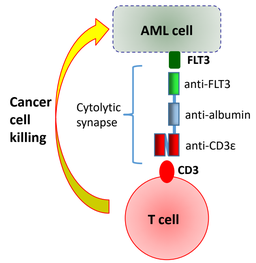
FLT3-targeting TriTACs are T cell engagers for treatment of acute myeloid leukemia

Richard Austin, Wade Aaron, Manasi Barath, Evan Callihan, Patrick Chew, Michael Cremin, Scott Gatto, Golzar Hemmati, Avneel Hundal, Che-Leung Law, Bryan Lemon, Jessica O'Rear, Purbasa Patnaik, Morgan Thompson, Lihn To, Holger Wesche, Kevin Wright, Yinghua Xiao, Stephen Yu, Timothy Yu. Harpoon Therapeutics, South San Francisco, CA

RATIONALE

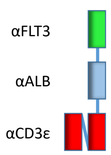
- FLT3 is membrane bound receptor tyrosine kinase
- FLT3 is expressed in various types of hematopoietic cells, including monocytes and myeloid precursors
- FLT3 RNA is present in >95% of AML samples (TCGA data)
- FLT3 mutations are oncogenic and are found in ~30% of AML[1]
- Small molecules targeting mutant FLT3, while not curative, have validated FLT3 as an AML target[1]
- A FLT3-targeting T cell engager will target wild-type and mutant FLT3



[1]Kazi & Rönnstrand Physiol Rev 2019; 99:1433–1466

BACKGROUND

Design of FLT3-targeting TriTACs



anti-FLT3 single domain antibody targets cells expressing FLT3

anti-albumin single domain antibody extends half-life

anti-CD3ε scFv engages T cells

MW ~53 kDa

Incorporated FLT3 antibodies representing 3 distinct CDR3 families (G3, G4, G8)

PRODUCTION

Process

CHO Pools (host cell used for manufacturing)

↓

Protein A chromatography

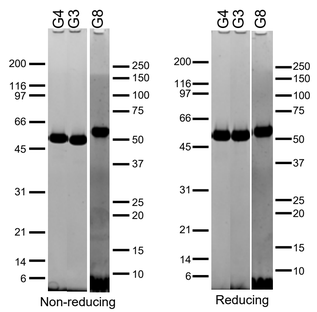
↓

Ion exchange chromatography

↓

Formulation

Purity

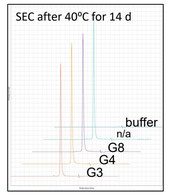


SDS-PAGE analysis of highly purified FLT3 TriTACs

BIOPHYSICAL CHARACTERIZATION

FLT3 TriTACs maintain high monomer content when stressed

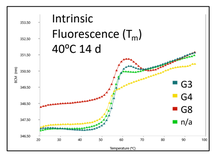
- Analytical Size Exclusion Chromatography (SEC) shows purified FLT3 TriTACs have high monomer content (T_0)
- TriTACs were stressed by 5x freeze-thaw cycles, incubation at 40°C for 14 days, or incubation at 4°C for 14 days
- Analytical SEC shows that after being stressed TriTACs maintain monomer content with no notable increase in dimer or low molecular weight species

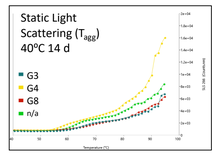


FLT3 TriTAC	% Dimer				% Monomer				% Low Molecular Weight			
	T_0	5x freeze-thaw	40°C 14 d	4°C 14 d	T_0	5x freeze-thaw	40°C 336 h	4°C 14 d	T_0	5x freeze-thaw	40°C 14 d	4°C 14 d
G3	0.9	0.9	0.9	0.9	97.9	97.9	97.4	97.9	1.2	1.2	1.6	1.2
G4	0.6	0.4	0.5	0.5	97.9	98	97.5	98	1.5	1.6	2	1.5
G8	0.9	0.7	0.9	0.6	97.3	97.7	97	97.8	1.7	1.6	2.2	1.6

