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Abstract #49

LYL119, a Preclinical ROR1-Targeted CAR T-Cell Product Incorporating Four Novel Reprogramming Technologies Designed to Enable Functional Cell Therapy for Solid Tumors

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Background

- T-cell exhaustion and lack of durable stemness (defined as the ability of cells to proliferate, persist, and self-renew) are key barriers to effective T-cell therapy in solid tumors[1-2]
- Lyell has developed multiple genetic and epigenetic T-cell reprogramming technologies to overcome these barriers:

Genetically reprogramming T cells through c-Jun overexpression resists exhaustion and results in increased proliferation, sustained cytokine production, and durable antitumor activity[1,3–4]

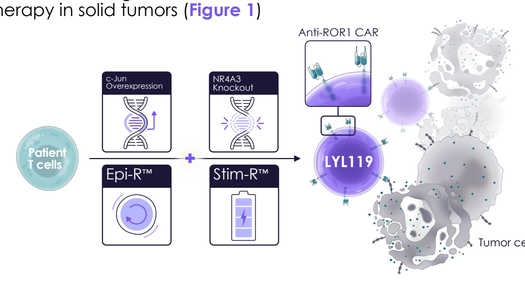
Genetically reprogramming T cells through *NR4A3* gene KO in combination with c-Jun overexpression further enhances resistance to exhaustion and improves antitumor activity[5]

Epigenetic reprogramming with Lyell's Epi-R™ manufacturing protocol preserves stem-like qualities by controlling T-cell proliferation and differentiation with optimized proprietary cell culture media and other manufacturing steps[1,6–8]

Epigenetic reprogramming with Lyell's Stim-R™ technology (a synthetic biomimetic designed to precisely and physiologically present T-cell activation signals during manufacturing) further improves T-cell polyfunctionality, persistence, and antitumor activity[9]

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

These four T-cell reprogramming technologies are combined in LYL119, an investigational ROR1-targeted CAR T-cell product enhanced with c-Jun over-expression, NR4A3 KO, and Epi-R and Stim-R technologies to overcome barriers to successful T-cell therapy in solid tumors (**Figure 1**)



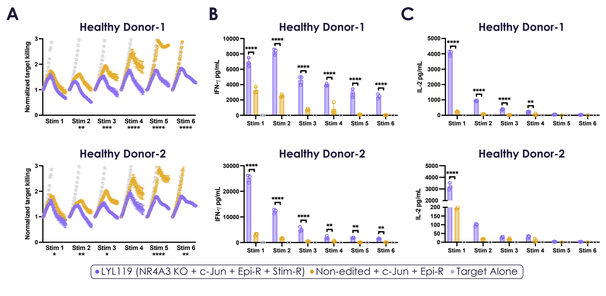
Methods

- Healthy-donor ROR1 CAR T cells were manufactured at research or clinical scale using the Epi-R manufacturing protocol, activated with Stim-R technology or a standard reagent, and transduced with a lentiviral vector encoding c-Jun, the ROR1 CAR, and an optimized variant of truncated EGFR (EGFRopt). NSCLC patient-donor ROR1 CAR T cells were manufactured at research scale.
- T cells were electroporated with a single guide RNA targeting human *NR4A3* complexed with SpyFi™ Cas9 nuclease (Aldevron®)
- ROR1 CAR T-cell cytotoxicity, cytokine production, phenotype, and single-cell transcriptomic and epigenetic profiles were evaluated in vitro following antigen restimulation assays designed to promote T-cell exhaustion
- Antitumor activity and transcriptomic analysis of ROR1 CAR T cells were evaluated in vivo using a ROR1-expressing H1975 human NCLSC xenograft model in NSG MHCI/II dKO mice

Results

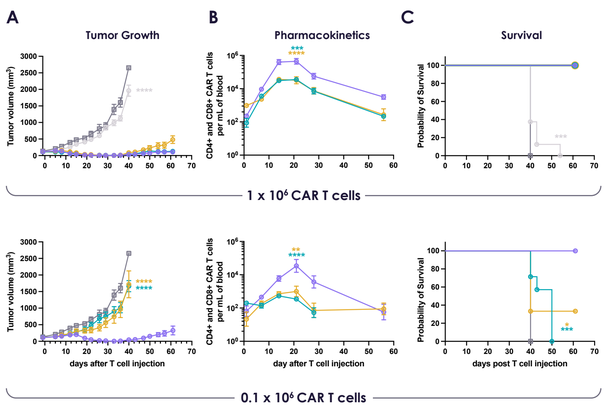
LYL119 demonstrated superior in vitro and in vivo activity compared to ROR1 CAR T cells reprogrammed with 2 or 3 technologies (Figures 2 and 3)

