Tumor-activated immune cell engagers designed to drive synthetic immunity against cancer demonstrated protease-dependent activation and robust anti-tumor activity in preclinical models

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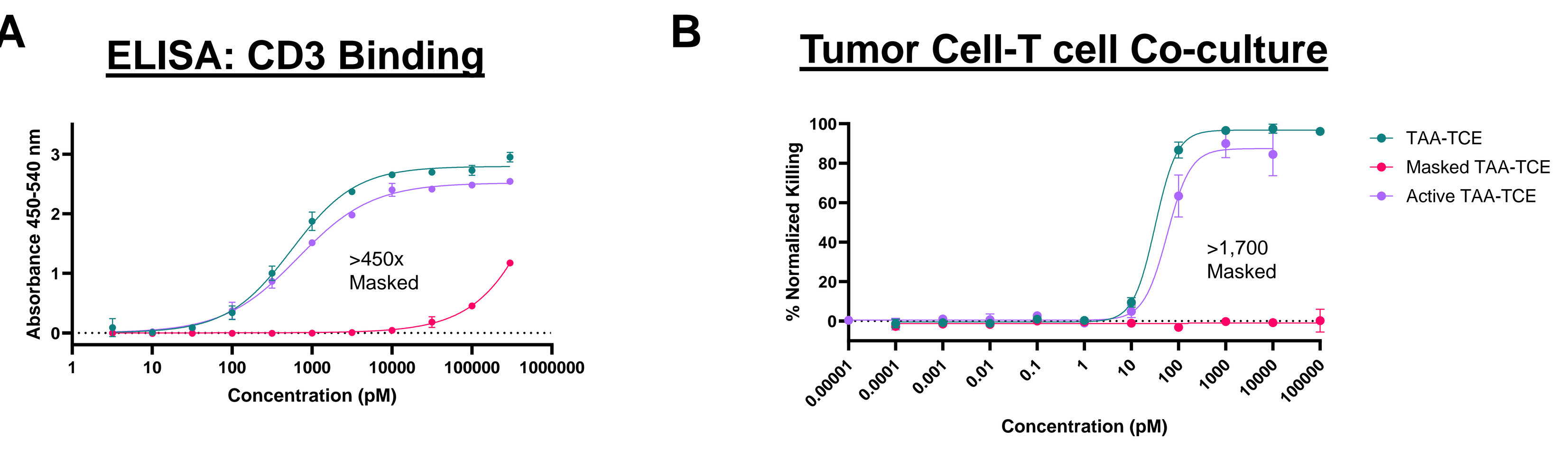
Background

Bispecific T cell engagers (TCEs) targeting tumor-associated antigens (TAAs) represent a validated therapeutic strategy, but they have seen limited success in solid tumors to date. Key challenges include on-target, off-tumor toxicity from TAA expression in healthy tissues, cytokine release syndrome from broad systemic T cell activation, and an immunosuppressive solid tumor microenvironment (TME) hindering anti-tumor immunity. To address these limitations, we developed **SELECTIVE EFFECTOR-ENHANCED CELL ENGAGERS** (SEECR), a novel class of tumor-activatable, immune cell engaging bispecific molecules designed to remain minimally active in peripheral tissues while activating selectively upon cleavage by proteases enriched in the TME. SEECR-T molecules were designed to simultaneously engage TAA on cancer cells, CD3 on T cells and incorporate a costimulatory domain to enhance T cell activation and functionality.

Regular T cell Engager (TAA-TCE)
- **Engages TAA and CD3**
- **Systemically active**
- **No costimulatory signaling**

