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Abstract #285

LYL797 Reprogrammed ROR-1 CAR T cells Demonstrate Limited Exhaustion, Maintenance of Stemness and Tumor Infiltration with Evidence of Tumor Lysis in Patients with Solid Tumors

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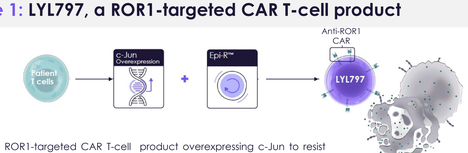
Background

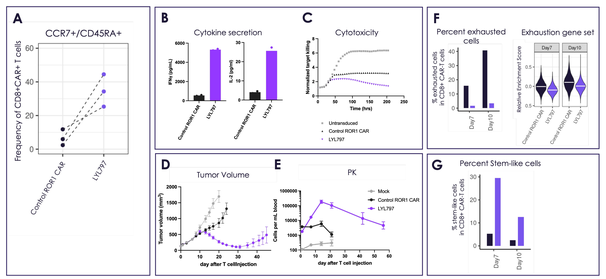
- Chimeric antigen receptor (CAR) T-cell therapy is effective in treating certain hematologic malignancies. Solid tumors present barriers to effective cellular therapy including an immune suppressive tumor environment leading to T-cell exhaustion[1] and the need for durable stemness in T-cell products[2].
- LYL797 is a ROR1-targeted CAR T-cell investigational drug designed to address these barriers using c-Jun overexpression to delay exhaustion[3] and Epi-R™ manufacturing protocol to preserve T-cell stemness[4-5] (Figure 1-2).
- Initial translational data from a multi-center Phase 1 dose-escalation trial in patients with relapsed/refractory ROR1+ triple-negative breast cancer (TNBC) and non-small cell lung cancer (NSCLC) are presented (NCT05274451).

Figure 1: LYL797, a ROR1-targeted CAR T-cell product



LYL797 is a ROR1-targeted CAR T-cell product overexpressing c-Jun to resist exhaustion following antigen encounter and manufactured using Epi-R protocol to maintain features of T-cell stemness.

Figure 2: Non-clinical LYL797 products demonstrate enhanced stemness, in vitro and in vivo activity, and reduced exhaustion compared to Control ROR1 CAR T cells[5]



(A) Proportion of CCR7+/CD45RA+ cells in CD8+CAR+ T cells in Control ROR1 CAR T-cell products and LYL797 non-clinical CAR T cells from 3 healthy donors by flow cytometry. After 3 rounds of antigen restimulation with A549 NSCLC tumor cells at a 1:1 E:T ratio, LYL797 maintains higher cytokine secretion (B) and cytotoxicity (C) compared to Control ROR1 CAR T cells. LYL797 demonstrates enhanced anti-tumor activity (D) and in vivo expansion (PK) (E) at the 2.5 x 10^6 CAR T-cell dose in a H1975 xenograft NSG MHCI/II dKO mouse model. Compared to Control ROR1 CAR T-cells, LYL797 CAR T cells are less exhausted (F) and more stem-like (G) after persistent antigen stimulation in vitro. Control ROR1 CAR T-cells were manufactured using a standard process without Epi-R and do not overexpress c-Jun. Data adapted from [5].

Methods

- Patients with ROR1+ TNBC and NSCLC were consented, enrolled, underwent apheresis, and received autologous LYL797 cells after lymphodepletion with fludarabine and cyclophosphamide. LYL797 cell doses evaluated include 50 x 10^6, 75 x 10^6, 100 x 10^6, 150 x 10^6 and 300 x 10^6 CAR+ cells.
- Phenotype of LYL797 drug products was assessed using flow cytometry and single-cell RNA-seq (scRNA-seq).
- Peripheral blood (PB) (Days 11 and 22) and tumor biopsies (range Day 21-30) were collected post LYL797 infusion, and CAR T-cell pharmacokinetics (PK) by digital droplet PCR (ddPCR), phenotype (flow cytometry and scRNA-seq), and tumor infiltration (multiplexed in situ hybridization [mISH]) were assessed.

