Epigenetic reprogramming (Epi-R™) yields an NY-ESO-1 T-cell receptor product (LYL132; GSK4427296) with improved stemness, metabolic fitness, and functional activity in the presence of persistent antigen exposure

Abstract No. 1115

Veena Krishnamoorthy, Jia Lu, Purnima Sundar, Chad Chapman, Brenda Jesernig, Yogin Patel, Stefan Siebert, Chang-Chih Wu, Martin Wohlfahrt, Byoung Ryu, Melissa DeFrancesco, Queenie Vong, Rachel Lynn, Shobha Potluri, Suman Kumar Vodnala

Lyell Immunopharma, Inc, South San Francisco, CA, and Seattle, WA

Objective

To evaluate the preclinical effects of Epi-R on the proportions and characteristics of less-differentiated T-cell populations in adoptive cell therapy (ACT) manufactured at research- and large-scale.

Background

- Traditional T-cell expansion results in progressive differentiation of T cells to an effector phenotype (T_{EFF}), characterized by a brief gain of effector functions, followed by apoptosis and loss of long-term anti-tumor potential (**Fig. 1**)
- Stemness of a T-cell product (ie, the proportion of stem memory T cells [T_{SCM}]) correlates with antitumor efficacy and response durability in solid tumor models.[1] Therefore, enriching an ACT product for T cells with durable stemness may be a key to improving clinical efficacy in patients with solid tumors
- Manipulation of the cellular metabolic state is a key strategy to enhance stem-like qualities and improve clinical efficacy, as the metabolic state of a T-cell influences its anti-tumor potential[2-4]
  - Aerobic glycolysis with rapid nutrient consumption promotes differentiation to an effector phenotype, leading to a sub-optimal therapeutic effect
  - Caloric restriction with enhanced mitochondrial spare respiratory capacity results in long-term in vivo T-cell persistence and solid tumor eradication
- Epigenetic reprogramming (Epi-R™) to alter the phenotypic T-cell profile of an ACT is a promising approach to improve clinical efficacy in solid tumors
- Letetresgene autoleucel (GSK3377794) is GSK's first-generation NY-ESO-1–specific T-cell receptor (TCR) T-cell therapy with demonstrated clinical activity in synovial sarcoma (SS); it targets NY-ESO-1/LAGE-1A+ tumors using a genetically modified, high-affinity TCR
  - Although it is one of the only TCR T-cell therapies to show activity in patients with solid tumors to date, it is imperative to continue to improve both response rates and durability for patients receiving ACT[5-9]
- LYL132 (GSK4427296) is a second-generation NY-ESO-1–specific TCR T-cell therapy based on letetresgene autoleucel that also incorporates Lyell's Epi-R technology, an optimized expansion process and epigenetic reprogramming media designed to improve metabolic fitness and preserve T cells with stem-like qualities in the manufactured product (**Table 1**)

Figure 1: Upon antigen encounter or TCR stimulation, co-stimulatory signals drive progressive differentiation of T cells, potentially resulting in apoptosis depending on the strength and quality of the signals

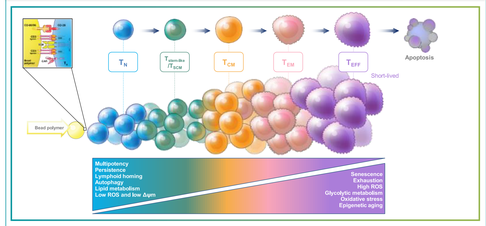


Table 1: Expression of surface markers that define functional properties of naive (T_N), stem-like ($T_{stem-like}$), central memory (T_{CM}), and effector memory (T_{EM}) T cells[10-13]

Phenotype	T_N	$T_{stem-like}$	T_{CM}	T_{EM}
CD27	++++	+++	++	+
CD62L	++++	+++	++	+
TCF7	++++	+++	++	+
CCR7	++++	+++	++	-
CD28	++	+++	+++	+
CD45RA	++	++	-	+/-
CD45RO	-	-	+	+
CD39	-	-	++	+++

(-) denotes no expression, (+) low expression, (++) intermediate expression, (+++) high expression, and (++++) very high expression.

Results

Differentiation and metabolic profile of LYL132

We evaluated LYL132 made from 5 healthy donors and 4 patients with SS at research-scale (**Fig. 2**) and 2 healthy donors at large-scale (**Fig. 3**). We assessed the differentiation profiles of T cells ($T_{stem-like}$ vs more differentiated subsets) in LYL132 compared to first-generation products and/or controls (**Figs. 2, 3**). Metabolic fitness of the products was assessed by examining genetic signatures associated with oxidative phosphorylation, fatty acid oxidation, hypoxia, and glycolysis, and maturation of protein complexes associated with the autophagy pathway (LC3b I and LC3b II) was examined by immunoblot (**Fig. 3**).