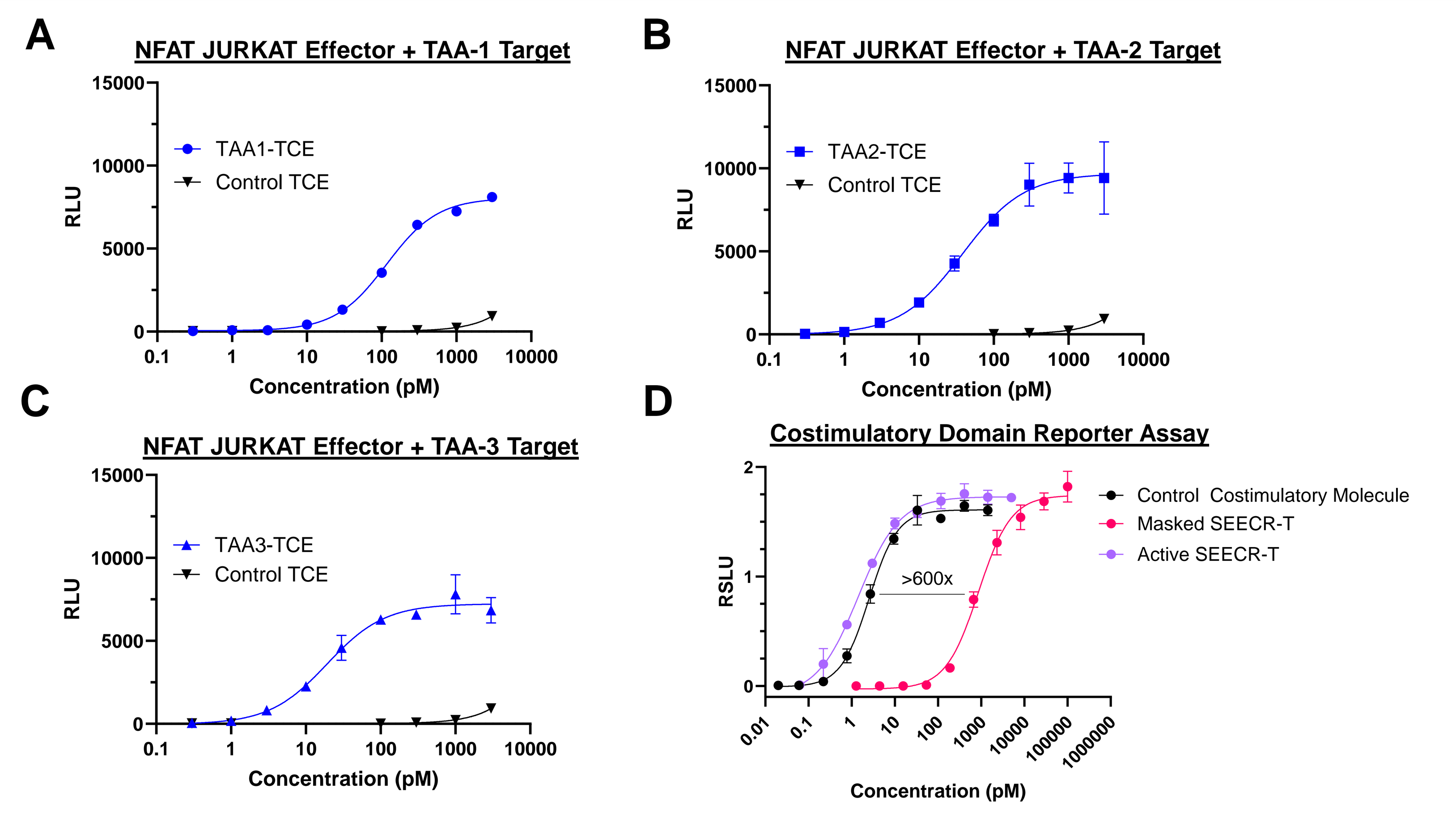
SEECR-T
- **Engages TAA and CD3**
- **Tumor activated**
- **Provides costimulatory signal**

