EDIT-202, a multiplexed AsCas12a-edited iPSC-differentiated iNK, displays a mature phenotype, high KIR expression, and ADCC towards multiple solid tumor lines

Poster 313

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OBJECTIVE

To determine if a correlation could be made between the phenotype of EDIT-202 cells and their potency *in vitro*. In addition, EDIT-202 cells were screened against solid tumor lines from multiple indications with different antibodies.

INTRODUCTION

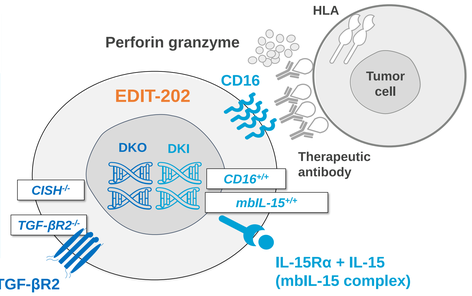
It has been demonstrated that chimeric antigen receptor T-cell (CAR-T) therapy can achieve durable remissions in hematologic malignancies.[1] However, CAR-T therapies have limited efficacy in solid tumors and are often associated with severe toxicity,[2] highlighting the need for safer, more efficacious cell therapies. Natural killer (NK) cells with high-affinity cluster of differentiation 16 (CD16) represent an attractive alternative therapy option to CAR-Ts because of their natural cytotoxic ability and low toxicity. Therefore, we have generated an off-the-self, allogeneic, feeder-free, induced pluripotent stem cell (iPSC)–derived iNK cell (EDIT-202) for targeting solid tumors.

Engineered AsCas12a and SLEEK™ enable the generation of iNK cells with enhanced functions against solid tumors

***CISH*$^{-/-}$*/TGFβR2*$^{-/-}$ double knock-out (DKO)**

Enhanced survival and/or expansion of iNK cells
Increased **IL-15** signaling through:
- Knock-out of **CISH**

Increased tumor control
Resistance to **TGF-β** inhibition through:
- Knock-out of **TGF-βR2**



***CD16*$^{+/+}$*/mbIL-15*$^{+/+}$ double knock-in (DKI)**

Enhanced survival and/or expansion of iNK cells
Increased **IL-15** signaling through:
- Knock-in of **mbIL-15 complex**

Enhanced ADCC
Constitutive overexpression of **CD16** through:
- Knock-in of **CD16**

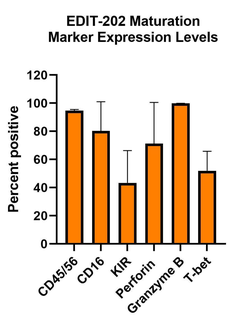
ADCC, antibody-dependent cellular cytotoxicity; CISH, cytokine-inducible SH2-containing protein; HLA, human leukocyte antigen; IL-15, interleukin-15; IL-15Rα, interleukin-15 receptor alpha; iNK, induced pluripotent stem cell-derived natural killer; mbIL-15, membrane-bound IL-15; SLEEK™, SeLection by Essential-gene Exon Knock-in: TGF-β, transforming growth factor beta; TGF-βR2, transforming growth factor-beta receptor 2.

METHODS

- Flow cytometry was performed to determine expression levels of the indicated markers. Perforin, granzyme b, and T-bet required intracellular staining.
- To determine the IC_{50}, a 3D tumor spheroid-killing assay was used with SKOV-3 target cells. Killer cell immunoglobulin-like receptor (KIR) and perforin levels were correlated using linear regression analysis. Non-linear regression analysis was used to correlate KIR and perforin levels with IC_{50}.
- Flow cytometry was used to determine the expression levels of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) in different cancer cell lines. A biosimilar (trastuzumab or cetuximab) antibody was used to bind the indicated antigen and a conjugated secondary antibody (AF488) was used to bind the biosimilar antibody.
- Cryopreserved EDIT-202 cells were plated with the indicated target cell lines and incubated for 48 hours. A lactate dehydrogenase (LDH) release assay was used to determine EDIT-202-induced cytotoxicity. An AlphaLISA® IFN-γ (human) detection kit was used to detect interferon gamma (IFN-γ) in the supernatant of the LDH assays used in Figure 4.

