Masked T Cell Engagers Designed to Drive Potent Synthetic Anti-Tumor Immunity with Favorable Tolerability

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SCAN ME

Abstract 972

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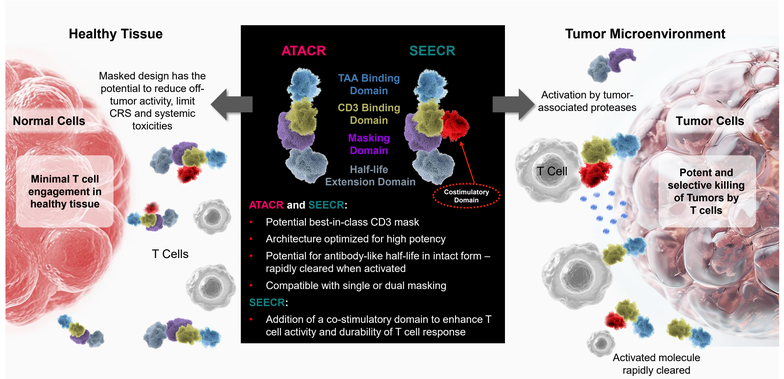
BACKGROUND

- T cell engagers (TCEs) represent a promising modality for cancer immunotherapy with clinically meaningful clinical activity in hematological malignancies and select solid tumors.
- However, many compelling TCE targets have remained out of reach due to toxicity limitations.
- TCEs simultaneously bind to CD3 on T cells and tumor-associated antigens (TAA) on tumor cells inducing an immunological synapse that results in T cell-mediated killing of tumor cells.
- Masked TCEs have the potential to unlock many TAA targets considered undruggable due to toxicity concerns.
- Xilio's masked T cell engager programs include bispecific molecules designed using its advanced tumor-activated cell engager (ATACR) format, which consists of a T cell engager with a masked CD3 targeting domain, and tri-specific molecules designed using its selective effector-enhanced cell engager (SEECR) format.
- The SEECR format builds upon the ATACR format by adding co-stimulatory signaling designed to further enhance potency and durability of T cell response.

DESIGN



ATACR

Advanced Tumor-Activated Cell Engager

"ATACR" Format *(masked cell engager)*

Designed to release a potent, short half-life T cell engager upon tumor-selective activation

Optimized for speed + simplicity

SEECR

Selective Effector-Enhanced Cell Engager

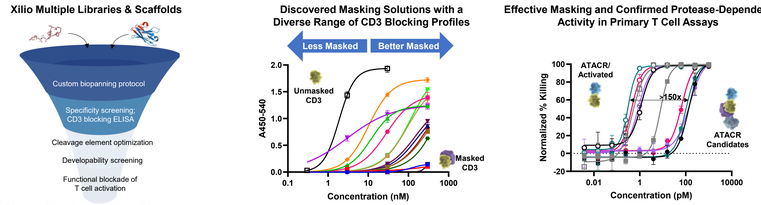
"SEECR" Format *(masked cell engager + co-stimulatory domain)*

Builds on ATACR format and adds a co-stimulatory domain designed to further enhance potency and durability of T cell response

Optimized for differentiation + enhanced activity

Program	Tumor Types	Xilio Masked T Cell Engager Format
PSMA	Prostate	ATACR
CLDN18.2	Gastric, Pancreatic, Esophageal	ATACR
STEAP1	Prostate, Lung, Colorectal	SEECR

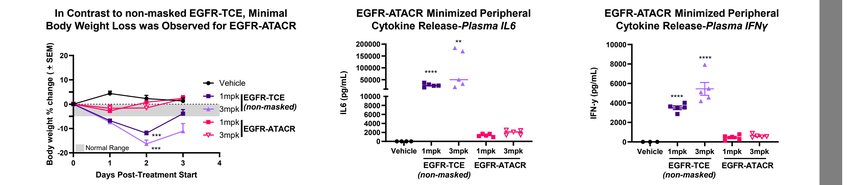
COMPREHENSIVE MASK DISCOVERY AND OPTIMIZATION RESULTED IN CD3 MASKING SOLUTION WITH BEST-IN-CLASS POTENTIAL

