TriTAC-XR is an extended-release T cell engager platform designed to minimize cytokine release syndrome by reducing Cmax in systemic circulation

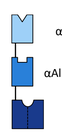
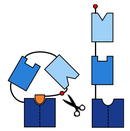
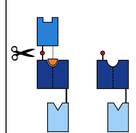
Kathryn S. Kwant, Sony S. Rocha, Katrina Stephenson, Golzar Hemmati, Timothy Yu, Raphaela Rose Banzon, Wade H. Aaron, Evan Callihan, Jessica O'Rear, Eric Bragg, Willis Kwong, Hubert Situ, Avneel Hundal, Stephen Yu, Taggra Jackson, Kevin J. Wright, Yinghua Xiao, Maria Rosalyn Dayao, Subramanian Thothathri, Linh To, Richard Austin, Holger Wesche, Bryan Lemon, S. Jack Lin.
Harpoon Therapeutics Inc., South San Francisco, CA.

Introduction

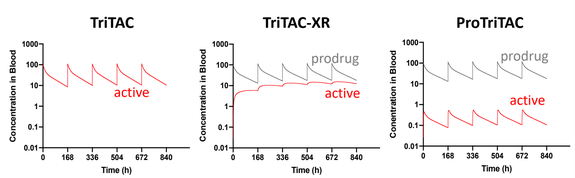
- T cell engagers are potent drugs but often require careful management of cytokine release syndrome (CRS)
- Subcutaneous dosing is a proven strategy to reduce CRS, but is limited by its increased immunogenicity risks
- Subcutaneous dosing is hypothesized to mitigate CRS by reducing the maximum drug concentration (Cmax) and preserve efficacy by maintaining the same minimum drug concentration (Cmin) as intravenous dosing
- A T cell engager dosed intravenously but designed to mimic subcutaneous PK properties could alleviate CRS without increasing immunogenicity
- TriTAC-XR molecules are engineered T cell engager prodrugs that become slowly activated in circulation by systemic proteases
- The extended-release mechanism enables slow build-up of active drug and reduced Cmax/Cmin ratio in circulation

Platform Comparison

Harpoon's T Cell Engager Platforms

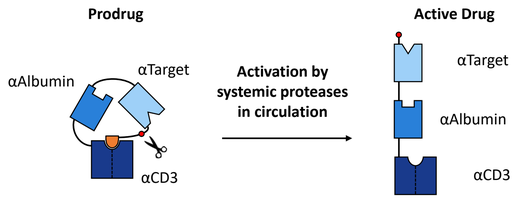
	TriTAC	TriTAC-XR	ProTriTAC
Drug Species			
Prodrug activation	N/A	Systemic proteases	Tumor proteases
Active drug half-life	Long	Long	Short

TriTAC-XR is predicted to have a distinct PK profile



Constitutively Active	Temporal control	Spatial control
• Least complex = robust • Solid tumors and heme malignancies	• Maximize systemic exposure and minimize CRS • Solid tumors, heme malignancies, and non-oncology diseases	• Minimize systemic exposure (active in tumor) • Solid tumors with broadly expressed tumor antigens

TriTAC-XR Platform Concept

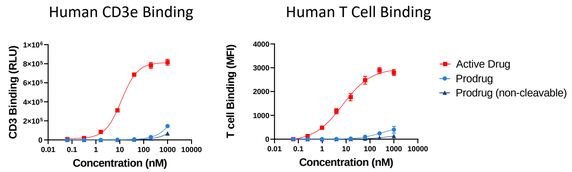


Hypothesis:

- Cmax leads to toxicity, and Cmin is required for efficacy
- Slow build-up of active drug and a reduced Cmax/Cmin ratio in circulation will reduce CRS and expand therapeutic index of T cell engagers

In Vitro Proof of Concept

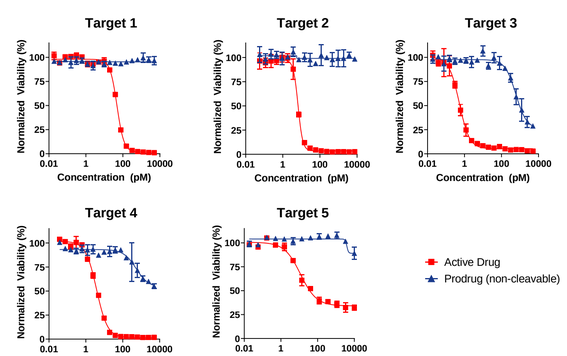
Biological activity in vitro is dependent on protease activation



ELISA was used to measure binding to recombinant human CD3e

Flow cytometry was used to measure binding to human T cells

Differential functional T cell killing observed across all targets tested



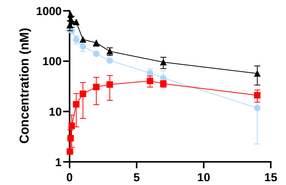
Co-cultures of resting human T cells and target cells were treated with TriTAC-XRs at 10:1 effector:target cell ratio in the presence of 15 mg/ml human serum albumin and tumor cell viability was determined after 48 or 72 hours.