Protease-dependent activation of CD3 domain and T cell engagement in vitro



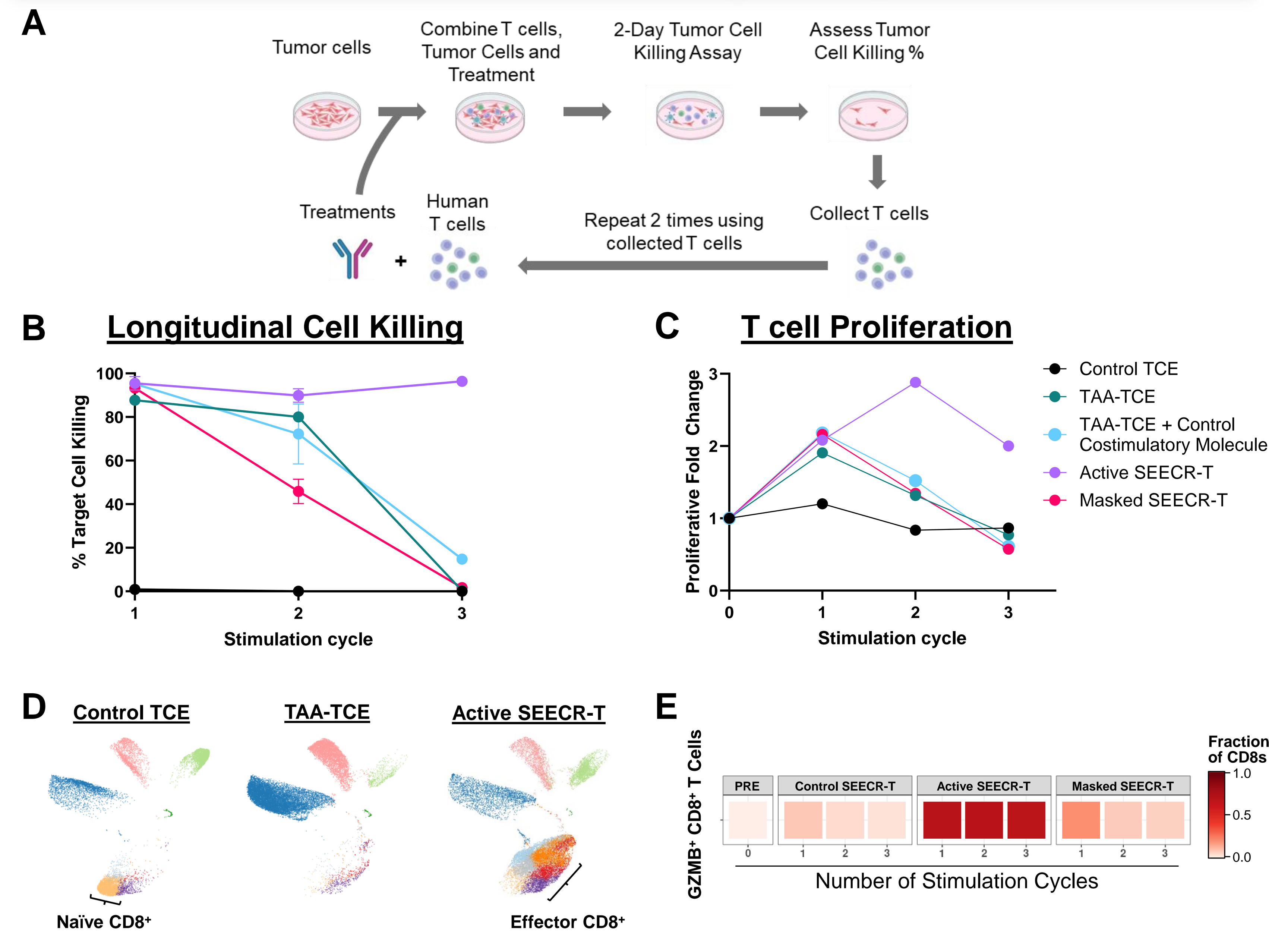
Demonstrated masking of CD3 in TAA-TCE molecule. Masked TAA-TCE was activated by incubation with protease in vitro (Active TAA-TCE) (**A**) Protease dependent CD3-binding demonstrated via TAA-TCEs bound to immobilized CD3 in an ELISA. (**B**) Protease-dependent tumor cell killing. Active TAA-TCEs led to killing in co-culture assay. A375 tumor cells were cultured overnight before addition of expanded T cells at a 5:1 E:T. Test articles were titrated into the wells and then plates were incubated for 2 days at 37°C. Effector cells were washed away and then remaining viable tumor cells were measured.

SEECR-T format is designed to be TAA-agnostic and enabled incorporation of masked costimulatory domain in vitro