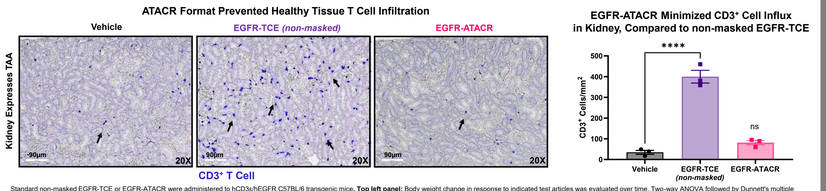


Left panel: Comprehensive mask discovery campaign yielded multiple masking solutions. **Middle panel:** CD3ε ELISA to screen the binding of new masked molecules in the scFv format. **Right panel:** Primary human T cells were co-cultured with cancer cells. Tumor cell killing in response to indicated titrations of test articles was evaluated using a luciferase-based cell viability readout.

VALIDATED EFFECTIVE MASKING AND ACTIVATION *IN VIVO*

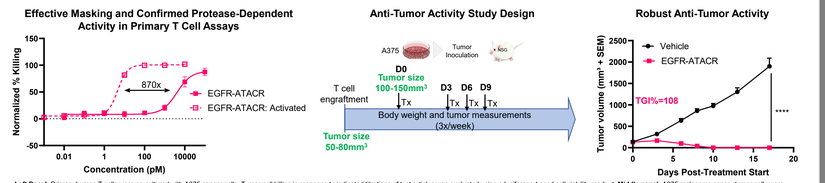
No Meaningful Cytokine Release Syndrome or On-Target/Off-Tumor Toxicity with EGFR-ATACR in a Fully Cross-Reactive Immune-Competent Setting Compared with Non-Masked TCE *In Vivo*





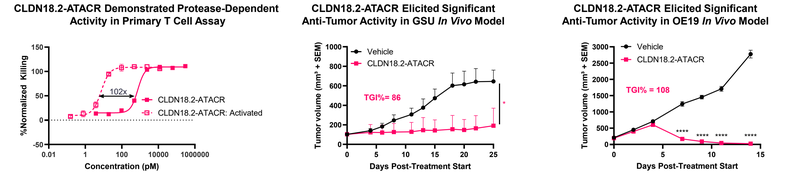
Standard non-masked EGFR-TCE or EGFR-ATACR were administered to hCD3ε/hEGFR C57BL/6 transgenic mice. **Top left panel:** Body weight change in response to indicated test articles was evaluated over time. Two-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical analysis: p<0.001(***). **Top middle panel:** Plasma IL-6 and **Top right panel**: IFNγ at 6 hours (measured using MSD of mice treated with standard non-masked EGFR-TCE or EGFR-ATACR). Significance by one-way ANOVA post Dunnett's multiple comparisons: p<0.01(**); p<0.0001(****) respectively. **Bottom left panel:** CD3 IHC representative image from mice treated with Vehicle, standard non-masked EGFR-TCE or EGFR-ATACR. **Bottom right panel:** CD3 IHC quantification. Data analyzed by one-way ANOVA with post hoc Dunnett's multiple comparisons tests: p<0.0001(****).

EGFR-ATACR Format Exhibited Anti-Tumor Activity in a Humanized Mouse Model

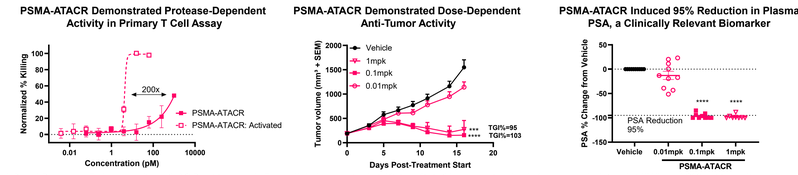


Left Panel: Primary human T cells were co-cultured with A375 cancer cells. Tumor cell killing in response to indicated titrations of test articles was evaluated using a luciferase-based cell viability readout. **Middle panel:** A375 melanoma cancer tumor cells were inoculated in NSG mice engrafted with human T cells; animals received IV doses of EGFR-ATACR or Vehicle. **Right panel:** Anti-tumor activity change in response to indicated test articles was evaluated over time. Two-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical analysis: p<0.0001(****).