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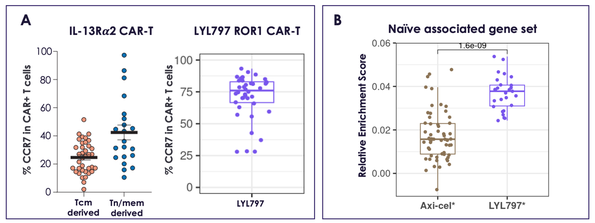
Results

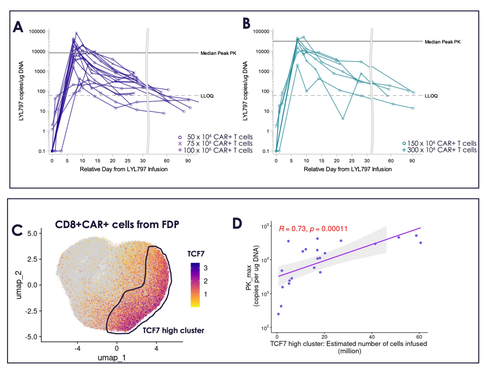
Figure 3: Epi-R manufacturing generates LYL797 clinical CAR T-cell products with an enhanced stem-like profile compared to other clinical CAR T-cell products



(A) Frequency of CCR7+ cells in either Tcm-derived or Tn/mem-derived IL-13Rα2 CAR-T products as reported by Brown et al [9] and in LYL797 ROR1 CAR T cells by flow cytometry (n=43). (B) Relative naïve-associated enrichment score in CD8+CAR+ T cells in either Axi-cel (n=59) or LYL797 (n=27) drug products. The average enrichment score of naïve-associated gene set [10] across all CD8+CAR+ T cells per product was calculated using scRNA-seq data. Data for "YESCARTA" products are described in Li et al [11]. Enrichment score was computed by AddModuleScore function in Seurat [15]. P-value is from Wilcoxon rank-sum test. LYL797 product analysis utilized data available as of Sep 23, 2024.

- The frequency of Naïve/Central Memory T cells in CAR T-cell products have been shown to be associated with improved efficacy[7-8].
- LYL797 CAR+ T-cells have a higher frequency of CCR7+ cells compared to IL-13Rα2 CAR T-cells[9].
- LYL797 CAR+ T-cells have a higher enrichment of a naïve-associated gene set[10] compared to Axi-cel CD19 CAR T cell products[11].

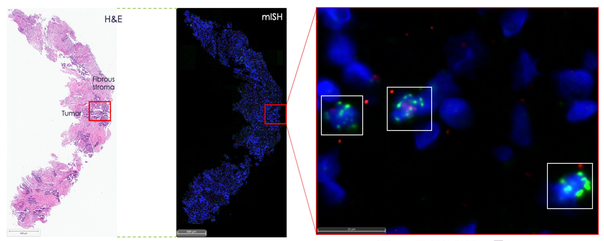
Figure 4: LYL797 peak expansion in peripheral blood (PK) is dose-dependent and is correlated with the number of stem-like CD8+ T cells infused



Time plots of individual LYL797 PK concentration by ddPCR for (A) Dose levels 50 –100 x 10^6 CAR+ T cells (median C_{max} = 8313.25 copies/µg DNA, n=18) and (B) Dose levels 150 x 10^6 and 300 x 10^6 CAR+ T cells (median C_{max} = 31794.6 copies/µg DNA, n=7). Peak cell expansion occurs between Days 7-14 post infusion (median T_{max} = 8 days, n=25). (C) Naïve/stem-like cluster (black outline) with high expression of TCF7 was identified in CD8+CAR+ LYL797 products (FDP) from scRNA-seq data analysis. The TCF7-high subset ranged from (7.8 - 63.8%, median = 31.6%) across all LYL797 products. (D) Correlation between the estimated number of infused CD8+CAR+ TCF7-high cells and PK at peak expansion (n=23). Correlation coefficient (R) and p-value (p) were calculated using the Spearman correlation test. LYL797 PK analysis utilized data available as of Sep 27, 2024.