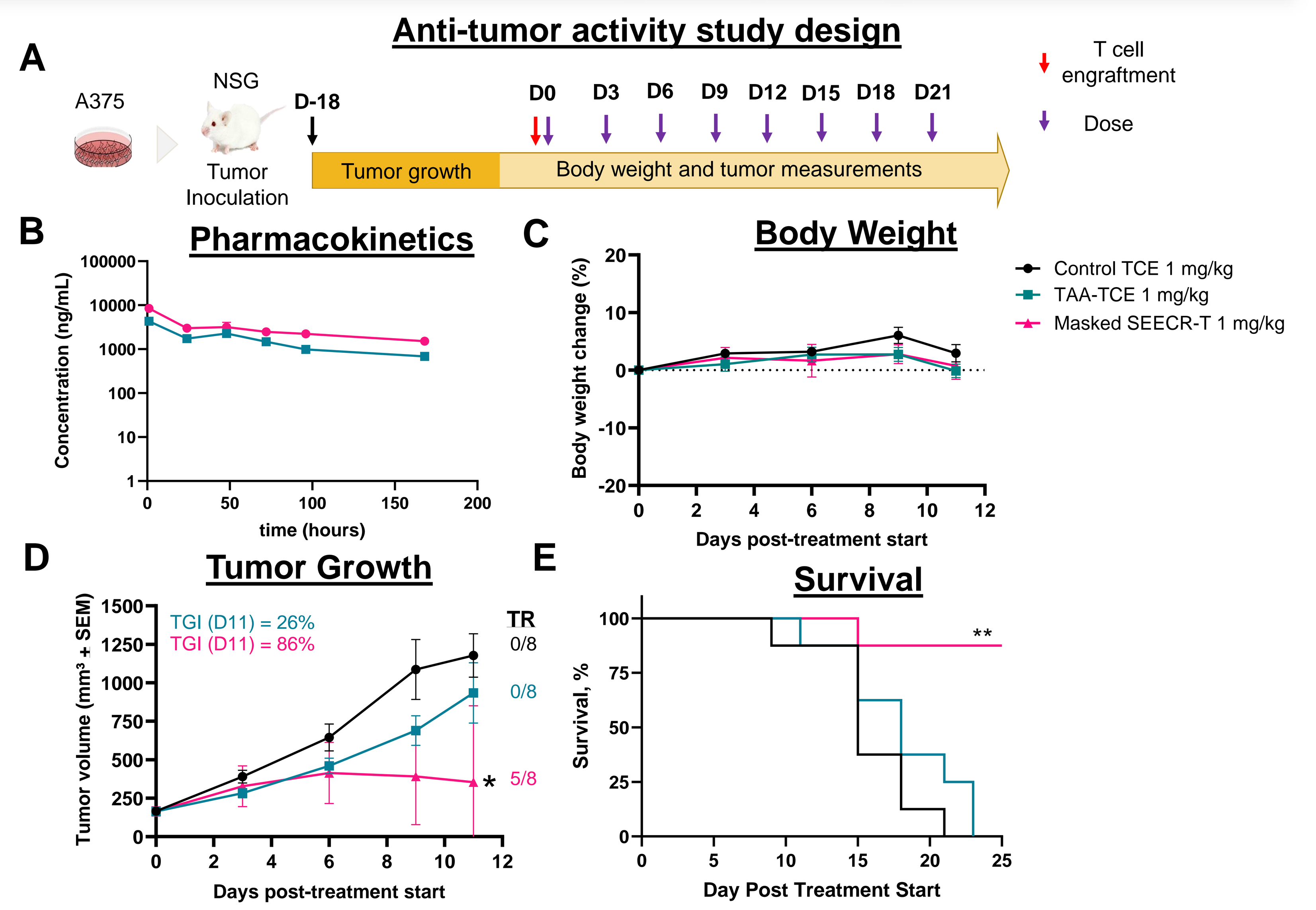
Multiple TAA-TCEs targeting different TAAs were tested for T cell activation. (**A, B, and C**) Potent activation of Jurkat NFAT Luciferase cells combined with A375 tumor cells that expressed the appropriate TAA. (**D**) Masked SEECR-T molecule that incorporated an additional costimulatory domain besides TAA and CD3 binding arms was tested in a costimulatory domain reporter assay. Masked SEECR-T molecule was activated by treatment with a protease resulting in active SEECR-T molecule. Masked SEECR-T, active SEECR-T, or costimulatory molecules alone were added to the relevant reporter gene cell line and activity was measured.

