

Genetic Editing of iNK Cell Therapies to Enhance Tumor Killing Capacity

iPSC-Derived Immunotherapies Congress 2022

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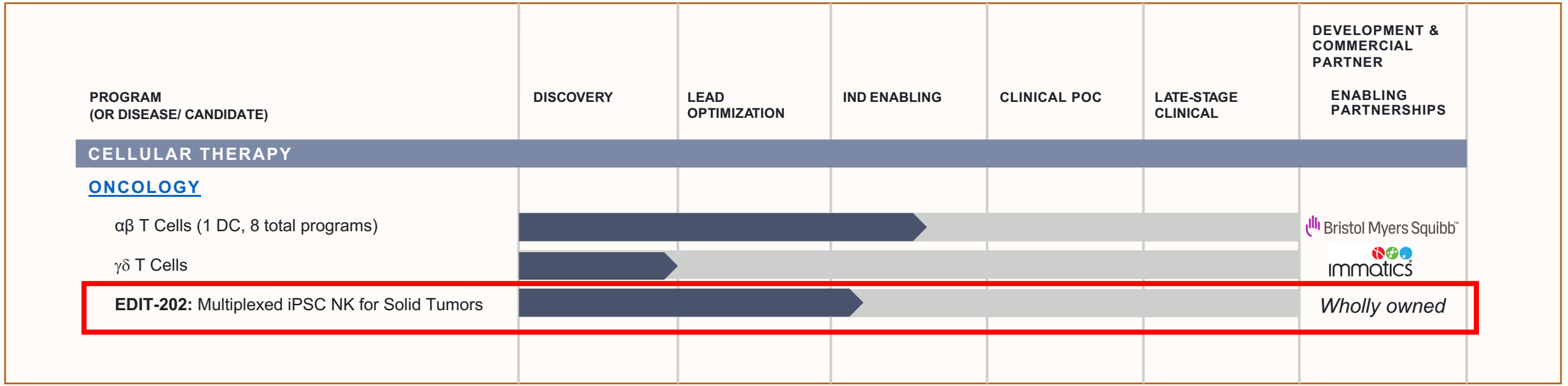
Disclosure

I am an employee and shareholder of Editas Medicine

iPSC-derived, Cas12a-edited NK cells are one of Editas' therapeutic approaches for oncology using our proprietary editing technologies



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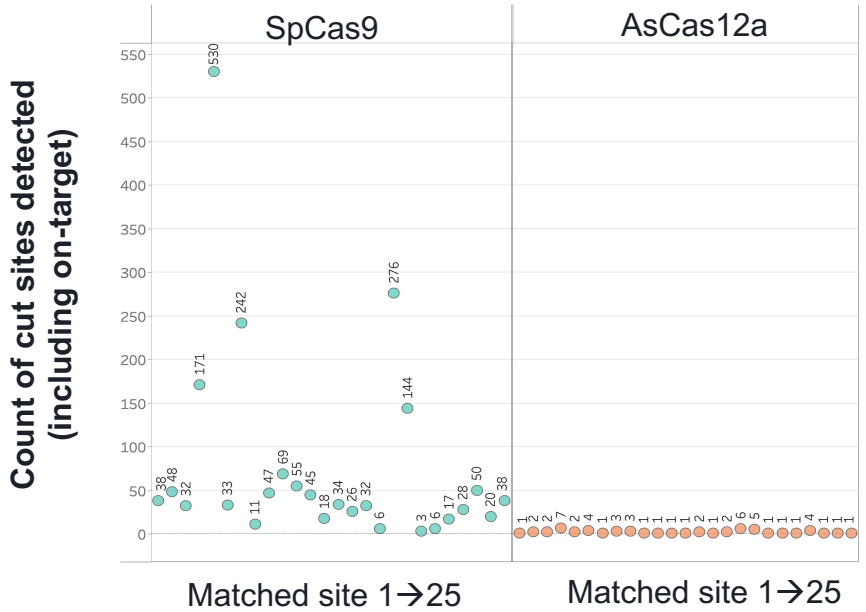
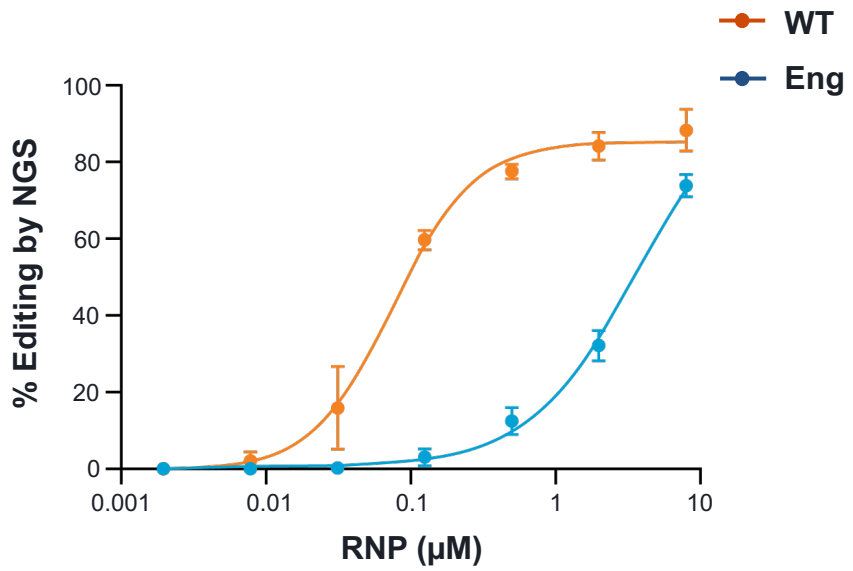
Engineered AsCas12a provides a high degree of gene knock-out specificity and efficiency

AsCas12a has greater specificity over SpCas9[1]



Digenome-Seq with 25 matched sites

AsCas12a engineering results in a 2-Log potency improvement[2]



AsCas12a, Acidaminococcus sp CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; Eng, engineered; NGS, next generation sequencing; RNP, ribonucleoprotein; SpCas9, staphylococcus aureus CRISPR-associated protein 9; WT, wildtype.

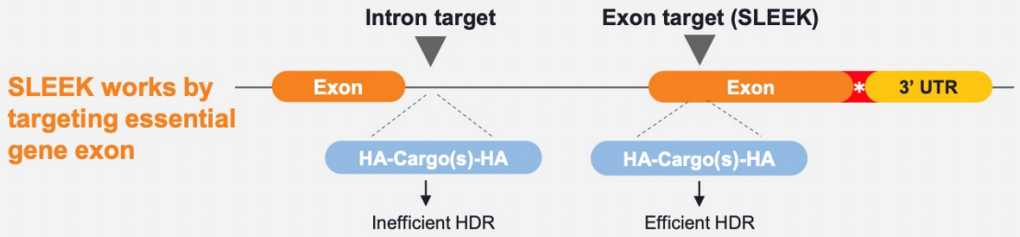
1. Gotta G *et al*. Poster presented at Cold Spring Harbor Laboratory; Laurel Hollow, New York, 10–13 October 2019.
2. Zuris J. Oral presentation at American Society of Gene & Cell Therapy (ASGCT); Washington, DC, 16–19 May 2022.

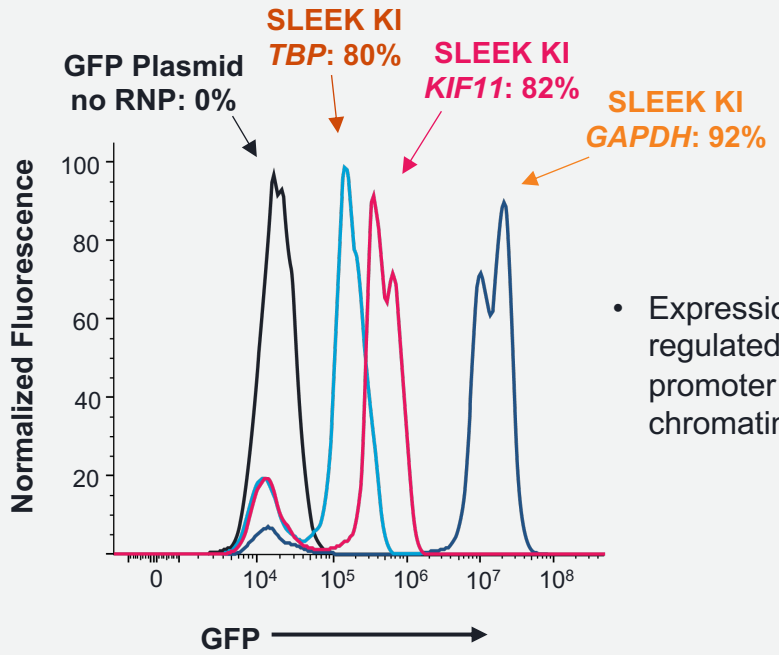
SLEEK technology enables efficient and robust gene knock-in

SLEEK™ GENE EDITING

Se**L**ection by **E**ssential-gene **E**xon **K**nock-In

- Enables >95% knock-in efficiency
- High-level, tunable cargo expression
- Near-homogeneous editing
- Efficient multicistronic cargos
- Simplifies iPSC clone selection process
- Robust, lineage-independent, expression of functional cargo in iPSCs





- Expression of cargo regulated by endogenous promoter strength and chromatin accessibility

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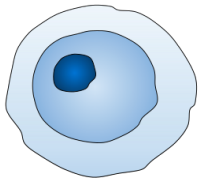
GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFP, green fluorescent protein; HDR: homology directed recombination; HA, homology arm; iPSC, induced pluripotent stem cell; KI, knock-in; KIF11, kinesin family member 11; RNP, ribonucleoprotein; TBP, TATA binding protein; UTR, untranslated region.

