Non-viral targeted knock-in of a KRAS G12D specific TCR, CD8α/β, and chimeric cytokine receptor in the TRAC locus outperforms lentiviral-based engineering of T cells

Abstract

T cells engineered with T cell receptors (TCRs) enable targeting of clonally-expressed oncogenic driver mutations and have potential to induce durable responses in patients with solid tumors. Viral vectors, including lentiviral vectors (LVV), have been a standard modality to deliver transgenes for T cell therapies but are severely limited in their manufacturing time, cost, and cargo size. In contrast, non-viral targeted gene knock-in (KI) overcomes these limitations, substantially reducing manufacturing complexity. Here, we compare primary human T cells engineered using LVV or KI processes to express a TCR recognizing a KRAS G12D mutant peptide presented on HLA-A*11:01, the CD8αβ co-receptor, and a chimeric interleukin receptor (ILR). We developed and optimized a non-viral manufacturing process that uses a novel CRISPR-Cas12a system to knock-in the transgene cassette within the TRAC locus and to simultaneously knock out the endogenous TCR. Our non-viral KI platform edits primary T cells with high efficiency, and we show that KI engineered TCR T cells performed equivalently or better in functional assays relative to LVV-engineered cells. Together, these data support the utility of a non-viral gene KI approach and its planned incorporation into clinical development.

Fig 1: Lentiviral delivery is limited by cargo size

Fig 2: Non-viral Knock-in can achieve high transgene integration frequency even with large transgenes

Fig 3: KI TCR T cells showed improved tetramer binding

Fig 4: KI TCR T cells show high functional avidity to the KRAS G12D peptide

Fig 5: KI TCR T cells show robust cytotoxicity in vitro

Fig 6: KI cell show superior anti-tumor activity in a xenograft model in vivo

Fig 7: EF-1α and Promoter-less construct designs for KI

Fig 8: EF-1α promoter drives stronger transgene expression

Fig 9: EF-1α promoter resulted in higher functional avidity

Fig 10: EF-1α promoter drives superior anti-tumor activity in an in vivo model

References

See SITC Abstract #355 for AFNT-212 product overview

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