Designing a ROR1 specific CD3 bispecific T-cell Engager to decouple cytotoxicity from cytokine release while maintaining T cell motility and serial killing

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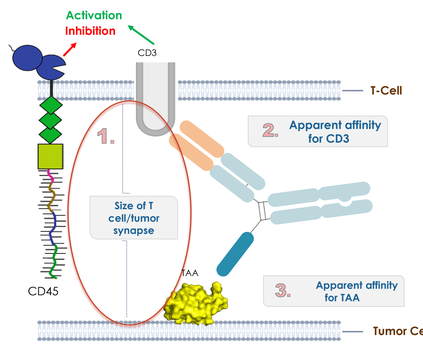
Xueyuan Zhou, Jeffrey Takimoto, Nikhita Khanna, Evren Alici, Paul Song and Brian Rabinovich

FUSEBIO THERAPEUTICS Boston, MA

Background

- T cell engagers (TCEs) can be powerful therapeutics because they directly bridge T cell to tumor cells
- Cytokine Release Syndrome (CRS) and neurotoxicity remain major adverse events
- T cell activation induced cell death (AICD) and exhaustion likely hinder durability and induction of memory
- There remains an unmet need for a format that maintains efficacy associated with current generation highly potent TCE but with a larger therapeutic window that induces durable responses and can be given in an outpatient setting
- Affinity for CD3, the TAA and the distance of the T cell/tumor synapse are key variables for modulating TCE activity
- FuseBio platform focuses on only 10 geometric formats and minor affinity tuning to quickly identify a TCE drug candidate
- TAA specific VHH with non-canonical disulfide bonds offer greater stability for developability and a modular design
- We have chosen ROR1 receptor tyrosine kinase like orphan receptor 1 (ROR1) as our first TAA to target because it is expressed broadly on both solid and liquid tumors including CLL, MCL, DLBCL, TNBC, lung cancer, head and neck cancer and ovarian cancer

How we decouple efficacy from toxicity & "exhaustion"



1. Size of the T cell/tumor synapse
   - Targeting domain on TAA
   - Geometry of TCE
   - ➢ A large dynamic synapse that partially excludes CD45 provides the optimal signal for sufficient degranulation to kill, maintain perforin/granzyme for multiple kills and prevent AICD
2. Affinity for CD3 and TAA
   - Time that synapse persists
   - ➢ Tight bond between TAA and CD3 that persists long enough to partially exclude CD45 from synapse but not long enough to reduce motility (trap the T cells), induce AICD or promote exhaustion

Limited screening of FuseBio TCE geometries allows for identification of hits with an array of decoupling ratios

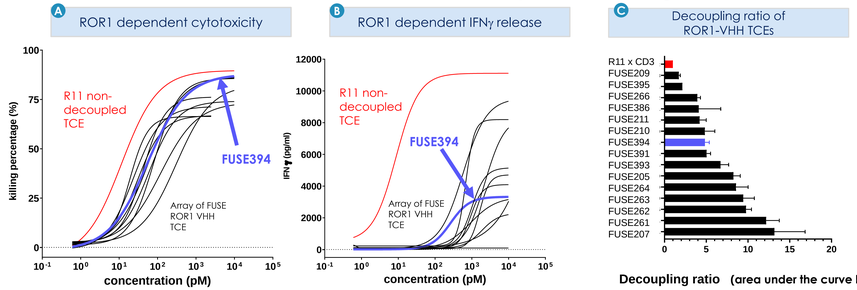


Figure 1. Screening for TCE that decouple cytotoxicity from cytokine release. ROR1 specific VHH were incorporated into multiple TCE geometries and assessed for their ability to induce PBMC mediated cytotoxicity of ROR1+ MDA-MB-231 and IFNγ release at 48 hours. The ratio of under the curve values for (A) versus (B) normalized to the R11 non-decoupled TCE is shown in C. Hit TCE's were chosen for highest maximum killing with IFNγ release that is <50% that of the non-decoupled control and a potency (EC50) that is 30 -100 fold weaker than the non-decoupled control. As such, FUSE394 was chosen as the lead TCE (blue).