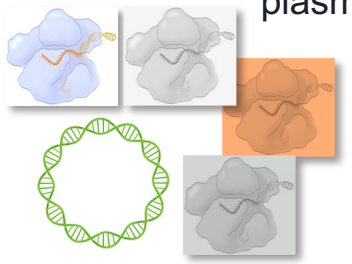
After editing, iPSC clones are screened for selected edit configuration

Unedited GMP iPSC



+

RNPs and/or plasmid(s)



Transfection

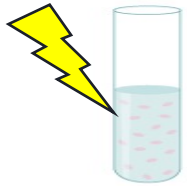
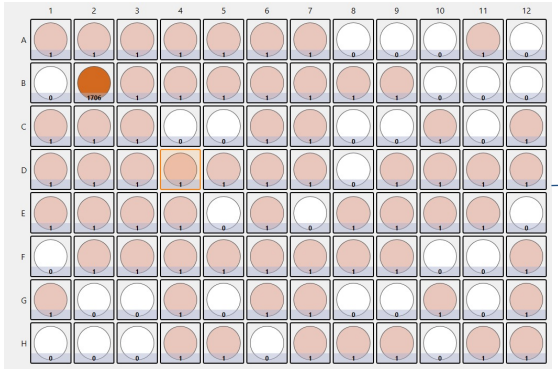
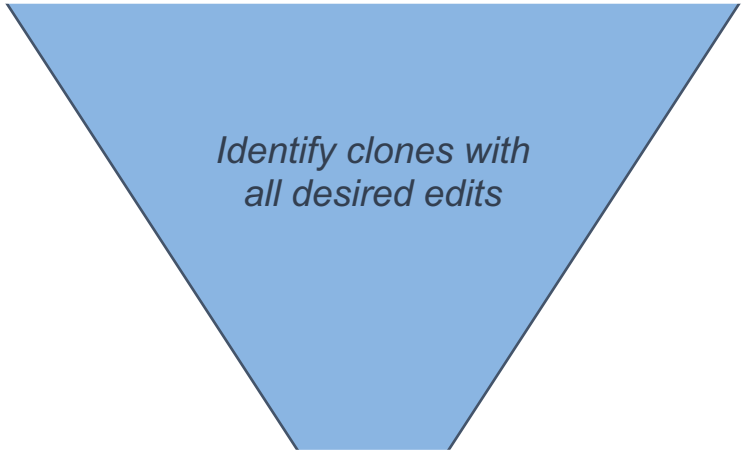


Plate single cells



Clones grow out

Resulting clones analyzed by multiplexed next-gen sequencing



Scale up and additional clone screening:
stemness, karyotyping, on/off-target analysis, structural rearrangements (UDiTaS)

Select clones evaluated for differentiation capacity to target cell type and final clone selection

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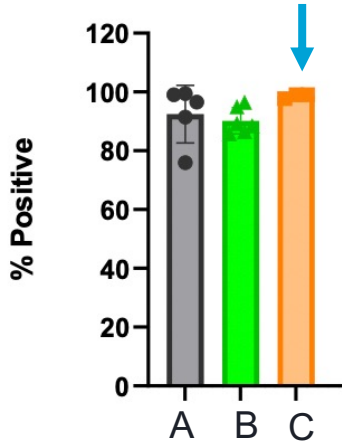
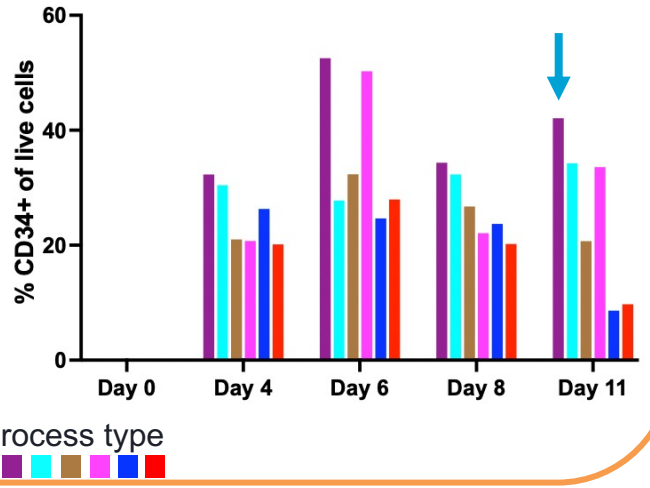
iPSC clones are differentiated to iNK cells using a feeder-free process that recapitulates natural NK phenotypic markers

iPSC → Heme progenitor → Mature iNK

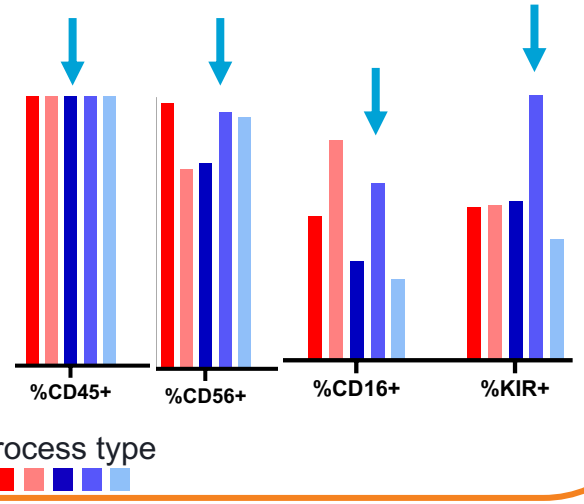
Tra-160+



CD34+



CD16+
pan-KIR+



Process parameters have been optimized for differentiation phenotype and function

CD, cluster of differentiation; GMP, good manufacturing practice; iNK, IPSC-derived natural killer cell; iPSC, induced pluripotent stem cell; KIR, killer cell immunoglobulin-like receptors; NK, natural killer; RNP, ribonucleoprotein; Tra, trastuzumab.

EDIT-202 is edited to resist TGFβ mediated suppression (TGFBR2 KO) and drive enhanced IL-15 responses (CISH KO)

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

Enhanced survival and/or expansion of iNK cells

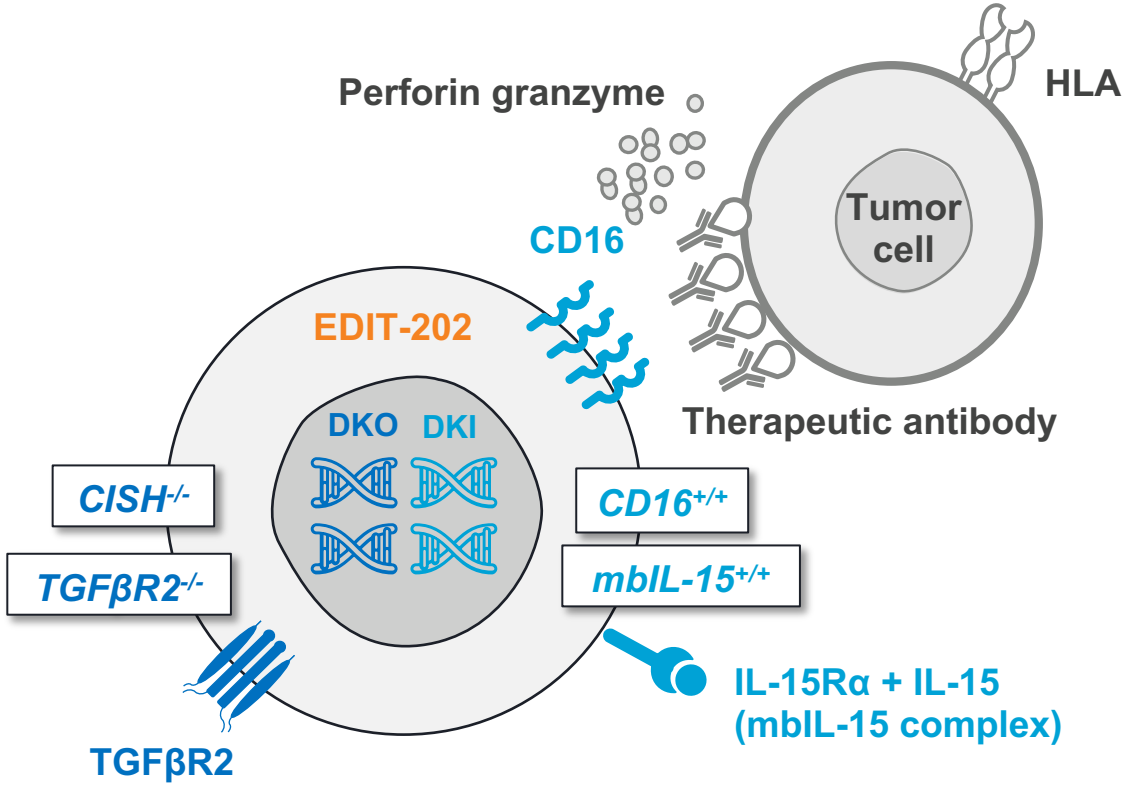
Increased **IL-15** signaling through:

- Knock-out of ***CISH***

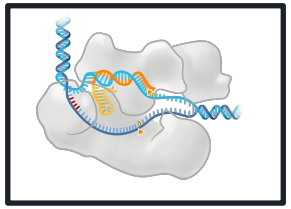
Increased tumor control

Resistance to ***TGFβ*** inhibition through:

- Knock-out of ***TGFβR2***



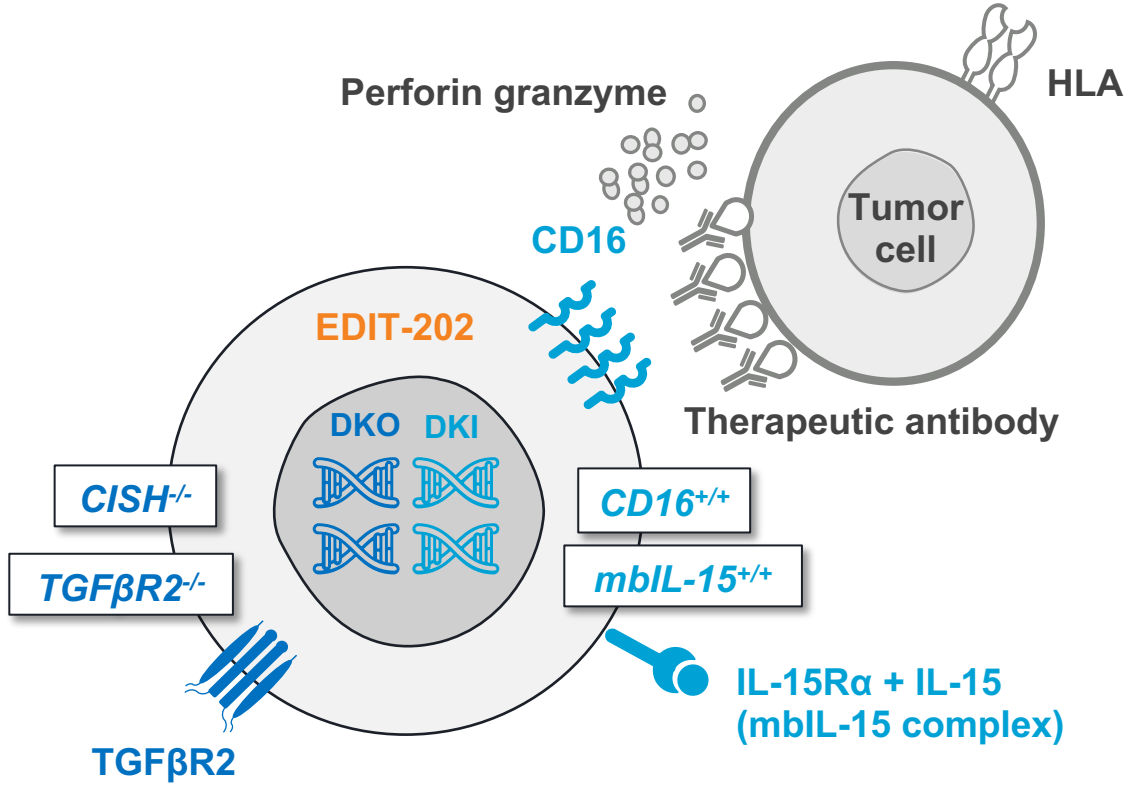
Eng. AsCas12a



AsCas12a, Acidaminococcus sp CRISPR-associated protein 12a; CD, cluster of differentiation; 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; TGFβ, transforming growth factor beta; TGFβR2, transforming growth factor beta receptor 2.

Zuris J. Oral presentation at Cold Spring Harbor Laboratory's Genome Engineering: CRISPR Frontiers; Laurel Hollow, New York, 20 August 2021.

EDIT-202 is edited to provide a high level of CD16 expression and provide autocrine IL-15 signaling



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

Enhanced ADCC

Constitutive overexpression of **CD16** through:

- Knock-in of ***CD16***

Enhanced survival and/or expansion of iNK cells

Increased **IL-15** signaling through:

- Knock-in of ***mbIL-15*** **complex**

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ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; 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; TGFβ, transforming growth factor beta; TGFβR2, transforming growth factor beta receptor 2.

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Zuris J. Oral presentation at Cold Spring Harbor Laboratory's Genome Engineering: CRISPR Frontiers; Laurel Hollow, New York, 20 August 2021.

EDIT-202, a 4x gene-edited product, resists TGFβ immune suppression, enhances CD16 expression and promotes IL-15 pathway activation

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

Enhanced survival and/or expansion of iNK cells

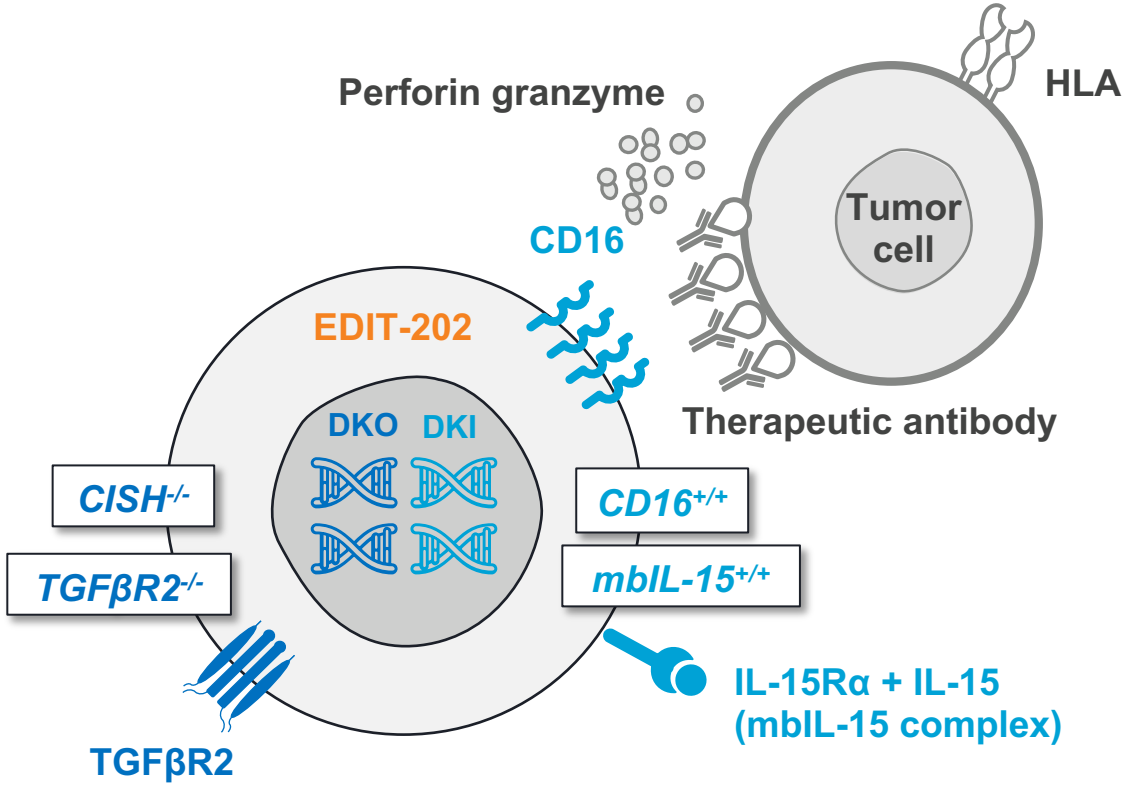
Increased **IL-15** signaling through:

- Knock-out of ***CISH***

Increased tumor control

Resistance to ***TGFβ*** inhibition through:

- Knock-out of ***TGFβR2***



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

Enhanced ADCC

Constitutive overexpression of **CD16** through:

- Knock-in of **cleavable *CD16***

Enhanced survival and/or expansion of iNK cells

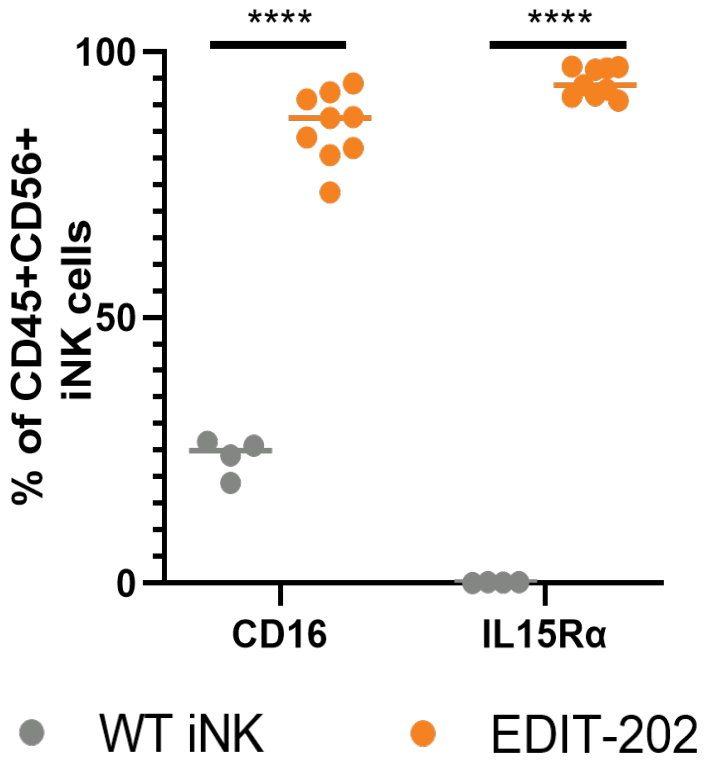
Increased **IL-15** signaling through:

- Knock-in of ***mbIL-15* complex**

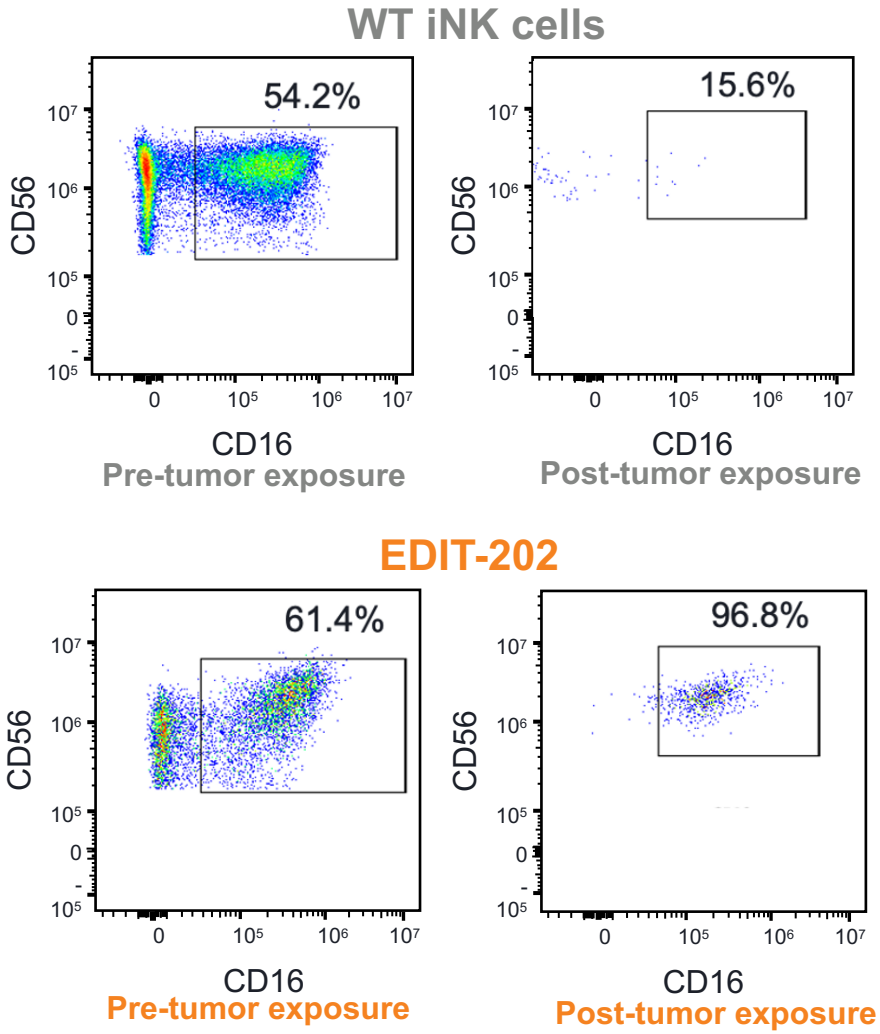
ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; CISH, cytokine-inducible SH2-containing protein; HLA, human leukocyte antigen; IL-15, interleukin-15; IL-15Rα, interleukin-15 receptor alpha; iNK, induced pluripotent stem cell-derived natural killer; mbIL-15, membrane-bound IL-15; TGFβ, transforming growth factor beta; TGFβR2, transforming growth factor beta receptor 2.

Zuris J. Oral presentation at Cold Spring Harbor Laboratory's Genome Engineering: CRISPR Frontiers; Laurel Hollow, New York, 20 August 2021

SLEEK KI provides high % iNK cells positive for IL15Rα and CD16



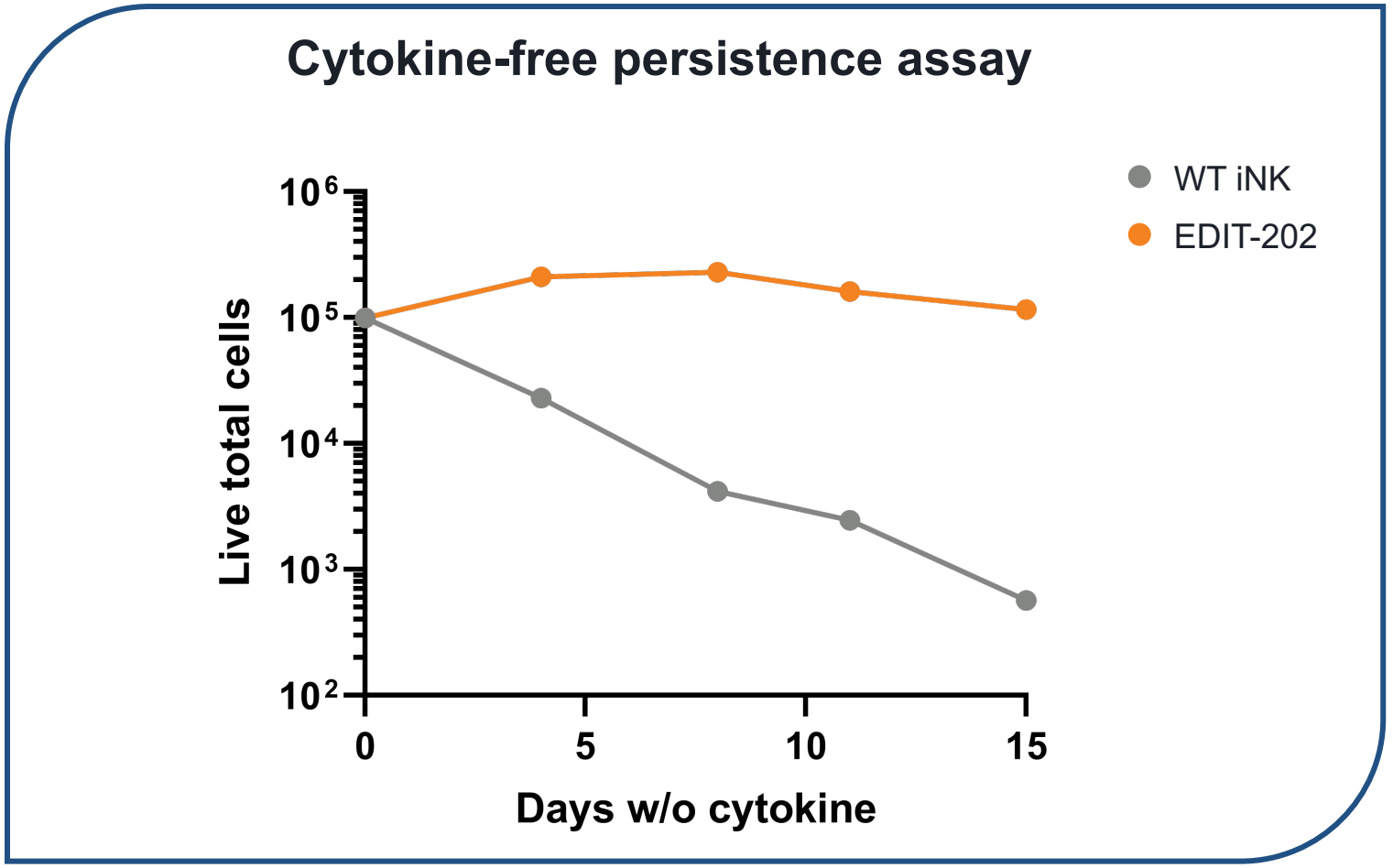
CD16 KI is resistant to down-regulation by tumor cell exposure



