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Abstract #283

Multiomic Profiling of LYL119: Reprogramming ROR1 CAR T cells With Reduced Exhaustion and Enhanced Memory Characteristics Is Associated With Increased AP-1 and Reduced NR4A Binding

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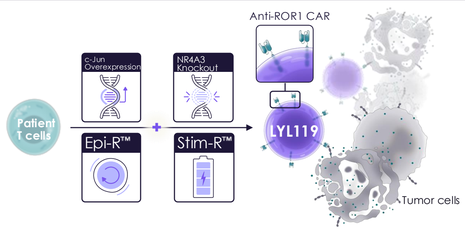
Background

Effective solid tumor cell therapy requires new strategies to improve T-cell activation, persistence, and durable function[1-2]. Lyell has developed LYL119, a ROR1-targeted chimeric antigen receptor (CAR) T-cell product enhanced with four T-cell reprogramming technologies to improve CAR T-cell functionality[3].

Figure 1: LYL119, a ROR1-targeted CAR T-cell product



LYL119 is a ROR1-targeted CAR T-cell product incorporating (1) c-Jun overexpression to counter exhaustion; (2) NR4A3 knockout to further resist exhaustion and enhance antitumor activity; (3) Epi-R™ manufacturing protocol to preserve T-cell stem-like qualities; and (4) Stim-R™ activation technology (a synthetic biomimetic designed to precisely and physiologically present T-cell activation signals during manufacturing) to improve T-cell potency.

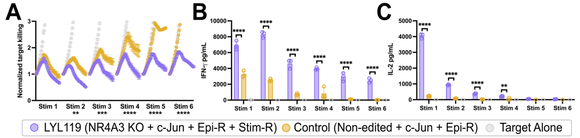
Methods

- LYL119 and control ROR1 CAR T cells (Non-edited + c-Jun + Epi-R) were manufactured with Epi-R and c-Jun overexpression. LYL119 was activated with Stim-R and incorporated NR4A3 editing. Control CAR T cells were activated with a standard reagent and were not edited.
- ROR1 CAR T-cell functional activity as well as transcriptional and epigenetic profiles (single-cell Multiome) were evaluated in vitro after a serial restimulation assay designed to induce exhaustion.
- Antitumor activity and transcriptional profile (CITE-Seq) of ROR1 CAR T cells were evaluated in vivo using a ROR1-expressing H1975 human NSCLC xenograft model in NSG MHCI/II dKO mice.

Results

LYL119 demonstrates superior in vitro and in vivo activity compared to control (Figures 2 and 3)

Figure 2: LYL119 demonstrates superior in vitro activity following serial antigen restimulation



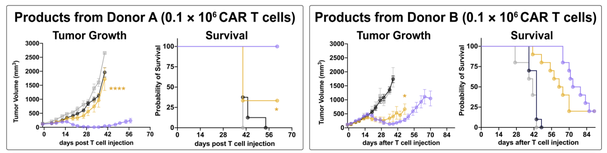
Serial antigen restimulation of ROR1 CAR T cells with A549 NSCLC tumor cells at an E:T ratio of 1:4. (A) Cytotoxicity, (B) IFN-γ and (C) IL-2 secretion from one out of two representative products is shown. Error bars represent mean ± SD of triplicate wells. Asterisks indicate significant differences comparing (A) the AUC, (B) IFN-γ, or (C) IL-2 levels of LYL119 and control CAR T cells.

References

1. Albelda SM. Nat Rev Clin Oncol. 2024;21(1):47-66
2. Krishna S, et al. Science. 2020;370:1328–1334
3. Lam V, et al. SITC 2023 Poster. Abstract 278
4. Sade-Feldman M, et al. Cell. 2018;175(4):998-1013.e20
5. Good CR, et al. Cell. 2021;184(25):6081-6100.e26
6. Caushi JX, et al. Nature. 2021;596(7870):126-132
7. Jansen CS, et al. Nature. 2019;576(7787):465-470
8. Zhao, J, et al. Cell discovery. 2020; 6(1): 22

Results

Figure 3: LYL119 eliminates xenograft tumor and prolongs animal survival in vivo at low CAR T-cell dose