Small modulations in distance geometry can have a large effect on TCE decoupling ratios

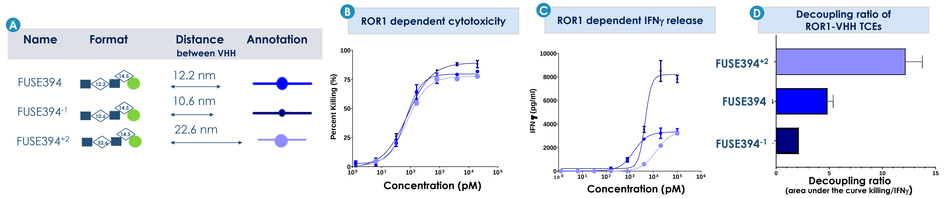


Figure 2. To determine whether small changes in TCE mediated synaptic distance between T cell and tumor cell impact the decoupling of cytotoxicity from cytokine release, variants of FUSE394 were generated. The distance between CDR3 of the first ROR1 specific VHH and anti-CD3 was 14.5 nm for all 3 variants. Compared to FUSE394, the distance between CDR3 of the second ROR1 specific VHH and FUSE394-1 and FUSE394+2 is 1.6 nm shorter and 10 nm longer, respectively (see A). The difference in length had no impact on PBMC mediated cytotoxicity of ROR1+ MDA-MB-231 (B) but was inversely correlated with IFNγ release (C). As such, compared to FUSE394, the decoupling ratios of FUSE394-1 and FUSE394+2 were ~2.5 fold lower and ~2.5 fold higher, respectively

FUSE394 decouples both the maximum and potency of cytokine release from cytotoxicity

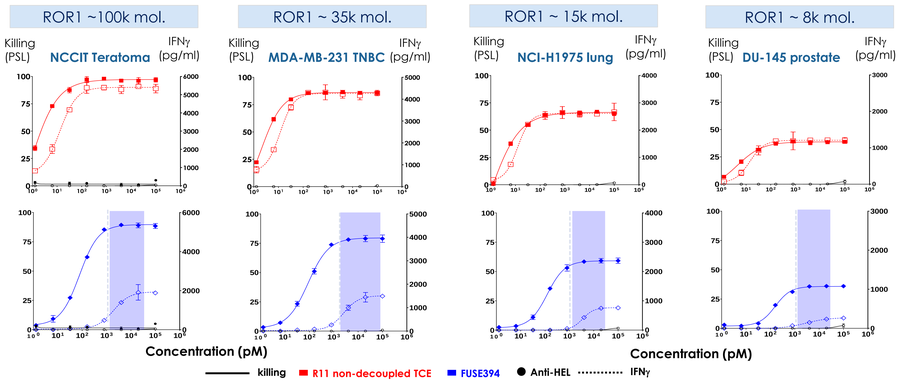


Figure 3. Parameters by which the R11 non-decoupled TCE control and FUSE394 induce PBMC mediated killing of ROR1+tumor cells and IFNγ release. Target cell lines with cell surface densities of ROR1 ranging from highest to lowest are shown from left to right. Maximum killing and IFNγ release overlapped for the R11 non-decoupled TCE control (upper row). In contrast, while maximum killing related to FUSE394 was similar to that of the non-decoupled control, both the potency and maximum IFNγ release were appreciably weaker/lower creating an "in-vitro" decoupling window of ~20,000 pM in which one can dose from little/no cytokine to maximum cytokine release while maintaining maximum killing across the full range of physiologically relevant cell surface densities of ROR1 (lower row).