CLDN18.2-ATACR AND PSMA-ATACR: DEMONSTRATED EFFICIENT MASKING AND *IN VIVO* POTENCY

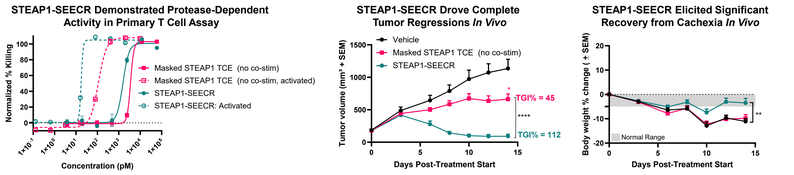


Left panel: Primary human T cells were co-cultured with GSU gastric carcinoma (stomach cancer) cell line. Tumor cell killing in response to indicated titrations of test articles was evaluated using a luciferase-based cell viability readout. **Middle panel:** GSU cells were inoculated in NSG mice engrafted with human T cells. Anti-tumor activity change in response to indicated test articles was evaluated over time. Two-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical analysis: p<0.05(*). **Right panel:** OE19 cells were inoculated in NSG mice engrafted with human T cells. Anti-tumor activity change in response to indicated test articles was evaluated over time. Two-way ANOVA followed by Šidák's multiple comparisons test was used for statistical analysis: p<0.0001(****).



Left panel: Primary human T cells were co-cultured with LNCaP prostate cancer cells. Tumor cell killing in response to indicated titrations of test articles was evaluated using a luciferase-based cell viability readout. **Middle panel:** C4-2 prostate cancel tumor cells were inoculated in NSG mice engrafted with human T cells. Anti-tumor activity change in response to indicated test articles was evaluated over time. Two-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical analysis: p<0.001(***); p<0.0001(****). **Right panel:** PSA percentage change in plasma over vehicle control. One-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical analysis for PSA: p<0.0001(****) against vehicle.

STEAP1-SEECR: CO-STIMULATORY DOMAIN ENABLED DEEP TUMOR REGRESSIONS AND PREVENTED TUMOR-ASSOCIATED CACHEXIA



Left panel: Primary human T cells were co-cultured with C4-2 prostate cancer cells. Tumor cell killing in response to indicated titrations of test articles was evaluated using a luciferase-based cell viability readout. **Middle and right panel:** C4-2 prostate cancer has been demonstrated to drive cachexia in vivo (Thalmann et al., The Prostate, 2000) in NSG mice engrafted with human T cells. Anti-tumor activity and body weight change in response to indicated test articles was evaluated over time. Two-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical analysis: p<0.05(*), p< 0.005(**), p<0.0001(****).

CONCLUSIONS

- Xilio's ATACR and SEECR formats represent next-generation TCEs designed to overcome the challenges associated with current, systemically active non-masked TCEs.
- By leveraging TME-specific protease activity for conditional activation, Xilio's ATACR and SEECR molecules maintained potent anti-tumor activity with evidence of reduced systemic toxicity in murine models.
- The incorporation of co-stimulatory signaling in the SEECR format enhanced anti-tumor activity in preclinical murine models, compared with TCE molecules lacking co-stimulation.
- Together, these preclinical data support the potential of our technology to expand the therapeutic window for TCEs and highlight the applicability of Xilio's masked formats across a diverse range of TCE targets.

ABBREVIATIONS: ANOVA: analysis of variance; CRS: Cytokine Release Syndrome; PSMA: Prostate-Specific Membrane Antigen; EGFR: Epidermal Growth Factor Receptor; CLDN18.2: Claudin 18 isoform 2; STEAP1: Six-Transmembrane Epithelial Antigen of the Prostate 1; TME: Tumor Micro-environment; CD: Cluster of Differentiation; ELISA: Enzyme-link Immunosorbent; NSG: NOG Scid Gamma; scFv: Single-chain Variable Fragment; IL: Interleukin; IFNγ: Interferon gamma; IHC: Immunohistochemistry; MSD: Mesoscale Discovery; PSA: Prostate-Specific Antigen; TGI: Tumor Growth Inhibition; Tx: Treatment; EO19: Gastric Adenocarcinoma; LNCaP: (Lymph Node Carcinoma of Prostate) is a human prostate cancer cell line

REFERENCES: 1. Uli Bialucha (2025). Design Illustration [AI-generated image], Luma-Photon and Playground_V3.

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