- 4 days *in vitro* E:T 2:1, SKOV3 target, 10ug/ml Trastuzumab
- Pre-gated on viable cells

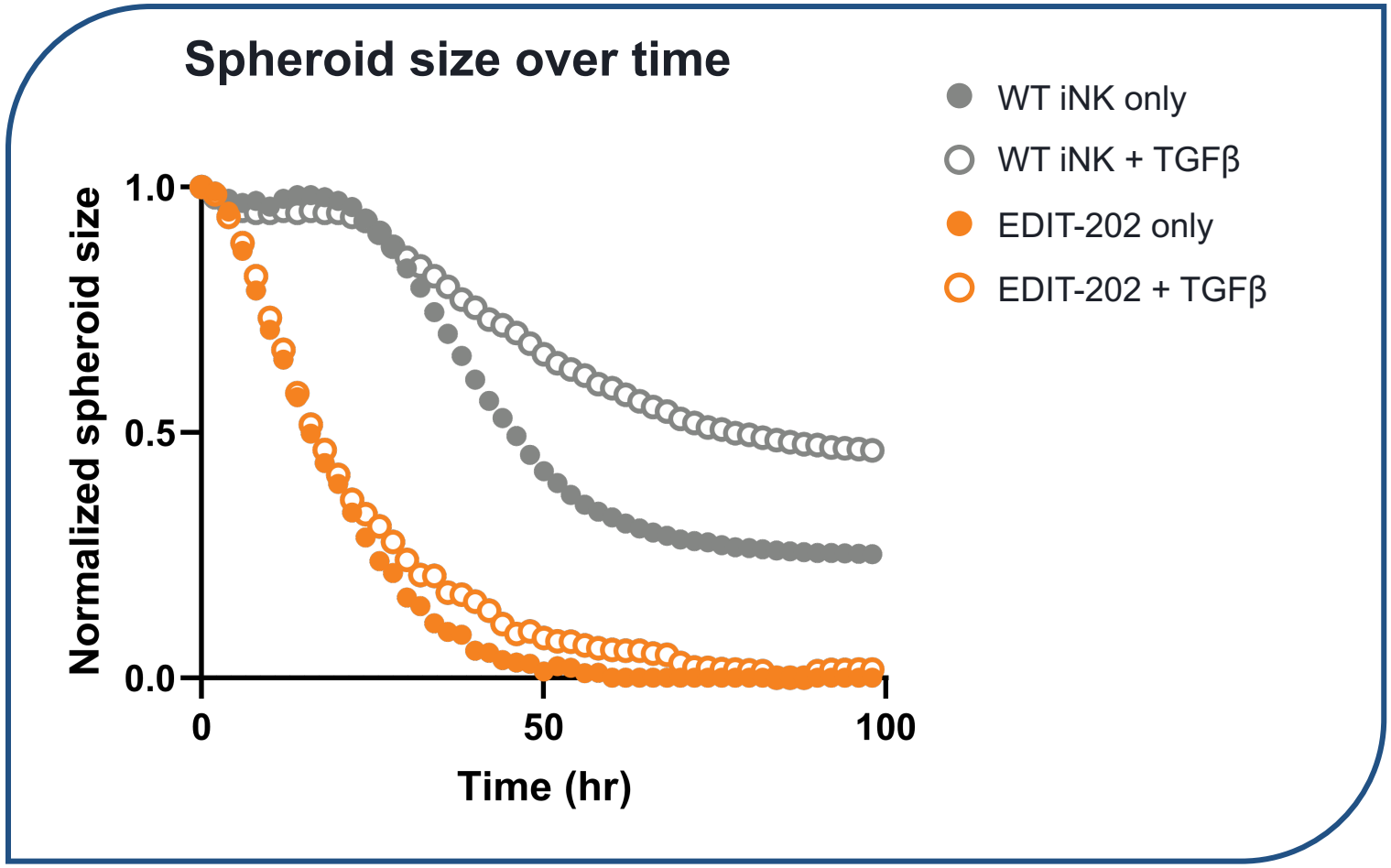
CD, cluster of differentiation; E, effector; IL-15Rα, interleukin-15 receptor alpha; iNK, induced pluripotent stem cell-derived natural killer; KI, knock-in; T, target; WT, wild type.

EDIT-202 persists without additional exogenous cytokines *in vitro*



iNK, induced pluripotent stem cell-derived natural killer; KI, knock-in, WT, wild type.

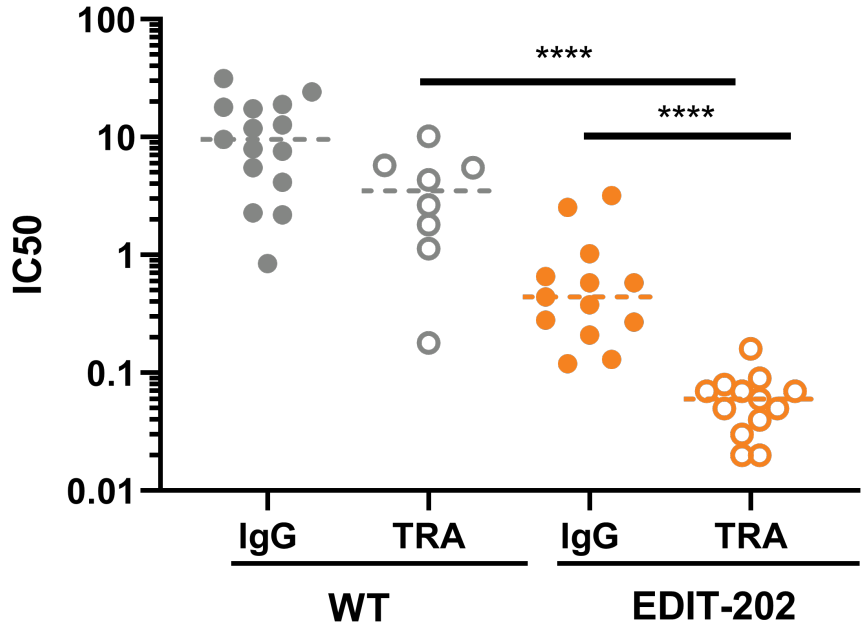
EDIT-202 shows enhanced killing of tumor spheroids in the presence of TGFβ



iNK, induced pluripotent stem cell-derived natural killer; TGFβ, transforming growth factor beta.