- LYL797 CAR T-cell expansion is observed across all doses tested, ranging from 50 x 10^6 to 300 x 10^6 CAR+ T cells.
- Participants infused at higher dose levels (150 x 10^6 and 300 x 10^6 cells) have higher median peak expansion in peripheral blood than those at lower dose levels (50 – 100 x 10^6 cells).
- A cluster of TCF7-high stem-like T cells was identified in CD8+CAR+ LYL797 clinical products. The number of TCF7-high stem-like CD8+CAR+ T cells infused is significantly correlated to PK at peak expansion.

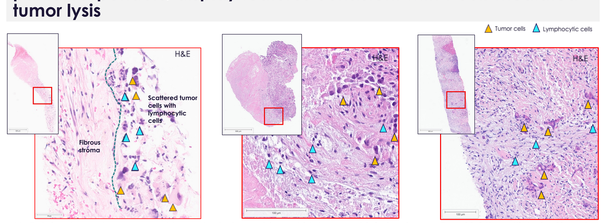
Figure 5: Tumor infiltration of LYL797 CAR+ T cells is observed in all patients evaluated using multiplex *in situ* hybridization (mISH) analysis



Representative mISH analysis showing LYL797 CAR T cell infiltration in a Day 29 post-infusion tumor biopsy (one representative of n=9 biopsies available as of May 29, 2024). H&E (left) and mISH (middle, right) were performed on serial sections. T cells were detected using RNAscope probes specific for human CD3e (TCR; green dots), and ROR1 CAR T were detected using RNAscope probes specific for the R12 CAR sequence in the LYL797 lentiviral vector (red dots). Biopsies were considered to have LYL797 CAR T cell infiltration when definitive TCR+CAR+ double-positive cells could be identified above background. H&E: hematoxylin and eosin; mISH: multiplex fluorescent *in situ hybridization*.

- ISH analysis shows definitive tumor infiltration of LYL797 CAR T-cells in all evaluable biopsy samples (n = 9).

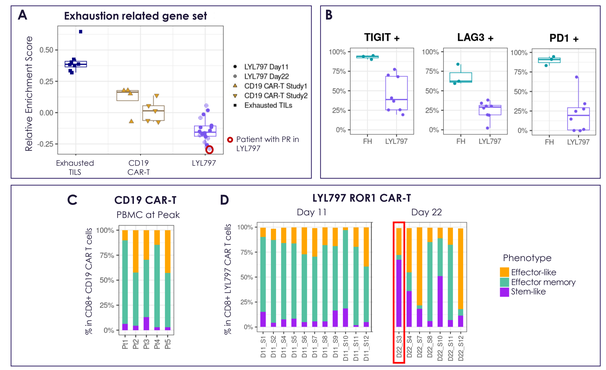
Figure 6: Multiple patients' tumor biopsies, including confirmed partial responders, display features consistent with T cell-mediated tumor lysis



H&E-stained samples were used to evaluate tumor biopsy histology post-infusion (range Day 23-26). Multiple (n=3) post-infusion tumor biopsies (of n=9 biopsies analyzed as of May 29, 2024) display histological features consistent with T cell-mediated tumor lysis, characterized by areas of T cell-rich lymphocytic inflammation (teal arrowheads) and scattered tumor cells (gold arrowheads).

- Histological features of T cell-mediated tumor lysis are consistent with observations in preclinical xenograft models

Figure 7: LYL797 CAR T cells demonstrate lower exhaustion and maintenance of stem- and memory-like phenotypes post-infusion compared to other CAR T-cell trials



(A) Relative enrichment score of exhaustion gene set[6], calculated as average enrichment score across cells per sample using scRNA-seq, in CD8+CAR+ cells from LYL797 Day11/22 peripheral blood samples (n=18), patient peripheral blood samples at peak expansion in two CD19 CAR T cell studies[12,13], and CD8+ exhausted TILs from TNBC patients[14]. Exhausted TILs refer to the t_CD8_CXCL13 cluster. Only patients with at least 300 cells in the t_CD8_CXCL13 cluster were included. (B) Frequency of TIGIT+, LAG3+, and PD1+ expression in CD8+CAR+ cells in LYL797 (n=8, purple) and a previously reported ROR1 CAR T cell trial[1] (FH, n=3, teal) in peripheral blood at peak expansion by flow cytometry. (C-D) Phenotyping of CD8+CAR+ T cells in peripheral blood from CD19 CAR T cell treated-patients[13] at peak expansion (C) or LYL797 Day11/Day22 post-infusion (D). Red circle in (A) and red box in (D) highlight a LYL797 patient with cPR. LYL797 post-infusion phenotype analysis utilized data available as of Sep 27, 2024.

