Discovery and preclinical characterization of novel TCR-mimetic T-cell engagers targeting TERT peptide-HLA complex for the treatment of solid and hematologic malignancies

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Crossbow therapeutics™

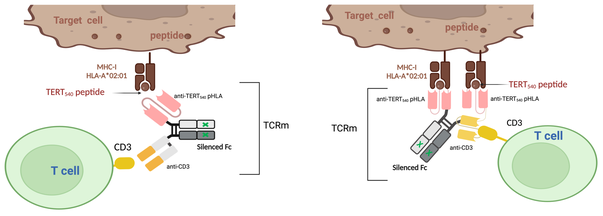
Poster #3507

Background and Rationale

- Telomerase reverse transcriptase (TERT) is an oncogenic driver that is highly expressed in 85-95% of tumors, including hematological malignancies and solid tumors
- TERT has a well-established role in tumorigenesis, and has low or undetectable expression in normal tissues, making it an attractive cancer target
- $TERT_{540}$ peptide is presented by HLA-A*02:01 on cancer cells
- Crossbow's TCR-mimetic bispecific antibodies (T-Bolt™ molecules) recognize peptide-HLA complexes and drive immune synapse formation via CD3 binding arm
- Two T-Bolt formats (1+1 and 2+1) are under evaluation and demonstrate sub-nM affinity and low-pM avidity for $TERT_{540}$ peptide-HLA complex (TERT pHLA)

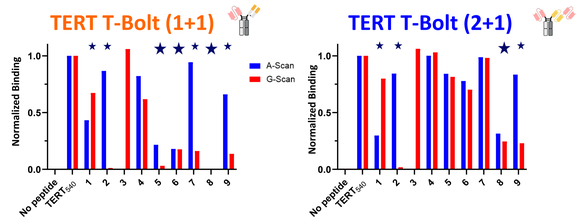
TERT T-Bolt (1+1) — One TERT pHLA binder and one CD3 binder

TERT T-Bolt (2+1) — Two TERT pHLA binders and one CD3 binder



Characteristic	Target	TERT T-Bolt (1+1)	TERT T-Bolt (2+1)
Affinity (K_D *in nM*)	TERT pHLA	~0.3	
	CD3	~5.0	
Avidity (K_D *in nM*)	TERT pHLA	N/A	~0.01

Ala/Gly Scan Identifies Multiple Residues on $TERT_{540}$ Peptide That Are Critical for Binding of T-Bolts



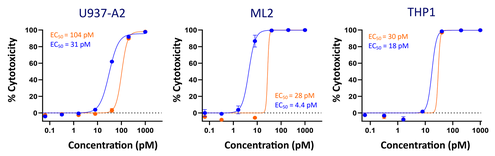
★ >=50% reduced binding compared to TERT peptide for both Ala and Gly scan
★ >=50% reduced binding compared to TERT peptide for either Ala or Gly scan

Test of T-Bolt binding to T2 cells loaded with 10 µM $TERT_{540}$ or A/G mutated peptide

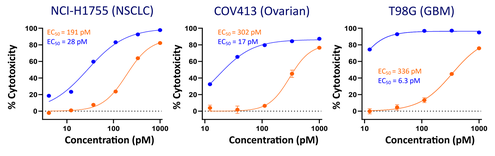
Data are normalized to the ability of the peptide to be presented by HLA-A*02:01

TERT T-Bolts Are Highly Potent Towards Target-positive Cancer Cell Lines *in vitro*

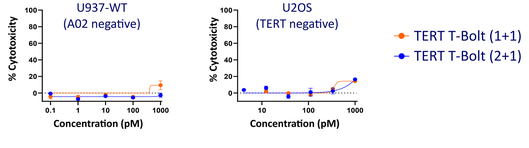
Target-positive Acute Myeloid Leukemia (AML) Cell Lines