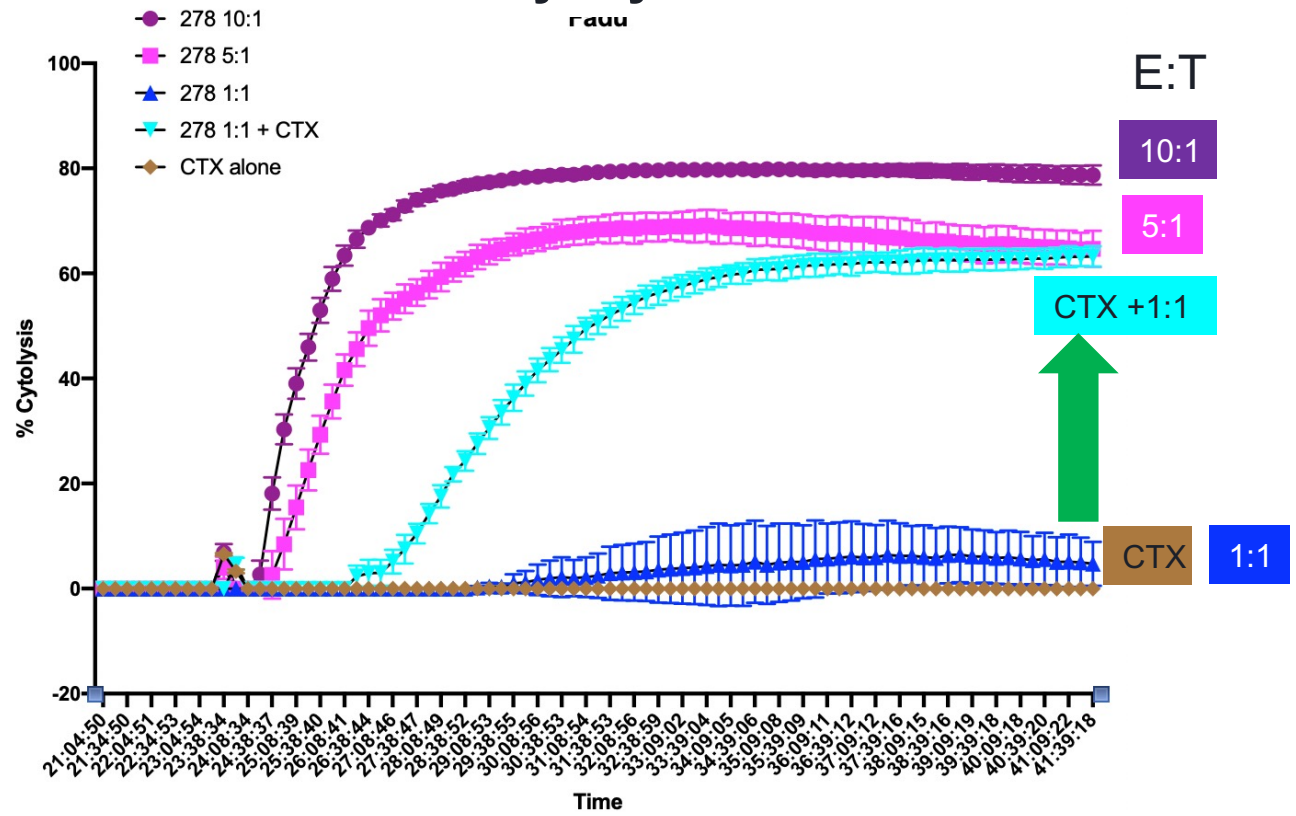
EDIT-202 synergizes with ADCC-competent monoclonal antibodies in vitro