- LYL797 transcriptional profile of known exhaustion genes is closer to CD19 CAR T cells than fully exhausted TILs[12-14]
- LYL797 demonstrates a lower frequency of TIGIT, LAG3, and PD-1 expression in CD8+CAR+ T cells at peak expansion compared to that in a previously reported ROR1-targeted CAR T cell clinical trial[1].
- LYL797 cells also retain a substantial proportion of stem-like and effector-memory-like subsets (median 82%) and a low proportion of terminal effector subsets (median 18%) post infusion, which are comparable to CD19 CAR T cells at peak expansion.
- LYL797 cells from Day 22 peripheral blood samples from a confirmed partial responder have the lowest exhaustion-related gene set score and highest proportion of stem-like cells compared to other patients.

Conclusions

- Translational data from an ongoing Phase 1 clinical trial of LYL797, a ROR1-targeted CAR T-cell product candidate, suggest T-cell reprogramming technologies can reduce T-cell exhaustion and enhance stemness of CAR T-cells resulting in CAR T-cell tumor infiltration with histologic evidence of tumor lysis.
- LYL797 Epi-R manufacturing protocol yields products with enhanced stem-like properties.
- Peak CAR T-cell expansion is dose-dependent and correlates with the number of infused TCF7-high stem-like CD8+CAR+ T cells.
- LYL797 CAR T-cells infiltrate solid tumors with evidence of T-cell mediated tumor lysis in patients with ROR1+ TNBC and NSCLC .
- LYL797 CAR T-cells demonstrate lower exhaustion and maintenance of stem- and memory-like phenotypes in peripheral blood post-infusion, suggesting c-Jun overexpression can delay CAR T-cell exhaustion in patients.
- These data mirror the non-clinical data[4-5], validating the preclinical models and demonstrating the value of c-Jun overexpression and Epi-R manufacturing technologies in LYL797 ROR1 CAR T.

References: 1. Specht J, et al. 2020; Oral presentation at EHA-EBMT 2nd European CAR T Cell Meeting. **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. AACR 2022 Poster. Abstract 2754. **5.** Park S, et al. ASGCT 2022 Poster. Abstract 661. **6.** Zhang Y, et al. Cancer Cell. 2021;39(12):1578-1593. **7.** Filosto S, et al, Blood Cancer Discov. 2024 Jan 8;5(1):21-33. **8.** Haradhvala, N.J., et al. Nat Med 28, 1848–1859 (2022). **9.** Brown, C.E., et al. Nat Med 30, 1001–1012 (2024). **10.** Gattinoni L, et al. Nat Med 2011 Sep 18;17(10):1290-7. **11.** Li X., et al. Cancer Cell, 2023 Nov 13;41(11):1835-1837. **12.** Shieh A, et al. Nat Commun. 2020;11, 219. **13.** Guerrero-Murillo M, et al. bioRxiv 2024.01.23.576878. **14.** Zhang Y, et al. Cancer Cell. 2021 Dec 13;39(12):1578-1593.e8. **15.** Hao Y, et al. Nature Biotechnology (2023).

Abbreviations: CAR, chimeric antigen receptor; TNBC, triple negative breast cancer; NSCLC, non-small cell lung cancer; scRNA-seq, single cell RNA sequencing; PB, peripheral blood; PK, pharmacokinetics; ddPCR, droplet digital polymerase chain reaction; ISH, multiplexed in situ hybridization; CM, central memory; UMAP, uniform manifold approximation and projection; cPR, confirmed partial response.

Ethics Approval: The study was approved by the Institutional Review Boards of each participating center.