- **Results of flow cytometry and RNA sequencing (RNA-Seq) showed an increased proportion of less-differentiated CD8+ and CD4+ T cells in LYL132 compared to first-generation products and/or controls expanded from most donors at large- and research-scale**
- **Genetic signatures of oxidative phosphorylation were upregulated in LYL132 TCR T-cell products compared to first-generation TCR T-cell products at both large- and research-scale**
- **RNA-Seq analysis demonstrated that genetic signatures of oxidative phosphorylation are upregulated in LYL132 compared to the first-generation product at both large- and research-scale**
- **Proportions of autophagy flux proteins were higher in LYL132 relative to the first-generation product**

Figure 2: Epi-R enhances the frequency of $T_{stem-like}$ cells in LYL132 manufactured at research-scale from samples donated by healthy individuals and patients with SS

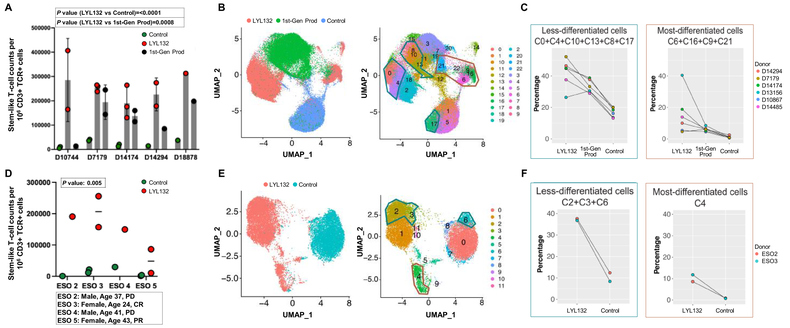
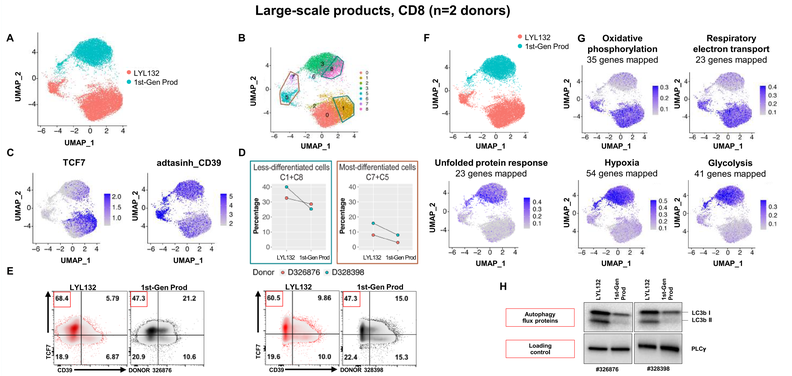


Figure 3: Epi-R enhances the frequency of $T_{stem-like}$ cells and improves metabolic fitness of LYL132 manufactured at large-scale



Functional analysis of LYL132

To assess proliferative capacity and cytotoxic function after persistent antigen exposure, we serially restimulated LYL132 and the first-generation product every 3-4 days with NY-ESO-1 antigen-expressing cells and evaluated T-cell expansion and proliferative capacity, cytotoxicity potential, and cytokine secretion (**Fig. 4**). T-cell phenotypes were evaluated after serial restimulation using transcriptional profiling by single-cell CITE-Seq (which captures both mRNA and protein) (**Fig. 5**).

- **LYL132 displayed a proliferative capacity similar to or higher than that of the first-generation products derived from both donors**
- **LYL132 showed persistent cytotoxicity and production of cytokines (interferon gamma [IFNγ] and interleukin-2 [IL-2]) in response to antigen-expressing cells**
- **After persistent antigen exposure, the proportion of CD8+ $T_{stem-like}$ cells in LYL132 was similar to or higher than that of the first-generation product**

Figure 4: LYL132 displays persistent anti-tumor efficacy, similar-to-improved proliferative capacity, and sustained cytokine production after serial antigen encounter compared to the first-generation product