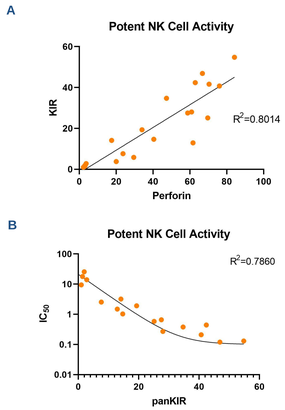
RESULTS

Figure 1: EDIT-202 cells express high levels of maturation markers such as KIR, Perforin, Granzyme B, and T-bet



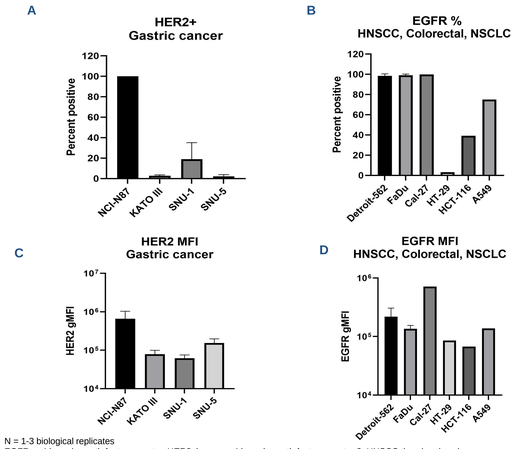
N=4 independent differentiations.
KIR, killer cell immunoglobulin-like receptor.

Figure 2: Maturation marker levels correlate with more potent NK cell activity



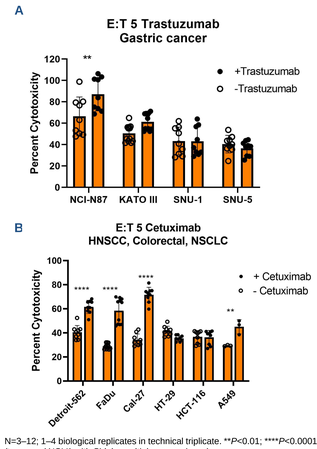
(A). N=21. **(B).** N=17; 4 independent differentiations.
IC_{50}, half maximal inhibitory concentration; KIR, killer cell immunoglobulin-like receptor; NK, natural killer.

Figure 3: Expression of EGFR and HER2 vary considerably across different cancer cell lines



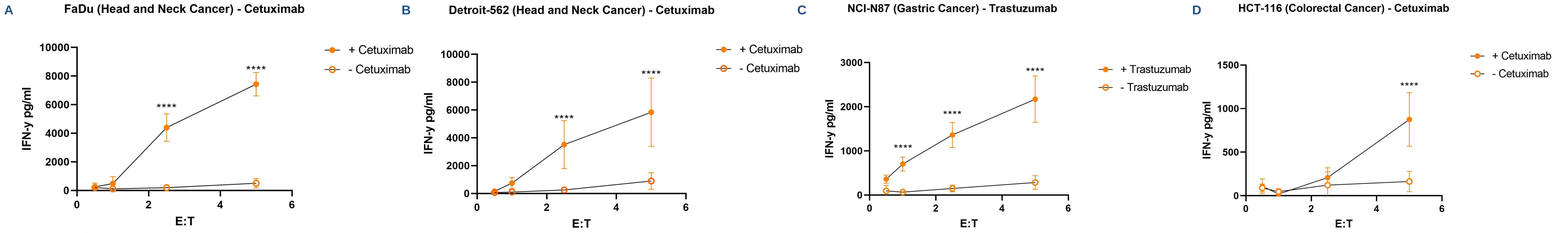
N = 1-3 biological replicates
EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HNSCC, head and neck squamous cell carcinomas; MFI, geometric mean fluorescence intensity; NSCLC, non-small cell lung cancer.

Figure 4: Cryopreserved EDIT-202 cells demonstrate activity against multiple solid tumor cell lines



N=3–12; 1–4 biological replicates in technical triplicate. **P<0.01; ****P<0.0001 (two-way ANOVA with Sidaks multiple comparisons).
HNSCC, head and neck squamous cell carcinomas; NSCLC, non-small cell lung cancer.

Figure 5: Cryopreserved EDIT-202 cells secrete more IFN-γ when combined with either trastuzumab or cetuximab



N=9–12; 3–4 biological replicates in technical triplicate. **** P<0.0001 (two-way ANOVA with Sidaks multiple comparisons).
IFN-γ, interferon gamma.

CONCLUSIONS

- EDIT-202 cells:
  - Display a mature phenotype, which is correlated with potent killing of tumor cells
  - Can be cryopreserved and still maintain their potent killing activity after thawing
  - Demonstrate significantly better cytotoxicity and IFN-γ secretion when combined with an ADCC-enabling antibody and target cells that express sufficient levels of antigen
  - Showed cytotoxic activity against ten different cancer cell lines from four different tumor types, using two different antibodies

DISCLOSURES

All authors are employees and stakeholders of Editas Medicine.

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