Increased potency and functional persistence *in vitro* of a next-generation NY-ESO-1–specific TCR therapy incorporating Gen-R™ genetic reprogramming technology

Abstract No. 232

Helle Jensen,[1] Rachel Fukuda,[1] Megan Murt,[1] Xiao Wang,[1] Lora Zhao,[1] Sheila Lou,[1] Purnima Sundar,[1] Christopher Navas,[1] Andrew Jimena,[1] Amanda Sims,[1] Travis Beckett,[1] Shobha Potluri,[1] Rowena Martinez,[1] David Chian,[1] Candace Sims,[1] Quinn Walker,[1] Abira Bandyopadhyay,[1] Breanna Pamintuan,[1] Bijan Boldajipour,[1] Chang-Chih Wu,[1] Martin Wohlfahrt,[1] Byoung Ryu,[1] Viola C. Lam,[1] Rachel C. Lynn,[1] Hajime Hiraragi,[1] Johannes Breuning,[2] Ashley Hamilton,[2] Fotini Kouri,[2] Jack Euesden,[2] Sara Brett,[2] and Blythe Sather[1]

[1]Lyell Immunopharma, 201 Haskins Way, South San Francisco, CA 94080. [2]GlaxoSmithKline, 980 Great West Road, Brentford, Middlesex, TW8 9GS, United Kingdom.

Background

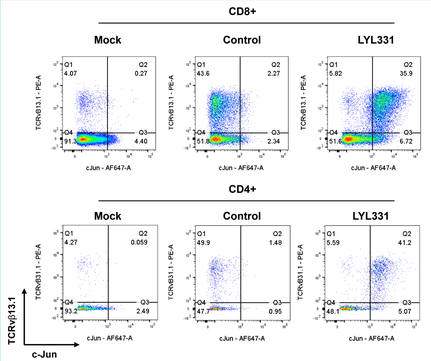
- New-York esophageal squamous cell carcinoma 1 (NY-ESO-1) is an immunogenic cancer testis antigen present in several types of tumors, including:
  - Synovial cell sarcoma (~70% to 80% of cases)[1-3]
  - Melanoma and common epithelial tumors (~25% of cases)[2,3]
  - Multiple myeloma (~30% of cases)[4]
- Letetresegene autoleucel (lete-cel; GSK3377794) is a T-cell therapy with demonstrated clinical activity in solid tumors[1,4]
  - Lete-cel comprises autologous, CD4+ and CD8+ lentiviral vector (LVV) transduced T cells engineered to express a high-affinity NY-ESO-1–specific T-cell receptor (TCR)[1,4]
  - Although it is one of the only TCR therapies to show activity in patients with solid tumors to date,[1,4] next-generation NY-ESO-1–specific TCR therapies are in development with the goal of further improving response rates and durability
- LYL331 (GSK4349560) is a next-generation NY-ESO-1-specific TCR therapy that incorporates Lyell's *ex vivo* genetic reprogramming technology to engineer T cells to overexpress c-Jun, a member of the activator protein (AP-1) family of transcription factors
  - Dysregulation of AP-1 family members has been implicated in chimeric antigen receptor (CAR) T-cell exhaustion[5]
  - Previous studies demonstrate that overexpressing c-Jun can delay functional exhaustion, thereby improving anti-tumor efficacy and persistence of CAR T cells in preclinical solid tumor models[5]
- Here, we show preclinical data for LYL331 evaluating the impact of c-Jun overexpression via Gen-R genetic reprogramming technology on primary and long-term T-cell functions *in vitro*

Results

Gen-R technology generated high, stable expression of c-Jun in LYL331

- Genetic reprogramming of LYL331 with Gen-R resulted in high levels and stable expression of c-Jun in both CD8+ and CD4+ T-cell populations compared to endogenous c-Jun expression in non-transduced (i.e., mock) or control (i.e., transduced with a NY-ESO-1 vector lacking c-Jun) T cells (**Figure 1**)

Figure 1: LYL331 displays high expression levels of c-Jun in combination with NY-ESO-1 TCR