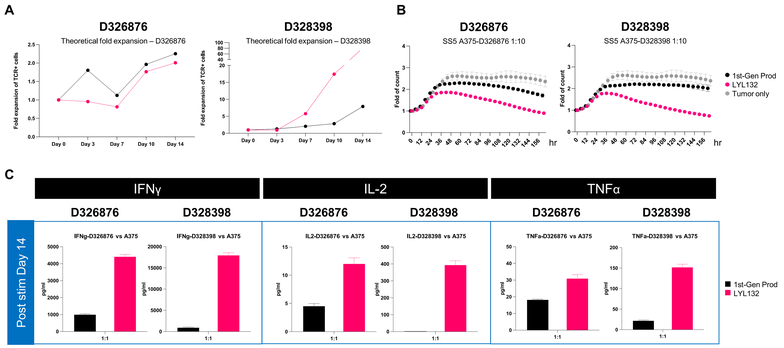
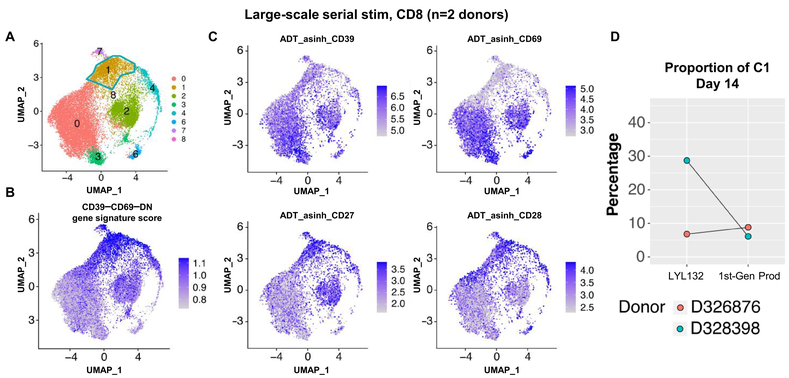


Figure 5: LYL132 maintains higher proportions of $T_{stem-like}$ cell populations after serial antigen encounter compared to the first-generation product



Conclusions

Using integrated phenotypic and transcriptomic analyses, we have demonstrated that epigenetic reprogramming of NY-ESO-1 TCR T-cell products with Epi-R (LYL132) improves stemness, metabolic fitness, and functional persistence relative to NY-ESO-1 TCR T-cell products developed without Epi-R.

- **STEMNESS:** The durable stemness seen with LYL132 provides evidence that our Epi-R technology can be used to generate autologous T-cell products with attributes known to correlate with anti-tumor efficacy and durable response
- **METABOLIC FITNESS:** Epi-R confers metabolic fitness, potentially by reducing glycolysis and driving metabolic reprogramming that may be required to preserve the stemness phenotype
- **FUNCTIONAL PERSISTENCE:** LYL132 demonstrated potent functional activity in vitro, with similar-to-higher functional capacity and greater persistence of $T_{stem-like}$ cell populations after serial antigen encounter relative to the first-generation NY-ESO-1 TCR T-cell product. LYL132 also maintained improved cytotoxicity and cytokine production throughout 4 rounds of antigen stimulation

Future Studies

LYL132 is being evaluated for safety and tolerability in a Phase 1, first-in-human, single-arm, dose-confirmation and -expansion study in synovial sarcoma/myxoid/round cell liposarcoma, Substudy 3 of GSK FTIH Master Protocol (NCT04526509).

Acknowledgments

We thank the GSK cell process development team for providing vector materials and Dr. Anusha Kalbasi from the University of California, Los Angeles for leukapheresis samples from patients with synovial sarcoma. Medical writing and editorial support were funded by Lyell Immunopharma, Inc, and provided by Melanie Styers, PhD, and Anuradha Kumari, PhD, of Verascity Science.

Abbreviations

ΔΨm, mitochondrial membrane potential; 1st-Gen Prod, first-generation product; ACT, adoptive cell therapy; C, cluster; CAR, chimeric antigen receptor; CCR7, CC-motif chemokine receptor 7; CD, cluster of differentiation; CD45RO, CD45 180-kDa isoform; CD45RA, CD45 200–220 kDa isoform; CR, complete response; IFNγ/IFNg, interferon gamma; IL, interleukin; LAGE-1A, L antigen family member 1a; LC3b I/II, light chain complex I/II; NY-ESO-1, New York esophageal squamous cell carcinoma 1 tumor antigen; PD, progressive disease; PLC, phospholipase C gamma; PR, partial response; RNA-Seq, RNA sequencing; ROS, reactive oxygen species; SS, synovial sarcoma; stim, stimulation; TCF7, transcription factor 7; T_{CM}, central memory T cell; TCR, T-cell receptor; T_{EFF}, effector T cell; T_{EM}, effector memory T cell; T_N, naive T cell; TNFα/TNFa, tumor necrosis factor alpha; T_{SCM}, stem memory T cell; $T_{stem-like}$, stem-like T cell.

Supplementary Materials

Please scan the Quick Response (QR) code for more detailed information about the methods and a full list of references. Copies of content obtained through the QR code are for personal use only and may not be reproduced without permission from the author of this poster: Veena Krishnamoorthy (vkrishnamoorthy@lyell.com)

Presented at ASGCT Annual Meeting 2022; May 16–18; Washington, DC, USA