Abstract 278

**PRECLINICAL DEVELOPMENT OF LYL119, A ROR1-TARGETED CAR T-CELL PRODUCT INCORPORATING FOUR NOVEL T-CELL REPROGRAMMING TECHNOLOGIES TO OVERCOME BARRIERS TO EFFECTIVE CELL THERAPY FOR SOLID TUMORS**

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**Background** Effective solid tumor cell therapy requires new strategies to improve T-cell activation, persistence, and durable function. We developed four complementary, stackable T-cell reprogramming technologies to enhance chimeric antigen receptor (CAR) T-cell therapy in solid tumors: 1) overexpression of the activator protein 1 (AP-1) family transcription factor c-Jun to delay T-cell exhaustion and improve functional activity; 2) nuclear receptor subfamily 4A member 3 (NR4A3) gene knock-out (KO) to further delay exhaustion and enhance functionality; 3) Epi-R™ manufacturing protocol to promote stem-like characteristics; and 4) Stim-R™ technology a synthetic biomimetic to improve T-cell potency compared with conventional activating reagents. LYL119 is a ROR1-targeted CAR T-cell product candidate that combines these technologies to create potent CAR T cells with durable function.

**Methods** Healthy donor T cells were manufactured at research or clinical scale with the Epi-R protocol, activated with Stim-R technology or a standard reagent, and transduced with a tri-cistronic lentiviral vector encoding ROR1 CAR, c-Jun, and truncated EGFR tag. The NR4A3 gene or a safe-harbor control gene was edited. CAR T-cell cytotoxicity, cytokine production upon repeated antigen stimulation assays designed to promote exhaustion. Finally, CAR T cells were evaluated in vivo using a ROR1-expressing H1975 human lung cancer xenograft model in mice.

**Results** LYL119 products were successfully manufactured at research and clinical scale with comparable product phenotype. We achieved ~90% genomic editing efficiency at the NR4A3 target gene resulting in 13-fold reduction in protein expression compared to a non-edited control. CAR T cells manufactured with Stim-R technology displayed an activated phenotype, as indicated by elevated CD25 expression, as well as high TCF-7 and CD127 expression suggesting maintenance of stem-like populations.

LYL119 demonstrated superior cytotoxicity and sustained cytokine production upon repeated antigen stimulation compared to various controls lacking one or more of the reprogramming technologies (figure 1). LYL119 also had reduced surface expression of inhibitory receptors and maintained higher expression of CD127 compared to non-edited CAR T cells manufactured using standard reagents, suggesting reduced T-cell exhaustion and improved maintenance of stem-like characteristics. Moreover, LYL119 showed robust antitumor efficacy in vivo across a 10-fold dose range, including a very low dose of 0.1x10⁶ CAR T cells (figure 2).

**Conclusions** These data suggest that LYL119, which combines c-Jun overexpression, NR4A3 KO, Epi-R protocol, and Stim-R technology, can limit exhaustion, maintain stem-like features, and has potential to provide effective and durable CAR T-cell antitumor activity in patients with ROR1+ solid tumors.

**REFERENCES**


![Abstract 278 Figure 1](http://jitc.bmj.com/content/2023/11/Suppl_1/A11731)
Abstract 278 Figure 2  Antitumor efficacy at three CAR T-cell doses in one representative experiment tested in an in vivo H1975 xenograft model (n=2 donors with 10 mice/group). LYL119 impaired tumor growth at a very low CAR T-cell dose (0.1x10^6). (* p < 0.05, ** p < 0.005, ****p < 0.0001, Tukey one-way ANOVA).

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