**Background** Letetregene autoleucel (lete-cel; GSK3377794) is a first-generation (1st gen) NY-ESO-1-specific T-cell receptor (TCR) therapy with demonstrated clinical activity in solid tumors.\(^1\)\(^-\)\(^2\) Next-generation T-cell therapies are in development with the goal of further improving response rates and durability. Gen-R is an *ex vivo* genetic reprogramming technology in which T cells are engineered to overexpress c-Jun, a member of the activator protein 1 (AP-1) family of transcription factors. Dysregulation of AP-1 family members is implicated in chimeric antigen receptor (CAR) T-cell exhaustion, and previous studies demonstrate that overexpressing c-Jun can delay functional exhaustion, thereby improving anti-tumor efficacy and CAR T-cell persistence in pre-clinical solid tumor models.\(^3\) LYL331 (GSK4349560) is a next-generation NY-ESO-1-specific TCR therapy that incorporates Gen-R technology. Here we show pre-clinical data for LYL331 evaluating the impact of c-Jun overexpression on primary and long-term durable T-cell functions in *vitro*.

**Methods** Donor T cells were transduced with a lentiviral vector encoding EF1α NY-ESO-1 (1st gen NY-ESO-1 TCR control) or EF1α c-JunWT NY-ESO-1 (LYL331) and characterized for primary functional activities (cytotoxicity, cytokine secretion, and proliferation) and NY-ESO-1 antigen sensitivity. In addition, we used an *in vitro* serial re-stimulation assay as a model for measuring features of T-cell exhaustion and evaluating long-durable functions.

**Results** LYL331 displayed stable and high expression of c-Jun and showed multiple enhancements to primary T-cell functions in *vitro* compared to the control, including superior cytotoxic activity, as well as increased cytokine secretion (IFNγ and IL-2), proliferative capacity, and sensitivity towards the NY-ESO-1 peptide. Enhanced proliferation was observed in both CD4+ and CD8+ T cell populations, indicating that overexpressing c-Jun can improve T-cell functions in CD4+ T cells transduced with an HLA class I restricted NY-ESO-1 TCR. In addition, while the control displayed characteristics of exhaustion (i.e., loss of cytotoxic activity and cytokine secretion and increased expression of multiple exhaustion markers) in the serial re-stimulation assay, LYL331 maintained the ability to kill and secrete cytokines and displayed reduced expression of exhaustion markers TIGIT, PD-1, and CD39.

**Conclusions** In addition to supporting the hypothesis that genetic reprogramming with Gen-R technology can delay the onset of exhaustion and improve the long-durable functions of LYL331, these data show that c-Jun overexpression can provide immediate benefits to the NY-ESO-1-specific TCR therapy during primary stimulation *in vitro*. Based on these promising pre-clinical data, LYL331 may have the potential to improve clinical responses in patients with solid tumor malignancies.

**REFERENCES**