FUSE394 preserves T cell motility, healthy morphology and serial killing

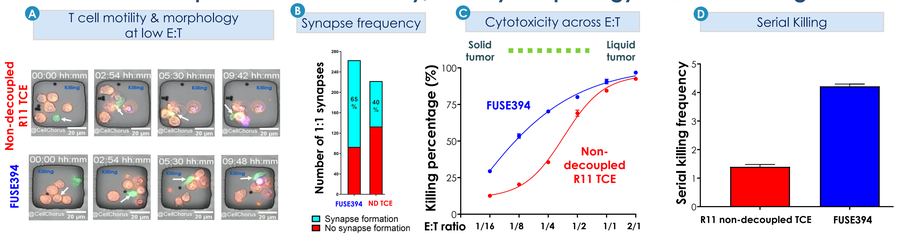


Figure 4. Expanded T cells were mixed with ROR1+ HBL-2 cells (A and B) or ROR1+ H1975 cells (C and D) at a ratio of 1:5 and a range of E:T ratios, respectively. For A and B, T cells and tumor cells were labeled green (see arrow) and red, respectively and time lapse microscopy ran for 20 hours. The non-decoupled TCE induced T cell/tumor synapses that persisted for >3 hours and were associated with T cell trapping. FUSE394 maintained T cell motility, healthy T cell morphology (A) and 50% more synapses (B) than the non-decoupled TCE. Consistent with this data, compared to the non-decoupled TCE, FUSE394 mediated a greater degree of tumor cell killing at low E:T ratios (C), which corresponded to a tumor cell killing frequency or "serial killing" of ~ 4 tumor cells/T cell in a 24 hour period (D).

FUSE394 induces the release of sufficient IFNγ to support bystander killing

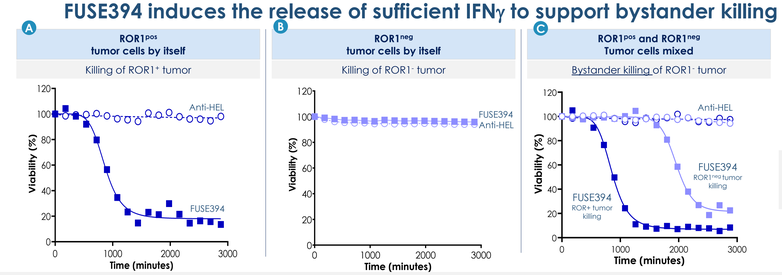


Figure 5. To assess bystander killing, ROR1+ MDA-MB-231 RFP cells (A; dark blue), T-47D ROR1neg eGFP cells (B; light blue) or a 1:1 mixture of the two (C) were co-cultured with expanded T cells in the presence of FUSE394 and viability measured over a 48 hour period. Anti-Hen Egg Lysozyme (HEL) was used as the negative control. Unless the tumors were mixed, FUSE394 induced T cell mediated killing of the ROR+ tumor only, with max cytotoxicity observed at ~20 hours. When tumors targets were mixed, we observed ROR1 independent bystander killing of the ROR1neg tumors with max cytotoxicity observed at ~ 45 hours. Bystander killing is IFNγ dependent and involves death receptor mediated apoptosis (e.g. FAS) and can play an important role in the treatment of ROR1 heterogenous tumors, a common feature of solid tumors. Similar results with ~5 hour faster kinetics were observed with the non-decoupled TCE (not shown)

FUSE394 mediates tumor growth inhibition

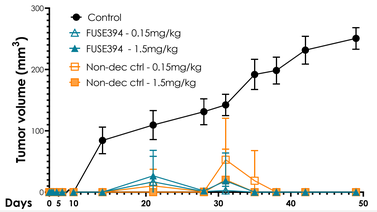


Figure 6. Tumor growth inhibition (TGI) in mice was assessed in NSG mice harboring subcutaneous ROR1+ NCCIT (human teratoma) tumors containing a 1:4 ratio of CD8+ T cells to tumor cells. Mice received two rounds of treatment (4 injections, Q48-hours at indicated dose). One between days 1-7 and the second between days 24-31. Long term TGI was observed in all groups receiving FUSE394 or the non-decoupled TCE control

Comparison of FUSE394 to ROR1 competition

	CAR-T	ADC	Standard TCE	Decoupled TCE
Optimized for Efficacy	✓	✓	✓	✓
Optimized for Safety	×	×/✓	×	✓
Potential outpatient therapeutic	×	×?	×	✓
T-cell survival/ reduced exhaustion	×/✓	NA	×	✓
Serial killing / Bystander killing	×	×/✓	✓	✓
No continuous infusion	×	✓	×	✓
Optimized for safe combination with other immunotherapies	×	×	×	✓

vs

- ✓ FuseBio's TCE platform allows for rapid/simplified identification of TCEs that decouple cytotoxicity from cytokine release and maintain T cell fitness from a small array of TCE geometries
- ✓ FUSE394 is a ROR1 specific decoupled TCE that may offer:
  - A larger therapeutic window and greater durability of anti-tumor activity compared to non-decoupled TCEs that induce activation induced cell death and cannot support immune memory
  - Greater safety for outpatient and combination therapy
  - Superior efficacy in solid tumors due to its ability to maintain T cell motility, induce serial killing while promoting sufficient IFNγ release to support bystander killing