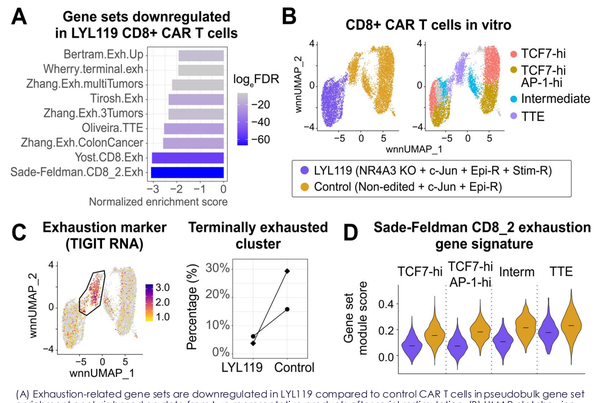
Tumor volume and animal survival at the 0.1 x 10⁶ CAR T-cell dose in a H1975 xenograft NSG MHCI/II dKO mouse model. Data from 2 of 3 independent animal studies are shown. Error bars represent mean ± SEM. Asterisks indicate significant differences compared to LYL119-treated animals.

LYL119 exhibits an improved phenotype associated with increased AP-1 motif accessibility and reduced NR4A motif accessibility following in vitro antigen restimulation (Figure 4 to 6)

After 15 days of restimulation with A549 NSCLC tumor cells, compared to Control, LYL119 displays:

- Lower proportion of terminally exhausted T cells and downregulation of exhaustion-related gene signatures across phenotypic cell subsets, associated with reduced NR4A motif accessibility (Figure 4 to 5)
- Upregulation of memory T-cell marker IL7R across phenotypic cell subsets, associated with increased AP-1 motif accessibility (Figure 6, A to D)
- Presence of a unique TCF7-hi stem-like cell subset with upregulation of stemness-related and effector-related gene signatures, indicating both persistence and cytotoxicity (Figure 6E)

Figure 4: LYL119 exhibits reduced exhaustion-related gene signatures in vitro



(A) Exhaustion-related gene sets are downregulated in LYL119 compared to control CAR T cells in pseudobulk gene set enrichment analysis based on data from two representative products after serial restimulation. (B) UMAP plot showing phenotypic annotation of cell subsets found by unsupervised clustering. (C) The terminally exhausted cluster enriched for TIGIT RNA expression is circled and is present at a lower proportion in LYL119. Symbols represent independent products. (D) The CD8_2 exhaustion gene set[4] is downregulated in LYL119 across phenotypic cell subsets. Data from one representative product after serial restimulation is shown in (B), left panel of (C), and (D).

Acknowledgement

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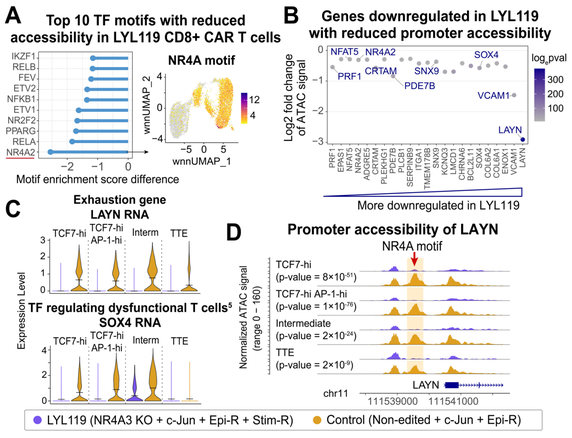
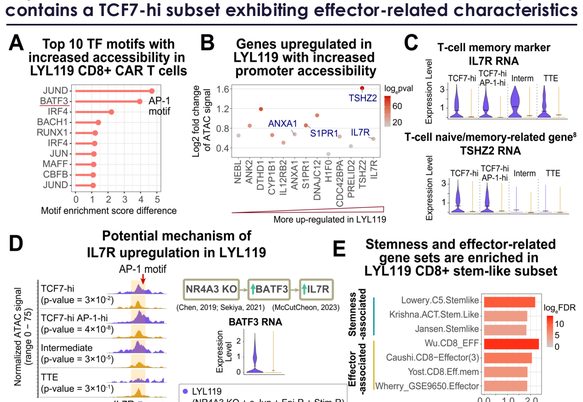
Figure 5: LYL119 exhibits decreased NR4A motif accessibility and reduced chromatin accessibility near exhaustion-related genes



(A) Top 10 transcription factor (TF) motifs with reduced enrichment score in LYL119 compared to control CAR T cells. The NR4A motif (named by NR4A2) is highlighted. (B) Genes significantly downregulated in LYL119 with reduced promoter accessibility. Exhaustion-related genes from gene sets shown in Figure 4A are highlighted. (C) LAYN and SOX4 RNA expression. (D) NR4A motif is present in LAYN's promoter peak (highlighted) with significantly reduced accessibility in LYL119 across phenotypic cell subsets. Data from one representative product after serial restimulation is shown in (A), (C), and (D).