SEECR-T demonstrated durable killing activity in A375 tumor cell re-challenge assay in vitro



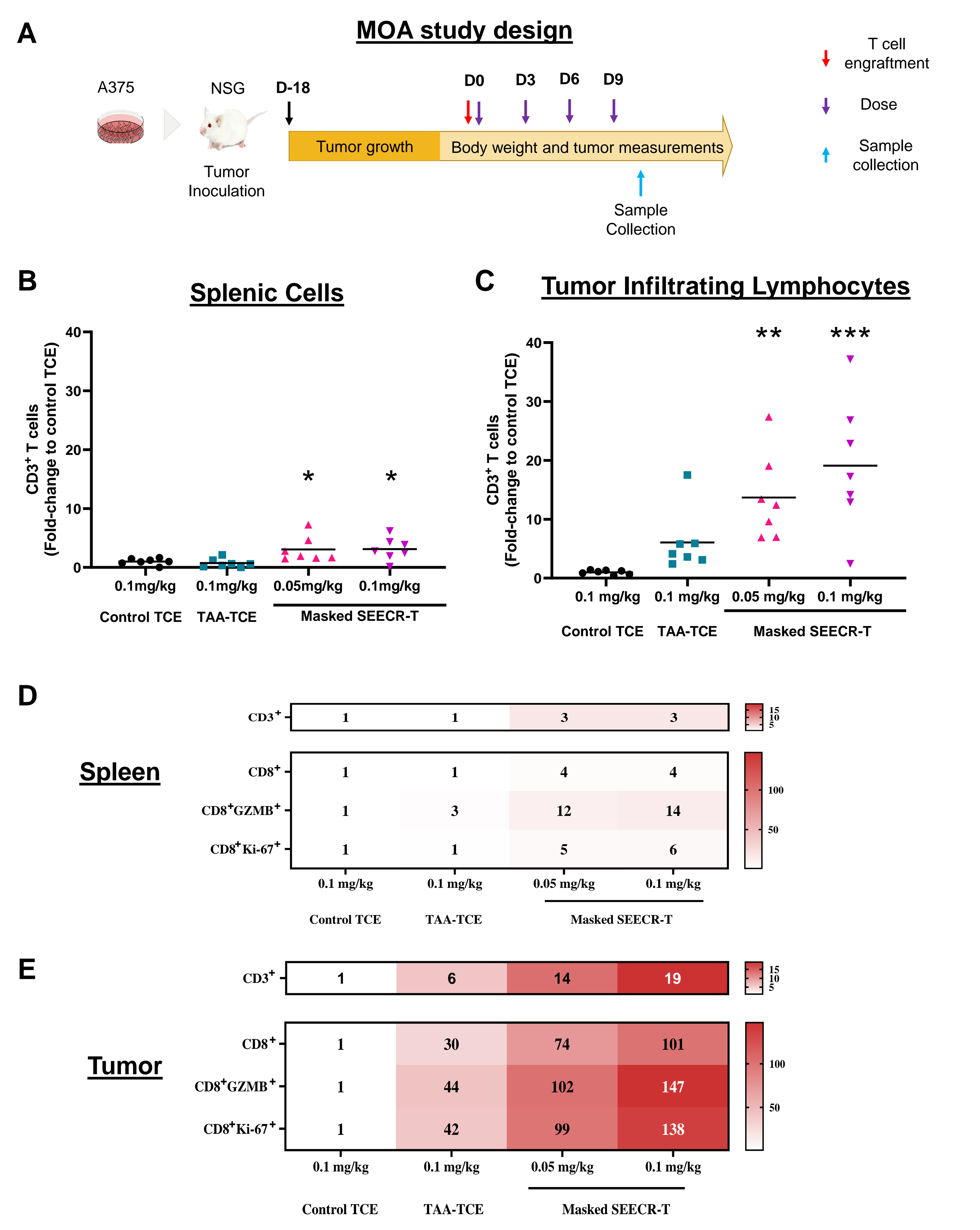
In vitro killing after repeated stimulation with SEECR-T molecules. (**A**) A375 tumor cells were plated for 4 hours followed by addition of T cells at a 2:1 E:T and test article. Cultures were maintained for 2 days; T cells were collected and killing was evaluated. T cells were counted and replated on tumor cells with fresh test article at a 2:1 E:T. This process was repeated for 3 total stimulations. (**B**) Active SEECR-T led to durable killing and (**C**) increased proliferation (Concentration Viable T cells / Original Plating Concentration Viable T cells). After cycle 3, cells were phenotyped using flow cytometry, and data were analyzed as follows: (**D**) XY-scatter plots showing the projections of 20,000 cells per sample onto the UMAP space. Each point corresponds to a cell; each panel corresponds to a different treatment group. Cell clusters were identified via *k-Means* clustering and then manually annotated based on the expression of selected markers of interest. Colors highlight different cell clusters. Arrows and text labels indicate cells belonging to clusters of interest. (**E**) After each cycle, cells were phenotyped using flow cytometry. Heatmap showing the proportion of cells belonging to *GZMB* positive cell clusters (fraction of CD8s) with respect to treatment molecules as well as the number of stimulation cycles. Color (red) intensity tracks with the fraction of CD8s positive for GZMB.

