technologies) (Study LP20-021) Scale bar: 100 µm

### Conventional ROR1 CAR T Cells Exhaust in Solid Tumors

- ROR1-targeted CAR T cells developed with conventional processes have different trajectories in the setting of hematologic vs solid tumor malignancies, with the latter exhibiting reduced overall effector function and progressing down an exhaustion pathway (**Figure 2**)
- A better understanding of this variance in CAR T-cell functional activity can be used to inform strategies for novel, more effective ACT for solid tumors

**Figure 2. Characteristics and Clinical Outcomes of Conventional ROR1-targeted CAR T cells in patients with Hematologic Malignancy (CLL) and Solid Tumors (NSCLC, TNBC)[5]**




A. Conventional ROR1 CAR T cells taken from patients with CLL demonstrated anticipated cellular expansion, low levels of exhaustion markers, and clinical activity.
B. T cells taken from the solid tumor patients treated with the same CAR T cells demonstrated anticipated expansion, however there were higher levels of exhaustion markers and poor clinical activity.

### Gen-R™: An Ex Vivo Genetic Reprogramming Technology to Overcome Exhaustion

- Exhausted T cells exhibit dysregulation of activation protein-1 (AP-1) complexes that can be countered by overexpression of the AP-1 family transcription factor c-Jun
- Gen-R technology results in the overexpression of c-Jun in T cells and rebalances AP-1 complexes in favor of activation and away from exhaustion pathways; this promotes maintenance of T-cell activation, proliferation, cytokine production, and cytotoxic activity in the exhausted T cell (**Figure 3**)[6]
- CAR T cells with Gen-R exhibit prolonged function and potency, decreased exhaustion markers and functional inhibition, and improved infiltration of solid tumors, resulting in greater overall tumor responses (**Figure 4**)

**Figure 3. Effects of c-Jun Overexpression via Gen-R on T Cell Activity**

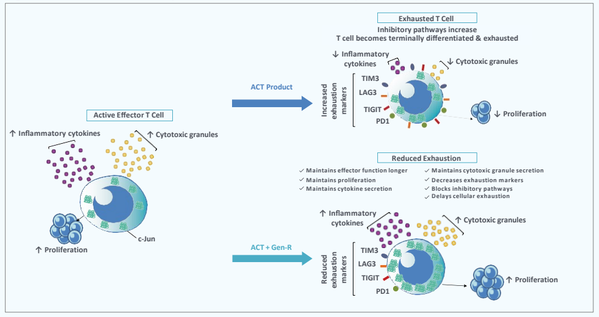
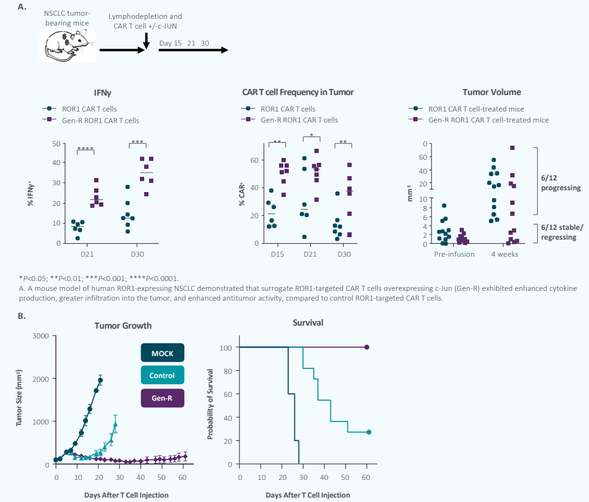



**Figure 4. Activity of ROR1-Targeted CAR T Cells With Gen-R in Human ROR1+ NSCLC Model**




*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.
A. A mouse model of human ROR1-expressing NSCLC demonstrated that surrogate ROR1-targeted CAR T cells overexpressing c-Jun (Gen-R) exhibited enhanced cytokine production, greater infiltration into the tumor, and enhanced antitumor activity, compared to control ROR1-targeted CAR T cells.




B. In a NSG xenograft model of a human ROR1+ lung cancer cell line (H1975), Gen-R ROR1 CAR T cells demonstrated enhanced anti-tumor efficacy and prolonged survival.

### Epi-R™: An Ex Vivo Epigenetic Reprogramming Technology to Improve Stemness

- Conventional ACTs consist of a mixture of extensively differentiated T cells; higher proportions of stem-like T cells have been associated with improved anti-tumor efficacy
- Epi-R is an optimized manufacturing process that is designed to intentionally and reproducibly generate populations of T cells with properties of durable stemness (**Figure 5**)
- T cells with durable stemness can proliferate, persist, and are able to provide prolonged anti-tumor functionality.