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



(A) Serial antigen restimulation with A549 NSCLC tumor cells at an E:T ratio of 1:4. (B) IFN-γ and (C) IL-2 secretion during antigen exposure described in (A). Two healthy independent donors at clinical scale are 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 non-edited + c-Jun + Epi-R conditions.

Figure 3: LYL119 has robust antitumor activity and proliferation in vivo



(A) Tumor volume, (B) peripheral blood ROR1 CAR T cells, and (C)animal survival at a 1 x 10^6 and 0.1 x 10^6 CAR T-cell dose range in a H1975 xenograft NSG MHCI/II dKO mouse model. Data from one representative research-scale donor of 3 independent animal studies is shown. Error bars represent mean ± SEM. Statistical analysis of peripheral blood ROR1 CAR T cell expansion was performed on Day 21 after T-cell injection. Asterisks indicate significant differences compared to LYL119–treated animals.

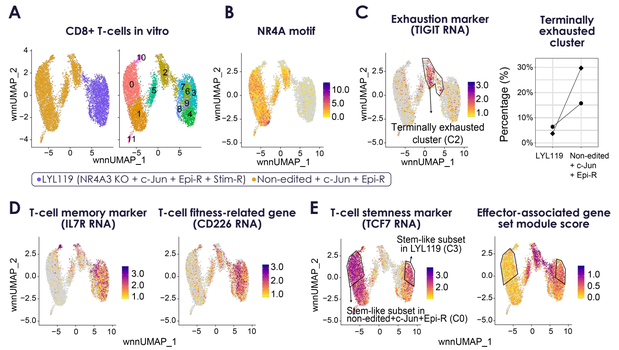
References

1. Park S, et al. AACR 2022 Poster. Abstract 2754
2. Krishna S, et al. *Science*. 2020;370:1328–1334
3. Lynn RC, et al. *Nature*. 2019;576:293–300
4. Park S, et al. ASGCT 2022 Poster. Abstract 661
5. Lam V, et al. SITC 2022 Poster. Abstract 243
6. Patel Y, et al. SITC 2022 Poster. Abstract 370
7. Harris BD, et al. SITC 2022 Poster. Abstract 340
8. Patel Y, et al. AACR Special Conference: Tumor Immunology and Immunotherapy 2022 Poster. Abstract A54
9. Li A, et al. SITC 2022 Poster. Abstract 252

LYL119 exhibited an improved phenotype following in vitro antigen restimulation as well as in an in vivo xenograft tumor model compared to ROR1 CAR T cells reprogrammed with c-Jun overexpression and Epi-R protocol (Figures 4 and 5)

- In vitro, LYL119 displayed (**Figure 4**):
  - Lower NR4A motif enrichment score across cells
  - Lower proportion of the cluster most enriched for terminal exhaustion gene signatures
  - Upregulation of memory T-cell marker IL7R and T-cell fitness-related gene CD226
  - Presence of a unique TCF7+ stem-like cell subset with elevated expression of effector-associated gene signatures, indicating both persistence and cytotoxicity
- In vivo, LYL119 exhibited (**Figure 5**):
  - Lower proportion of the cluster most enriched for terminal exhaustion gene signatures
  - Increased expression of IL7R and CD226
  - Lower proportion of a potentially suppressive FOXP3-hi T-cell cluster

Figure 4: LYL119 exhibits reduced exhaustion and enhanced T-cell memory/fitness-related gene expression in vitro



Single-cell Multiome data of CD8+ T cells within LYL119 and non-edited + c-Jun + Epi-R ROR1 CAR+ T cells after 15 days of restimulation with A549 NSCLC tumor cells. (A) UMAP plot derived from one clinical-scale donor showing identified T-cell clusters. (B) NR4A motif enrichment score. (C) The terminally exhausted cluster enriched for TIGIT RNA expression is circled and is present at a lower proportion in LYL119. Symbols correspond to CAR T cells derived from independent donors. (D) IL7R and CD226 RNA expression. (E) A TCF7-hi stem-like subset in LYL119 (circled, C3) shows elevated effector-related gene signatures compared to its counterpart in non-edited + c-Jun + Epi-R ROR1 CAR+ T cells (C0).