SEECR-T had antibody-like PK profile, was well-tolerated, demonstrated anti-tumor activity and prolonged animal survival



Pharmacokinetics (PK), tolerability, and anti-tumor activity of SEECR-T molecules were evaluated in the human A375 melanoma model in NSG mice engrafted with human T cells. (**A**) In the efficacy study, animals received IV doses of TAA-TCE (1 mg/kg, Q3Dx8), masked SEECR-T (1 mg/kg, Q3Dx8), or control TCE molecules (1 mg/kg, Q3Dx8). (**B**) TAA-TCE and masked SEECR-T demonstrated similar PK profiles. (**C**) All treatments were well tolerated, and no body weight loss was observed. (**D**) Masked SEECR-T molecule (IV, 8 doses) significantly inhibited tumor growth, achieving 86% TGI on Day 11 (Data presented as mean ±SEM, two-way ANOVA followed by post hoc Dunnett's test on Day 11, *P < 0.05). (**E**) The treatment with masked SEECR-T molecule improved median animal survival from 17 days to more then 27 days (Gehan-Breslow-Wilcoxon test, **P < 0.005).

SEECR-T induced tumor-selective pharmacodynamic effects in the A375 CDX tumor model



D – Spleen

	Control TCE 0.1 mg/kg	TAA-TCE 0.1 mg/kg	Masked SEECR-T 0.05 mg/kg	Masked SEECR-T 0.1 mg/kg
CD3⁺	1	1	3	3
CD8⁺	1	1	4	4
CD8⁺GZMB⁺	1	3	12	14
CD8⁺Ki-67⁺	1	1	5	6

E – Tumor

	Control TCE 0.1 mg/kg	TAA-TCE 0.1 mg/kg	Masked SEECR-T 0.05 mg/kg	Masked SEECR-T 0.1 mg/kg
CD3⁺	1	6	14	19
CD8⁺	1	30	74	101
CD8⁺GZMB⁺	1	44	102	147
CD8⁺Ki-67⁺	1	42	99	138

Systemic responses to TAA-TCE and masked SEECR-T molecules were evaluated in A375 tumor model in NSG mice engrafted with human T cells. (**A**) In the mechanism of action (MOA) study, animals received IV doses of TAA-TCE (0.1 mg/kg, Q3Dx4), masked SEECR-T (0.05 and 0.1 mg/kg, Q3Dx4), or control TCE molecules (0.1 mg/kg, Q3Dx4). Spleen and tumor samples were collected on Day 11 after treatment. (**B-C**) Flow cytometry analysis was performed to evaluate percentage of CD3⁺ T cells in spleen and tumor. The data are presented as a ratio to the control TCE. One-way ANOVA followed by Tukey's multiple comparisons test (*P < 0.05; **P < 0.01; ***P < 0.005). (**D-E**) Flow cytometry analysis was conducted to evaluate the percentage of effector CD8⁺ T cells as well as their functional activity (granzyme B expression and proliferation) in the spleen and tumor. The data are presented as ratios relative to the control TCE.

Summary

- Masked CD3 domain enabled protease-dependent activation of T cell engagement.
- SEECR-T was designed to minimize activity in peripheral tissues while activating selectively within the TME.
- SEECR-T demonstrated durable killing in an *in vitro* re-challenge tumor cell killing assay. Improvement in the effector function of SEECR-T was mediated by an increase of effector T cells.
- SEECR-T molecules demonstrated antibody-like pharmacokinetic characteristics in human A375 CDX model in NSG mice engrafted with human T cells.
- In a mouse model, SEECR-T was well-tolerated and demonstrated tumor-selective immune activation, tumor growth inhibition and improved survival.
- Based on these preclinical data, SEECR-T format may enable optionality regarding TAA-targeting, costimulatory domain, and masks.

E: effector; GZMB: granzyme B; IV: intravenously; RLU: Relative light units; RSLU: Relative subtracted light units; T: target; TAA: tumor associated antigen; TCE: T cell engager; TGI: tumor growth Inhibition; TME: tumor microenvironment; TR: tumor regression, defined as a reduction in tumor size compared to Day 0; SEM: standard error mean.