Figure 6: LYL119 exhibits enhanced memory-related gene expression associated with increased AP-1 motif accessibility and contains a TCF7-hi subset exhibiting effector-related characteristics



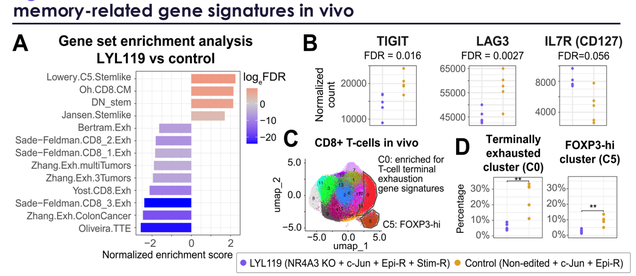
(A) Top 10 TF motifs with increased enrichment score in LYL119 compared to control CAR T cells. The AP-1 motif (named by BATF3) is highlighted. (B) Genes significantly upregulated in LYL119 with increased promoter accessibility. Stemness-related genes[6,7] are highlighted. (C) IL7R and TSHZ2 RNA expression. (D) AP-1 motif is present in the promoter peak of IL7R (highlighted) with increased accessibility in LYL119. One potential regulatory mechanism is shown. (E) The TCF7-hi stem-like subset in LYL119 shows elevated stemness-related and effector-related gene signatures compared to its counterpart in control. Data from one representative product after serial restimulation is shown in (A), (C), and (D).

LYL119 exhibits an improved phenotype in an in vivo xenograft tumor model compared to control (Figure 7)

On day 14 after T-cell injection (1 x 10⁶ CAR T-cell dose), tumor-infiltrating ROR1 CAR+ T cells from LYL119 exhibits:

- Downregulation of exhaustion-related gene signatures and lower proportion of terminally exhausted T cells
- Upregulation of memory/stemness-related gene signatures
- Lower proportion of a potentially suppressive FOXP3-hi T-cell cluster

Figure 7: LYL119 exhibits reduced exhaustion and enhanced T-cell memory-related gene signatures in vivo



(A) Pseudobulk gene set enrichment analysis results of exhaustion-related gene sets and T-cell stemness/memory-related gene sets based on data from 10 mice. (B) RNA expression of TIGIT, LAG3 and IL7R. (C) UMAP plot highlighting the cluster most enriched for T-cell terminal exhaustion gene signatures (C0) and a FOXP3-hi cluster (C5). (D) Proportion of C0 and C5. Symbols represent individual mice in each treatment group. Asterisks indicate significant differences.

Conclusions

LYL119, a ROR1-targeted CAR T-cell product enhanced with c-Jun overexpression, NR4A3 KO, Epi-R manufacturing protocol, and Stim-R technology exhibits:

- Superior in vitro activity compared to control CAR T cells and antitumor activity in vivo even at the low dose of 0.1 x 10⁶ cells.
- Reduced T-cell exhaustion and enhanced memory-related characteristics after antigen encounter in vitro and in vivo.
- Reduced accessibility of the NR4A binding motif and increased accessibility of the AP-1 binding motif in vitro.

These data suggest that enhanced LYL119 antitumor functions may be attributed to reduction of T-cell exhaustion and maintenance of T-cell memory characteristics. The improved phenotype potentially results from epigenetic changes favoring AP-1 family transcription factor binding while reducing NR4A binding. LYL119 is entering Phase I clinical development in patients with ROR1+ solid tumors and will initially enroll patients with platinum-resistant ovarian cancer or relapsed/refractory endometrial cancer.

Abbreviations:

AUC, area under the curve; AP-1, activator protein 1; BATF3: basic leucine zipper transcriptional factor ATF-like 3; CAR, chimeric antigen receptor; CD, cluster of differentiation; dKO, double knockout; E:T, effector-to-target; FOXP3, forkhead box protein P3; IFN-γ, interferon gamma; IL-2, interleukin 2; IL7R, interleukin 7 receptor; Interm, Intermediate; KO, knockout; LAG3, lymphocyte activation gene 3; LAYN, layilin; NR4A3, nuclear receptor subfamily 4 group A member 3; MHC, major histocompatibility complex; NSCLC, non-small cell lung cancer; NSG, NOD scid gamma; ROR1, receptor tyrosine kinase-like orphan receptor 1; SD, standard deviation; SEM, standard error of the mean; SOX4, SRY-box transcription factor 4; TCF7, transcription factor 7; TF, transcription factor; TIGIT, T cell immunoreceptor with Ig and ITIM domains. TSHZ2, teashirt zinc finger homeobox 2.

* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.