Pharmacokinetics in NHP

FLT3-targeting TriTAC and equivalent TriTAC-XR were engineered for in vivo proof-of-concept experiments:

- FLT3 is a membrane-bound receptor tyrosine kinase
- FLT3 is expressed on >95% of acute myeloid leukemia (AML) blasts and on various hematopoietic cells including monocytes and myeloid precursors
- Other T cell engagers targeting AML (including FLT3) have reported significant CRS despite step-up dosing with corticosteroids in clinical testing
- All 3 binding domains of FLT3 TriTAC and TriTAC-XR are cyno cross-reactive

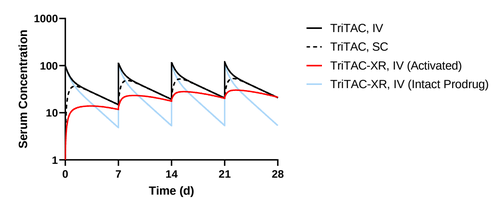
Slow build-up of Active Drug and reduction of Cmax/Cmin ratio demonstrated in a single-dose PK study in cyno monkeys



Dosing Frequency	Cmax/Cmin TriTAC-XR	Cmax/Cmin TriTAC
Weekly	1.2	10
Biweekly	2.4	28

N=2 animals each for TriTAC and TriTAC-XR at 1 mg/kg. TriTAC-XR intact prodrug is cleaved in systemic circulation, generating activated drug that slowly accumulates until reaching Cmax at 6 days post dose.

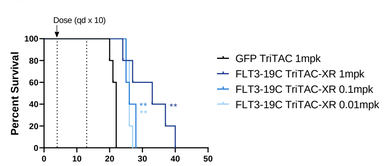
TriTAC-XR is expected to have PK profile superior to IV and SC dosed TriTAC



Weekly repeat dosing of intravenous (IV) and subcutaneous (SC) administered TriTAC and IV administered TriTAC-XR modeled from single dose NHP PK data. TriTAC-XR is predicted to have a lower Cmax (and therefore less toxicity) but similar Cmin (similar efficacy) to TriTAC dosed IV or SC.

Efficacy in Mouse

FLT3 TriTAC-XR extends survival in EoL-1 disseminated tumor model

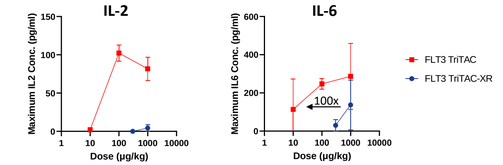


NSG mice (n=5) were implanted with EoL-1 human eosinophilic leukemia cells IV on d0 and human T cells on d2. FLT3 TriTAC-XR or non-targeting GFP TriTAC was dosed IP qdx10, starting on d4. FLT3 TriTAC-XR extended survival compared to GFP TriTAC by log-rank test (**, P<0.01).

Therapeutic Index in NHP

Expansion of therapeutic index demonstrated by assessing safety (cytokine release) and efficacy (depletion of FLT3-expressing cells) in cyno monkeys

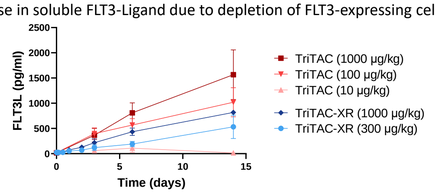
SAFTEY: TriTAC-XR offers 100x protection in cytokine release compared to TriTAC



Peak cytokine levels after a single dose of TriTAC or TriTAC-XR. TriTAC induced significant cytokines at 100-fold lower doses than TriTAC-XR.

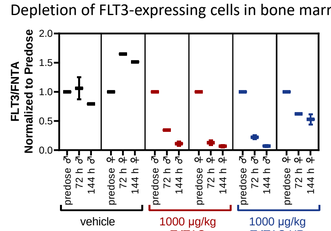
EFFICACY: TriTAC-XR has similar pharmacodynamic effects to TriTAC after single dose

Increase in soluble FLT3-Ligand due to depletion of FLT3-expressing cells



Soluble FLT3-Ligand (FLT3) is induced when FLT3 signaling is eliminated. Observed robust induction of FLT3L at 100 and 1000 µg/kg TriTAC and 300 and 1000 µg/kg TriTAC-XR.

Depletion of FLT3-expressing cells in bone marrow



RNA was isolated from bone marrow samples. RT-PCR was used to quantify FLT3 RNA levels.

Repeat dosing studies planned to more accurately compare TriTAC and TriTAC-XR efficacy in a clinically relevant setting

Summary

- TriTAC-XR is an extended-release T cell engager platform designed to mitigate cytokine release syndrome
- A single dose of FLT3 TriTAC-XR produced similar PD effects with significantly lower cytokines than a comparable TriTAC in cyno monkeys
- The expected safety improvement of TriTAC-XR could enable the treatment of non-oncology diseases in addition to solid tumors and heme malignancies

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