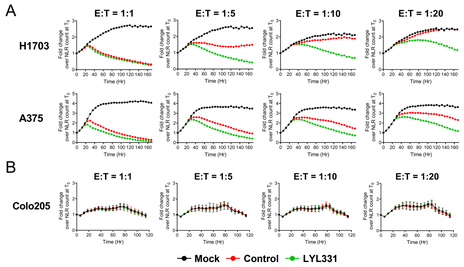
Human donor T cells were either left non-transduced (mock) or transduced with a LVV encoding EF1α_NY-ESO-1 (control) or EF1α_c-JunWT_NY-ESO-1 (LYL331). Surface NY-ESO-1 TCR expression was detected by flow cytometry using an anti-TCRvβ13.1 antibody (Ab), and intracellular c-Jun expression was detected using an anti-c-Jun Ab. TCRvβ13.1 is a naturally occurring TCR variant and was present on approximately 3% to 6% of non-transduced T cells from the donors evaluated in these studies. Flow cytometry plots show TCRvβ13.1 and c-Jun staining in gated CD8+ and CD4+ T cells. The TCRvβ13.1 gate was defined based on comparison to the mock sample, and the c-Jun gate was defined based on comparison to endogenous c-Jun in the control sample. Representative example from one donor is shown.

Results (cont)

Gen-R increased the potency of LYL331 against NY-ESO-1 antigen-positive tumor cell lines *in vitro*

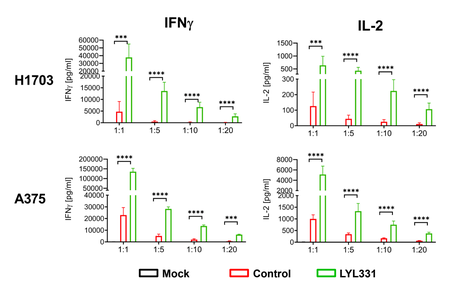
- LYL331 demonstrated more potent killing of the NY-ESO-1 antigen-positive A375 and H1703 tumor cell lines compared to the control (**Figure 2A**)
  - Both the kinetics of killing and the overall efficiency of tumor cell clearance were improved, particularly at lower effector to target (E:T) ratios (**Figure 2A**)
- No killing of the antigen-negative tumor cell line (Colo205) by LYL331 or the control was observed (**Figure 2B**)
- LYL331 secreted significantly higher levels of interferon gamma (IFNγ) and interleukin-2 (IL-2) in response to both the A375 and H1703 tumor cell lines compared to the control (**Figure 3**)

Figure 2: c-Jun overexpression enhances cytotoxic activity of LYL331 against NY-ESO-1 antigen–positive tumor cell lines compared to the control



Functional activity of LYL331 and control T cells was evaluated against NY-ESO-1 antigen-positive tumor cell lines (H1703 [NCI-H1703] and A375) or an NY-ESO-1 antigen-negative tumor cell line (Colo205, negative control) transduced to stably express NucLightRed (NLR) at E:T ratios of 1:1, 1:5, 1:10, or 1:20. (**A**) Cytotoxic capacity was assessed over a 168-hr time course of co-culture with H1703 or A375 NLR tumor cells using an IncuCyte™ S3 Live-Cell Analysis System. Killing curves are shown for the mock (black), control (EF1α_NY-ESO-1; red), and LYL331 (EF1α_c-JunWT_NY-ESO-1; green) samples from one representative donor. Data represent the mean value from duplicate measurements for each sample. (**B**) Growth kinetics of Colo205 NLR tumor cells co-cultured with mock, control, or LYL331 samples was assessed over a 114-hr time course using IncuCyte. Error bars represent mean ± standard deviation (SD) of samples from 3 donors.

Figure 3: c-Jun overexpression increases the secretion of IFNγ and IL-2 by LYL331 in response to NY-ESO-1 antigen–positive tumor cell lines compared to the control

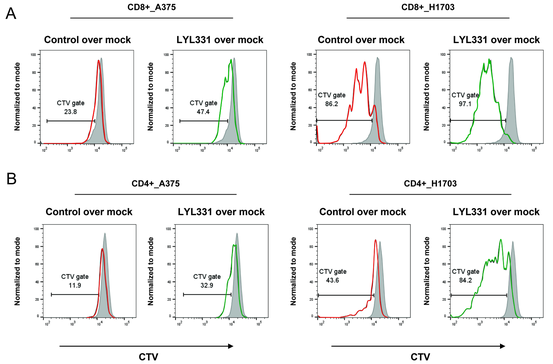


IFNγ and IL-2 secretion were evaluated by Meso Scale Discovery (MSD) analysis using supernatants collected from the IncuCyte assay (described in Figure 2A) after 24 hr of co-culture with H1703 or A375 NLR tumor cells at E:T ratios of 1:1, 1:5, 1:10, or 1:20. Bars for mock (black), control (EF1α_NY-ESO-1; red), and LYL331 (EF1α_c-JunWT_NY-ESO-1; green) T cells represent mean ± SD of samples from 3 donors. IFNγ graphs: ***P=0.0002 and ****P<0.0001; IL-2 graphs: ***P=0.0007 and ****P<0.0001 by ratio paired *t*-test analysis comparing the control and LYL331 using GraphPad Prism.