**Figure 5. Composition of Conventional T-cell Preparations vs Epi-R T-cell Preparations**

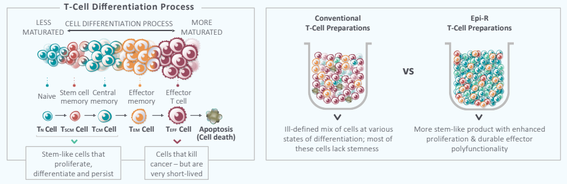
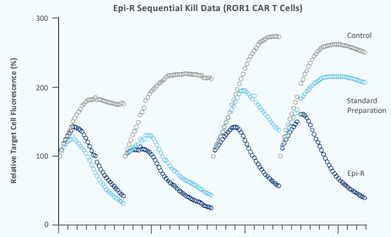
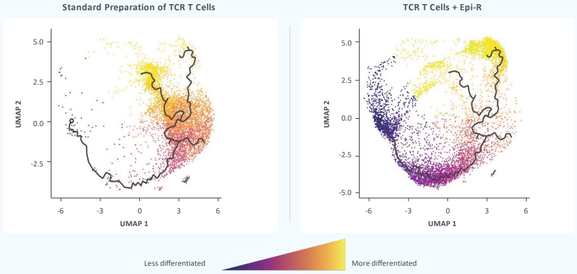



**Figure 6. Cytotoxicity of ROR1 CAR T Cells Produced With Epi-R vs. Standard Preparation CAR T Cells and Control T Cells Upon Serial Re-Exposure to Tumor Cells**




ROR1 CAR T cells + Epi-R, standard preparation CAR T, and untransduced T cells (control) were compared during repeated restimulation using ROR1+ lung cancer cells (NCI-H1975).

**Figure 7. Difference in Epigenetic States of TCR T cells + Epi-R vs Standard Preparation TCR T cells Following Repeat Antigen Stimulation**




Standard Preparation TCR T cells and TCR T cells + Epi-R were analyzed using single-cell RNA-Seq to visualize epigenetic state transitions in response to repeated antigen stimulation
- Standard Preparation TCR T cells were predominantly more differentiated (orange to yellow) following repeated stimulation
- TCR T cells + Epi-R produced less differentiated, more stem-like cells (blue to purple) and effector cells (yellow), and maintained stem-like qualities after removal from Epi-R conditions

### LYL797: A Novel ROR1-Targeted CAR T Cell Product Incorporating Gen-R and Epi-R Technologies

LYL797 incorporates proprietary genetic and epigenetic reprogramming technologies to overcome barriers to effective CAR T-cell therapy for solid tumors, namely CAR T-cell exhaustion and lack of stemness.

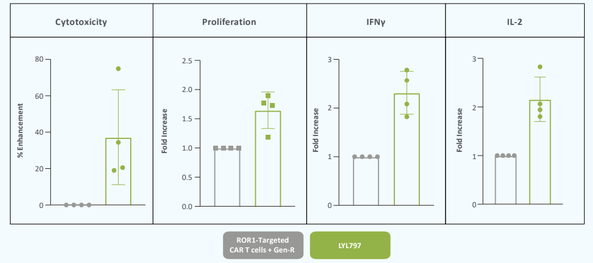
LYL797 construct contains:

- ROR1-specific CAR: a 4-1BB/CD3ζ co-stimulatory domain, an optimized spacer, and a single-chain variable fragment (scFv) derived from an R12 rabbit monoclonal antibody that recognizes and binds with high specificity to human ROR1
- A proprietary version of human EGFR ($EGFR_{tpl}$) used for tracking of the CAR T cells in the peripheral blood
- c-Jun (Gen-R)

### LYL797 Preclinical Data

In preclinical studies, ROR1-targeted CAR T cells reprogrammed with both Gen-R and Epi-R showed improved antitumor activity, proliferative capacity, and cytokine secretion compared to ROR1-targeted CAR T reprogrammed with Gen-R alone (**Figure 8**). These data support the use of both Gen-R and Epi-R technologies in the development of LYL797.

**Figure 8. Improvement in Functional Activity of LYL797 (Gen-R + Epi-R) versus ROR1-Targeted CAR T Cells Reprogrammed With Gen-R Alone (Control)**




LYL797 and ROR1-targeted CAR T cells + Gen-R (control) were repeatedly stimulated using ROR1+ lung cancer cells (NCI-H1975).
LYL797 showed increased cytotoxicity, proliferation, and IFNγ and IL-2 secretion compared to control, demonstrating the beneficial effects of Gen-R and Epi-R on CAR T-cell function.

## Conclusion

- Preclinical studies of Gen-R and Epi-R technologies suggest they overcome two barriers to ACT therapies in patients with solid tumors: T-cell exhaustion and lack of durable stemness
- Studies are ongoing to determine the mechanisms by which antitumor activity of LYL797 in ROR1-expressing solid tumor xenograft models is enhanced
- LYL797 will be evaluated for safety and efficacy in a phase 1, first-in-human, single arm, dose-escalation and -expansion study in advanced ROR1+ TNBC and NSCLC (NCT05274451)

## Abbreviations

AP-1, activator protein-1; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CR, complete response; D, day; IFN, interferon; IL, interleukin; NSCLC, non-small cell lung cancer; ORR, overall response rate; PR, partial response; TNBC, triple-negative breast cancer

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