IC50 at 100 hours



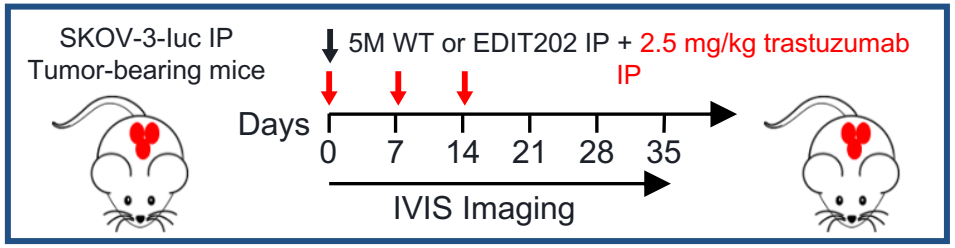
SKOV3 = ovarian cancer

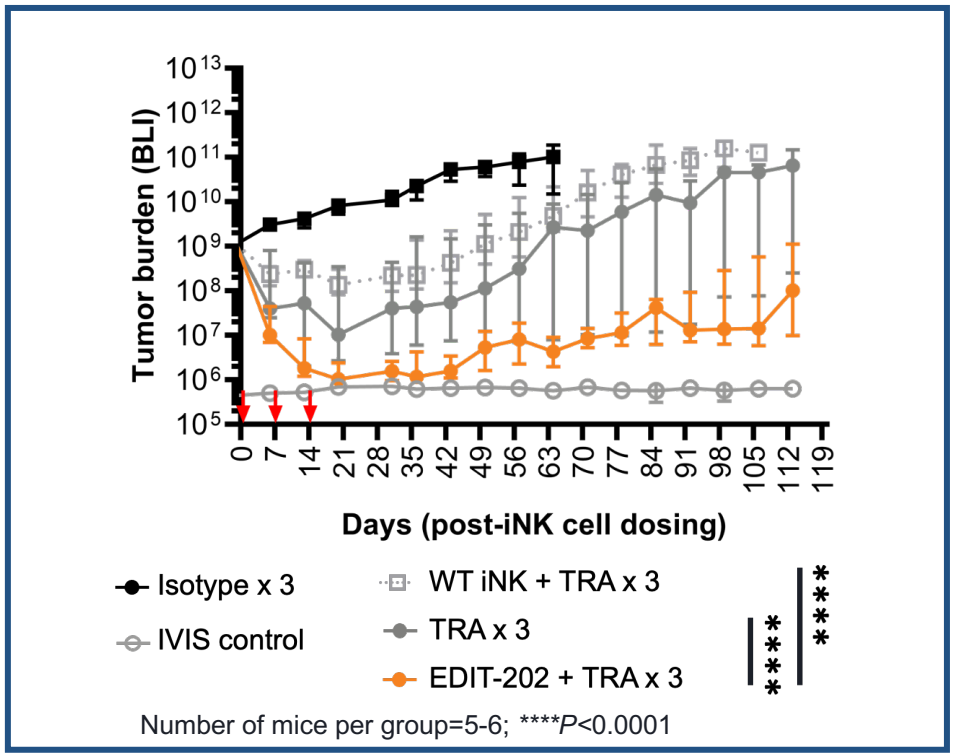
% Cytolysis over time

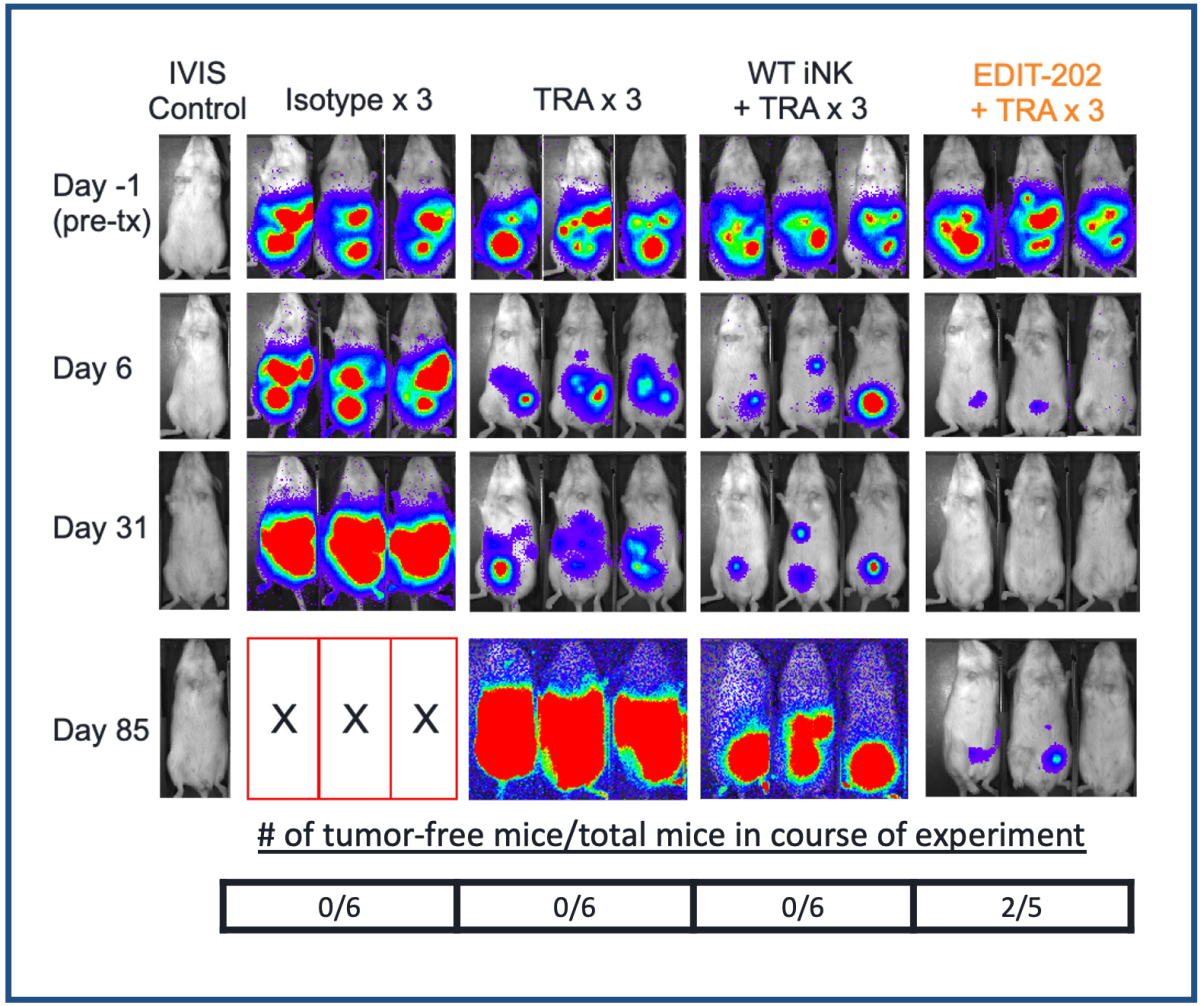


Fadu = head and neck cancer

EDIT-202 synergizes with ADCC-competent monoclonal antibodies *in vivo*

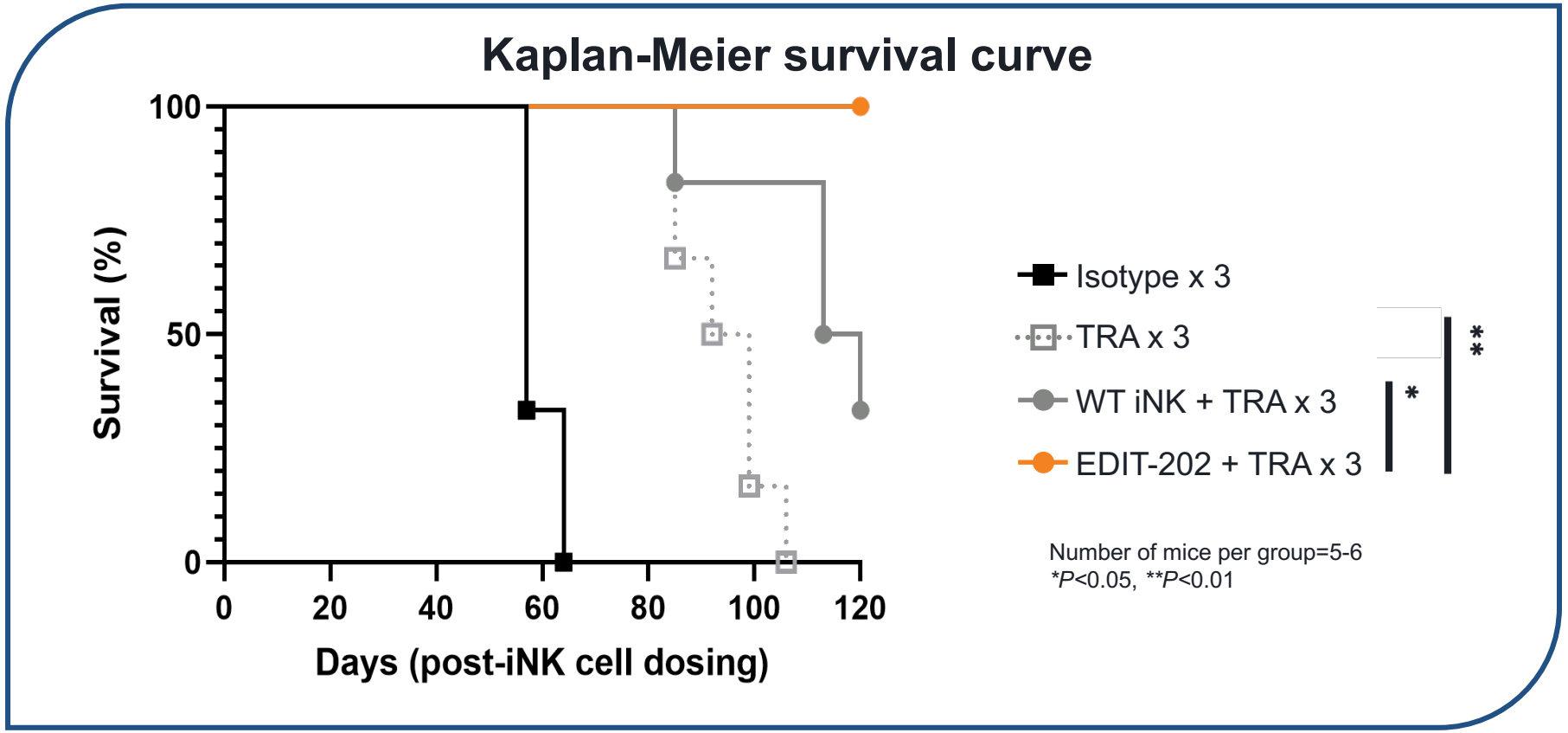






ADCC, antibody-dependent cellular cytotoxicity; BLI, bioluminescent imaging; iNK, induced pluripotent stem cell-derived natural killer; IP, intraperitoneal; IVIS, in vivo imaging system; SKOV-3-luc, luciferase (luc)-expressing SKOV-3 cell line; TRA, trastuzumab; WT, wild type.

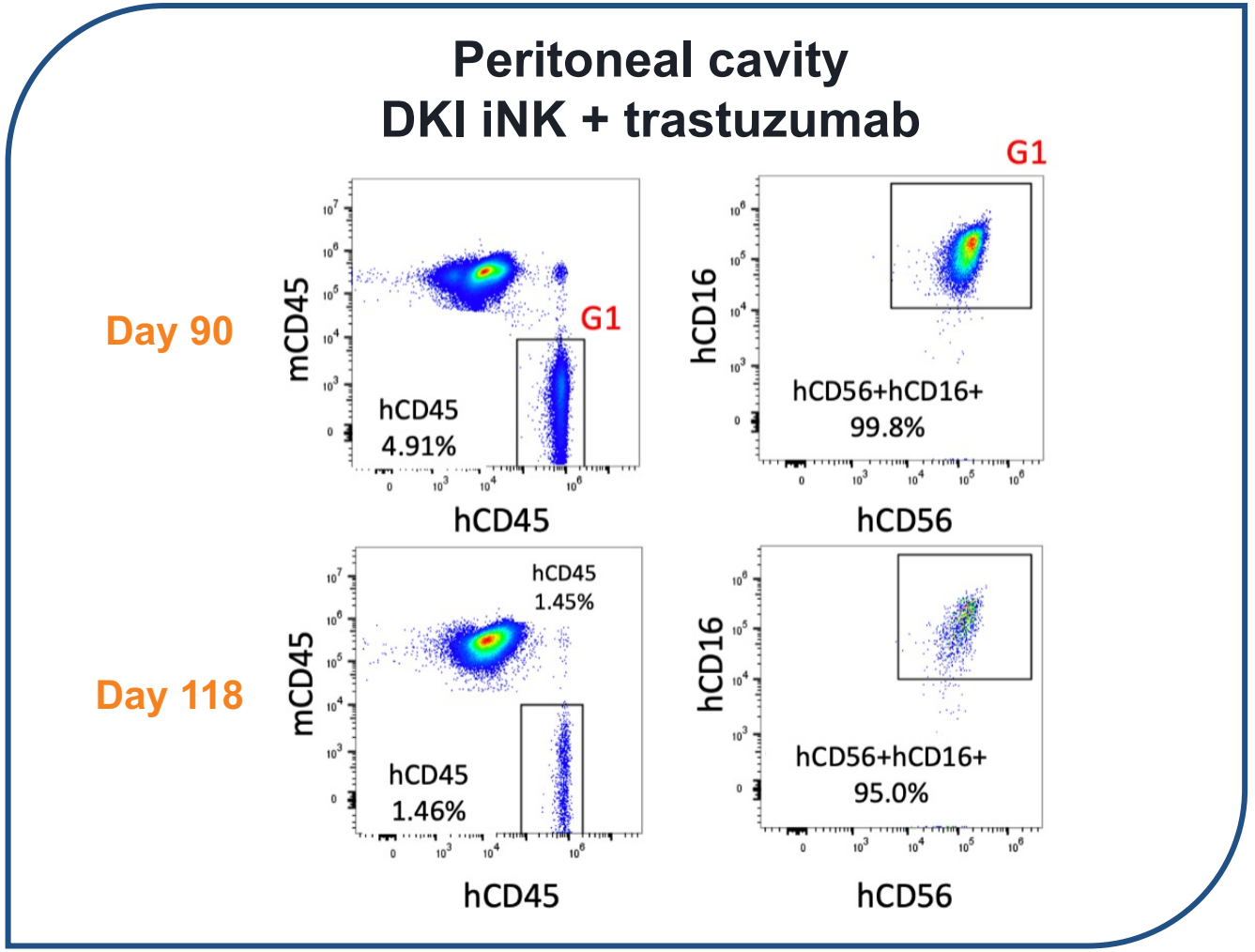
EDIT-202 plus TRA significantly increased survival over WT iNK cells + TRA in an ovarian SKOV-3-luc IP solid tumor model



EDIT-202 plus TRA increases survival to 100% compared with 0% survival with TRA only at day 120

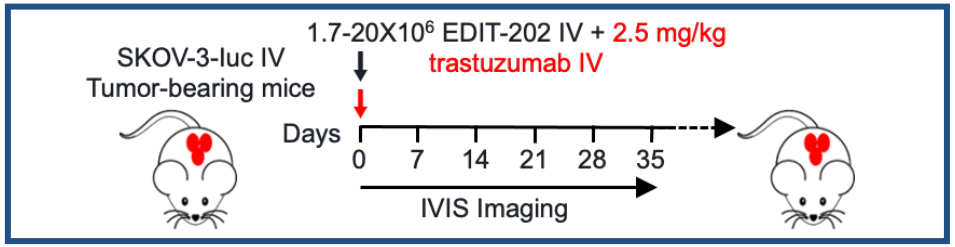
iNK, induced pluripotent stem cell-derived natural killer; IP, intraperitoneal; SKOV-3-luc, luciferase (luc)-expressing SKOV-3 cell line; TRA, trastuzumab; WT, wild type.