Gen-R enhanced the proliferative capacity of LYL331 CD8+ and CD4+ T cells

- Enhanced proliferative capacity of both LYL331 CD8+ (**Figure 4A**) and CD4+ T cells (**Figure 4B**) was observed in response to A375 and H1703 tumor cell lines when compared to the control
- These results demonstrate that genetic reprogramming with Gen-R can enhance the function of CD4+ T cells transduced with an HLA class I–restricted NY-ESO-1 TCR

Figure 4: c-Jun overexpression enhances the proliferative capacity of LYL331 in response to NY-ESO-1 antigen–positive tumor cells compared to the control

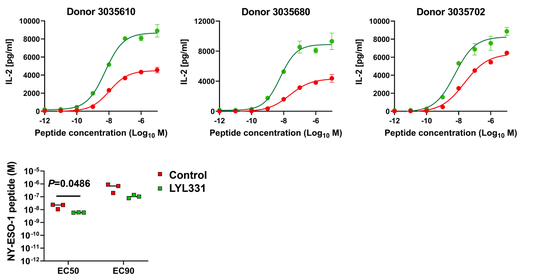


Proliferation of LYL331 and control T cells was assessed after 5 days of co-culture with the NY-ESO-1 antigen-positive tumor cell lines A375 or H1703 by CellTrace Violet (CTV) dilution and flow cytometry analysis. The proliferation of gated (**A**) TCRvβ13.1+ CD8+ or (**B**) TCRvβ13.1+ CD4+ T cells from one representative donor is illustrated by overlaying mock (gray) with the control (EF1α_NY-ESO-1; red) or LYL331 (EF1α_c-JunWT_NY-ESO-1; green) samples.

Gen-R increased the sensitivity of LYL331 towards the NY-ESO-1 peptide

- LYL331 displayed lower EC50 and EC90 values in response to NY-ESO-1 peptide–pulsed T2 cells compared to the control (**Figure 5**)

Figure 5: c-Jun overexpression increases the sensitivity of LYL331 towards the NY-ESO-1 peptide compared to the control

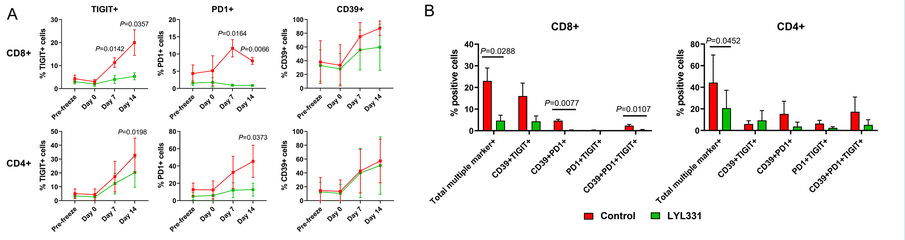


A T2 dose-response assay was used to assess the NY-ESO-1 antigen sensitivity of LYL331 and control T cells. T cells were co-cultured with T2 cells pulsed with 10^{-5} to 10^{-12} M NY-ESO-1$_{157-165}$ SLLMWITQC peptide at an E:T ratio of 1:5. IL-2 levels were measured in the supernatants after 24 hr of co-culture. (**A**) Curve fitting with non-linear regression was performed for the control (EF1α_NY-ESO-1 TCR; red) and LYL331 (EF1α_c-JunWT_NY-ESO-1; green) samples using GraphPad Prism. Data shown represent mean ± SD from 2 replicate samples per donor. (**B**) Mean EC50 and EC90 values were calculated from the donors shown in (A). *P* values ≤0.05 derived from ratio paired *t*-test analysis comparing the control and LYL331 are shown.

c-Jun overexpression via Gen-R can delay the onset of exhaustion and improve long-term function of LYL331

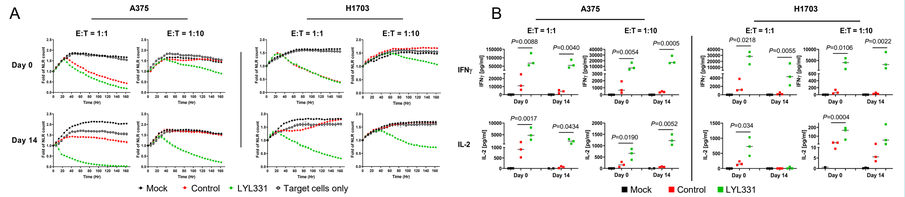
- LYL331 displayed a significant reduction in the expression of exhaustion markers (i.e., TIGIT, PD-1, and CD39) compared to the control after persistent antigen exposure (**Figure 6**)
- LYL331 maintained the ability to clear tumor cells and produce cytokines compared to the control after persistent antigen exposure (**Figure 7**)