Abbreviations:
AUC, area under the curve; CAR, chimeric antigen receptor; CD, cluster of differentiation; dKO, double knockout; EGFRopt, optimized variant of truncated epidermal growth factor receptor; E:T, effector-to-target; FOXP3, forkhead box protein P3; IFN-γ, interferon gamma; IL-2, interleukin 2; IL7R, interleukin 7 receptor; KO, knockout; LAG3, lymphocyte activation gene 3; NR4A3, nuclear receptor subfamily 4 group A member 3; NLR, NucLight Red; 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; TCF7, transcription factor 7; TIGIT, T cell immunoreceptor with Ig and ITIM domains.

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

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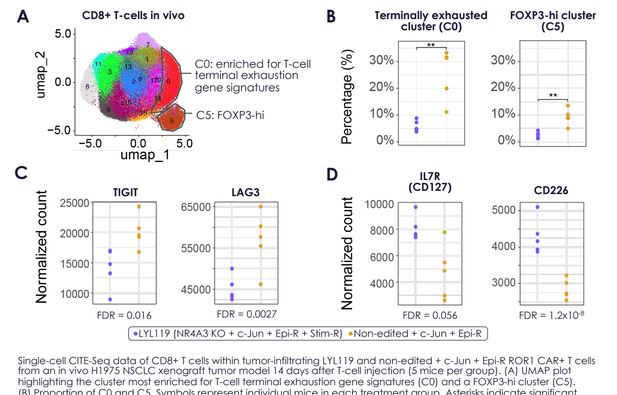
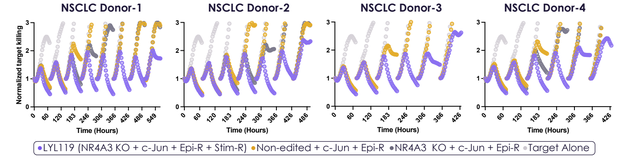
Figure 5: LYL119 exhibits reduced exhaustion and enhanced T-cell memory/fitness-related gene expression in vivo



Single-cell CITE-Seq data of CD8+ T cells within tumor-infiltrating LYL119 and non-edited + c-Jun + Epi-R ROR1 CAR+ T cells from an in vivo H1975 NSCLC xenograft tumor model 14 days after T-cell injection (5 mice per group). (A) UMAP plot highlighting the cluster most enriched for T-cell terminal exhaustion gene signatures (C0) and a FOXP3-hi cluster (C5). (B) Proportion of C0 and C5. Symbols represent individual mice in each treatment group. Asterisks indicate significant differences. (C-D) RNA expression of TIGIT, LAG3, IL7R and CD226.

Figure 6: NSCLC patient-derived LYL119 demonstrates superior cytotoxicity in vitro



Normalized target killing following sequential stimulation with H1975 NSCLC tumor cells at a 1:25 E:T starting ratio. Lysis of NLR-expressing tumor cells was quantified by measuring total NLR intensity and normalized relative to the starting intensity for each round of stimulation.

Conclusions

- LYL119, an investigational ROR1-targeted CAR T-cell product enhanced with c-Jun overexpression, *NR4A3* KO, Epi-R manufacturing protocol, and Stim-R technology exhibited:
  - Potent cytotoxicity and enhanced cytokine secretion upon antigen restimulation in vitro
  - Potent antitumor activity, superior ROR1 CAR T-cell expansion, and improved survival in a H1975 xenograft tumor mouse model
  - Enhanced memory and T-cell fitness-related gene expression with reduced T-cell exhaustion after antigen encounter in vitro and in vivo
- LYL119 produced from NSCLC patient cells demonstrated superior cytotoxic activity compared to patient-derived ROR1 CAR T cells reprogrammed with 2 or 3 technologies

These preclinical studies demonstrate the potential of LYL119 to provide effective and durable activity. LYL119 is being advanced into a Phase I clinical trial in patients with ROR1+ solid tumors.