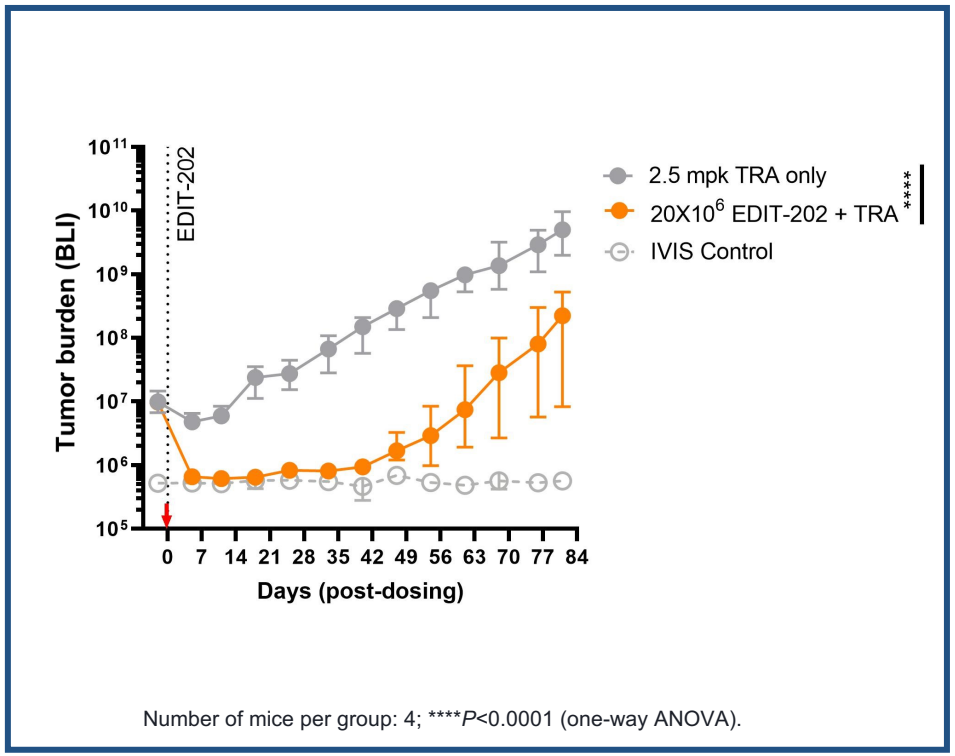
mbIL-15 and CD16 KI drive long term persistence and CD16 expression maintenance *in vivo*



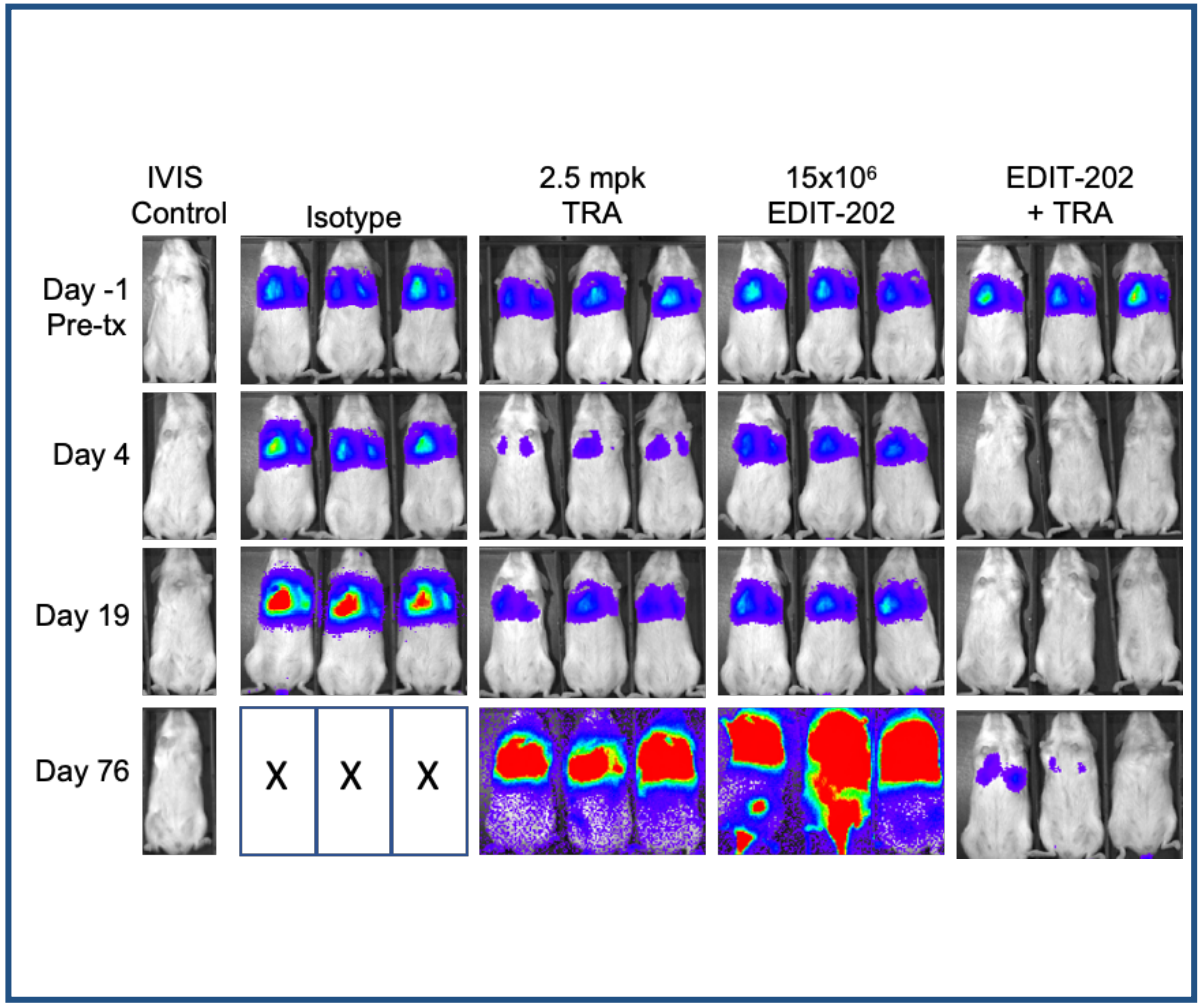
CD, cluster of differentiation; DKI, double knock-in; KI, knock-in; mbIL-15, membrane-bound IL-15.

EDIT-202 shows *in vivo* efficacy in a solid tumor lung model after IV administration





Number of mice per group: 4; ****P<0.0001 (one-way ANOVA).



BLI, bioluminescent imaging; IV, intravenous; IVIS, in vivo imaging system; SKOV-3-luc, luciferase (luc)-expressing SKOV-3 cell line; TRA, trastuzumab.

EDIT-202 is an iPSC-derived iNK cell therapy with prolonged cell persistence and significantly enhanced efficacy in solid tumors

- ✓High cell surface levels of CD16 and mbIL15
- ✓Enhanced natural cytotoxicity and ADCC-mediated killing against 3D SKOV-3 spheroids
- ✓Resistance to TGFβ induced immunosuppression due to TGFβR2 KO
- ✓Upregulated and continuous expression of CD16 after tumor exposure enabling serial tumor killing
- ✓Prolonged cytokine independent persistence due to mbIL15 KI
- ✓Potent anti-tumor efficacy in an *in vivo* ovarian SKOV-3-luc solid tumor model

These data support the development of EDIT-202 as a potential allogeneic cell-based medicine for treatment of solid tumors

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ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; iNK, induced pluripotent stem cell-derived natural killer; iPSC, induced pluripotent stem cell; KI, knock-in; KO, knock-out; mbIL-15, membrane-bound IL-15; SKOV-3-luc, luciferase (luc)-expressing SKOV-3 cell line; TGFβ, transforming growth factor beta; TGFβR2, transforming growth factor beta receptor 2.