Figure 6: LYL331 displays reduced expression of exhaustion markers compared to the control after persistent antigen exposure



An *in vitro* serial re-stimulation assay was used to assess T–cell exhaustion and long-term function of LYL331 (EF1α_c-JunWT_NY-ESO-1; green) and control (EF1α_NY-ESO-1; red) T cells. T cells were stimulated every 3 or 4 days with the NY-ESO-1 antigen–positive A375 tumor cell line for a total of 4 rounds of stimulation. Expression of exhaustion markers CD39, PD-1, and TIGIT was determined by flow cytometry. (**A**) %TIGIT, %PD1, and %CD39 expression is shown from gated TCRvβ13.1+ CD8+ and CD4+ T-cell populations at assay start, after 2 rounds of stimulation with antigen, and after 4 rounds of stimulation with antigen (i.e., on Days 0, 7, and 14, respectively). Pre-freeze data was obtained after completed production prior to cryopreservation. Data shown represent mean ± SD from 3 donors. (**B**) A multiple marker analysis using Boolean gating in FlowJo was performed based on the %TIGIT+, %PD1+, and %CD39+ data on Day 14 of the serial re-stimulation assay shown in (A). Bars represent mean ± SD from 3 donors. *P* values ≤0.05 derived from paired *t*-test analyses comparing the control and LYL331 are shown.

Figure 7: LYL331 demonstrates persistent anti-tumor efficacy and sustained cytokine production compared to the control



Mock (filled black), control (EF1α_NY-ESO-1 TCR; red), and LYL331 (EF1α_c-JunWT_NY-ESO-1 TCR; green) T cells were serially re-stimulated with the NY-ESO-1 antigen-positive A375 tumor cell line as described in Figure 6. The functional activity of LYL331 and the control was assessed against the H1703 and A375 NLR tumor cell lines at an E:T ratio of 1:1 and 1:10 at assay start (Day 0) and after 4 rounds of stimulation with antigen (Day 14). (**A**) The cytotoxic capacity was assessed over a 162-hr time course using IncuCyte. Growth curves for a sample containing only the tumor cells are shown for comparison (open black). Data represent mean ± SD from duplicate measurements for each sample from one representative donor. (**B**) IFNγ and IL-2 cytokine secretion levels were evaluated by MSD analysis using supernatants collected from the IncuCyte assay (described in Figure 7A) after 24 hr of co-culture with H1703 or A375 NLR tumor cells. Data represent mean cytokine levels for samples from 3 donors. *P* values ≤0.05 derived from ratio paired *t*-tests analyses comparing the control and LYL331 are shown.

References

1. D'Angelo SP et al. *Cancer Discov*. 2018;8(8):944–57.
2. Robbins PF et al. *J Clin Oncol*. 2011;29:917–24.
3. Robbins PF et al. *Clin Cancer Res*. 2015;21(5):1019–27.
4. Stadtmauer EA et al. *Blood Adv*. 2019;3(13):2022–34.
5. Lynn RC et al. *Nature*. 2019;576:293–300.

Acknowledgements

We thank the GSK cell process development team for providing vector materials. We thank Lori Pender for support in preparing the poster presentation. Medical writing and editorial support were funded by Lyell Immunopharma, Inc and provided by Melanie Styers, PhD, of Verascity Science.

Ethics statement

Experiments presented in this abstract relied on human donor material that was obtained from commercial vendors. These vendors use their own IRB-approved protocol and consent process.

Conclusions

- **These preclinical studies demonstrate that c-Jun overexpression via Gen-R genetic reprogramming technology can provide benefit to NY-ESO-1–specific TCR therapy during primary stimulation *in vitro***
- **Overexpression of c-Jun with Gen-R resulted in superior cytotoxic activity, as well as increased cytokine secretion (IFNγ and IL-2), proliferative capacity, and sensitivity towards the NY-ESO-1 peptide of LYL331 relative to the control**
- **c-Jun overexpression with Gen-R also delayed the onset of exhaustion and improved the long-term function of LYL331 relative to the control *in vitro***
- **Based on these promising preclinical data, LYL331 has the potential to improve clinical responses in patients with solid tumor malignancies**

Presented at SITC Annual Meeting 2022; Nov 8–12; Boston, MA, USA