Target-positive Solid Tumor Cell Lines

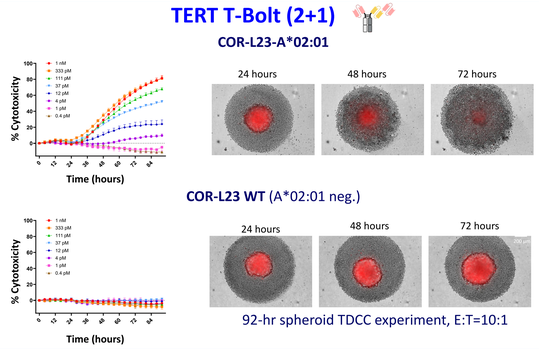


Target-negative (Control) Cell Lines



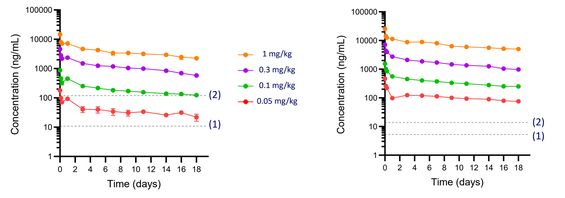
72-hr TDCC experiment combining CD3 and Target tumor cells at 10:1 ratio with dilution of T-Bolts

TERT T-Bolts Show Activity Against Solid Tumor Spheroids (COR-L23-A2, KRAS-mutant NSCLC)



92-hr spheroid TDCC experiment, E:T=10:1

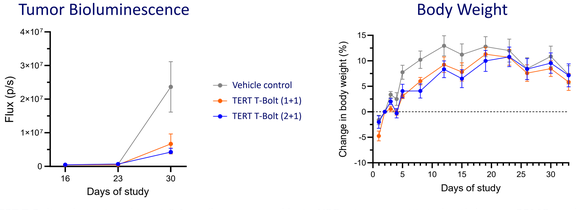
TERT T-Bolts Exhibit PK Profile Comparable to Conventional IgG-Like Antibodies



PK profile in huFcRN mice. Half-life of TERT T-Bolts is >10 days at all dose levels characterized

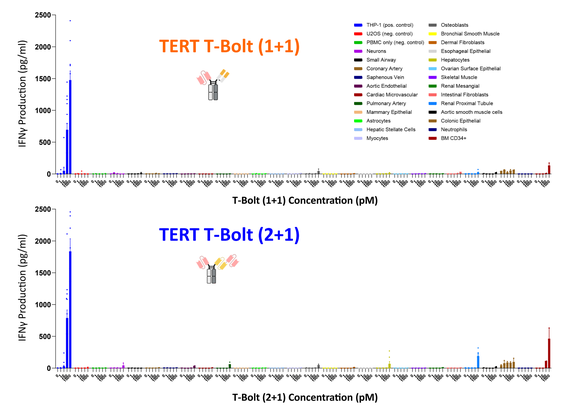
(1) Average EC_{90} for AML cell line TDCC; (2) Average EC_{90} for solid tumor cell line TDCC

TERT T-Bolts Demonstrate *in vivo* Efficacy in a Pilot Study of Disseminated AML (U937-A2) in NSG Mice



TERT T-Bolts administered i.v. at 0.1 mg/kg, once weekly, to NSG mice reconstituted with human PBMCs and injected with U937-A2 cells. Mice with fewer than 0.75% circulating human T-cells by flow cytometry were excluded from efficacy readout

TERT T-Bolts Do Not Induce IFNγ Production When Applied to a Broad Panel of Primary Cells



PBMCs were mixed with different primary cells or control cells at E:T=3:1 with T-Bolt dilution

IFNγ concentration in the supernatant was measured after 24-hr incubation

Response towards bone marrow CD34+ cells is consistent with known expression of TERT in hematopoietic progenitor cells and is being further evaluated

Summary and Conclusions

- We have generated TCR mimetics targeting the $TERT_{540}$ peptide/HLA-A*02:01 complex in a TCE format (TERT T-Bolts) and shown that they induce potent, target-dependent T-cell activation and T-cell mediated killing of TERT- and HLA-A*02:01-positive cancer cell lines
- The 2+1 format (two pHLA target-binding domains and one CD3-binding domain) results in greater potency against cancer cells, likely due to increased avidity for TERT pHLA
- Specificity profiling revealed no meaningful activity of TERT T-Bolts against T2 cells loaded with supra-physiological levels of computationally predicted HLA-A*02:01 cross-reactive peptides, or multiple unrelated HLAs (alloreactivity) (data not shown)
- Multiple studies are ongoing to define the *in vivo* and *ex vivo* efficacy of TERT T-Bolts in heme and solid tumors
- Our results highlight the therapeutic potential of TERT-targeting TCRm-based TCEs and support the continued development of TERT T-Bolts for hematologic malignancies and solid tumors