Stressed FLT3 TriTAC samples maintain thermal stability

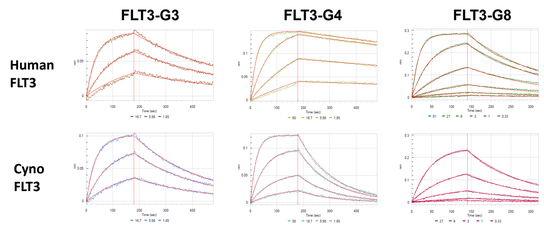
- The melting transition (T_m) and aggregation temperatures (T_{agg}) of purified FLT3 TriTACs and stressed FLT3 TriTAC samples were measured
- Across all samples, initial melting transitions were 53-57˚C with very low aggregation as exhibited by low counts detected at 266 nm





FLT3 TriTAC	T_m (C)				T_{agg}266nm (C)			
	T_0	5x freeze-thaw	40°C 14 d	4°C 14 d	T_0	5x freeze-thaw	40°C 14 d	4°C 14 d
G8	56.2	55.8	56.1	55.4	59.1	56.3	55.4	55.7
G4	56.5	55.9	55.8	56.7	58.5	55.6	56.2	55
G3	53.7	53.4	53.9	53.9	55.4	54.4	54.1	54.1

FLT3 TriTACs Bind Human and Cynomolgus FLT3

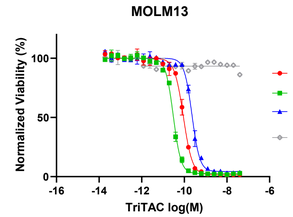


Biolayer interferometry was used to measure binding affinities for human, cyno and mouse FLT3

FLT3 TriTAC	Human KD (nM)	Cyno KD (nM)	Mouse KD (nM)
G3	1.9	2.7	7.6
G4	0.7	10	no binding
G8	7.7	10.3	16

IN VITRO PHARMACOLOGY

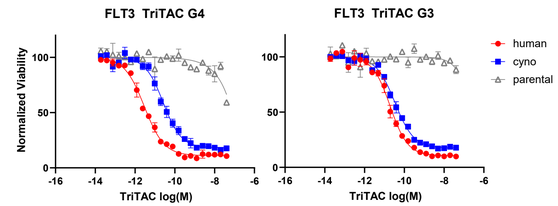
FLT3 TriTACs direct T cells to kill FLT3-expressing leukemia cell lines



Cell line	FLT3 TriTAC EC_{50} (pM) G3	G4	G8
EOL1	47	10	
MV-4-11	224	90	390
THP1	458	46	461
MOLM13	90	30	229
HL60	1518	551	5478

Co-cultures of resting human T cells and AML cells were treated with FLT3 TriTACs in the presence of 15 mg/ml HSA. Killing of the AML cells was observed after a 48-hour incubation.

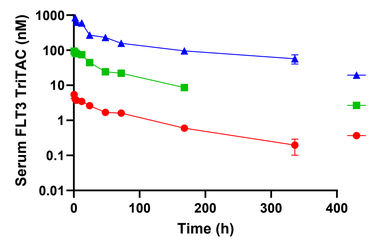
FLT3 TriTACs direct T cells to kill HCT116 cells engineered to express cyno or human FLT3



Co-cultures of resting human T cells and HCT116 cells engineered to express cyno or human FLT3 were treated with FLT3 TriTACs. After a 48-hour incubation, potent killing was observed with the cells expressing human or cyno FLT3 but not parental HCT116 cells.

PHARMACOKINETICS

A FLT3 TriTAC has a half-life of ~4 days in cynomolgus monkeys

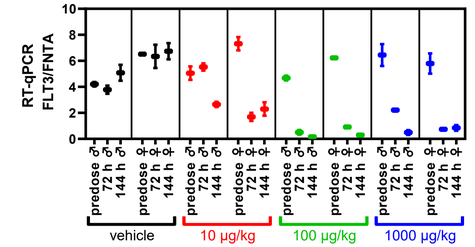


Dose (µg/kg)	T_{max} (h)	C_{max} (nM)	AUC_{168} (h*nM)	AUC_{INF} (h*nM)	Half-life (h)	Cl (µg/(h*nM))	Vz (µg/(nM))
10	0.5	5.44	279	373	85.9	0.0805	10.0
100	0.5	94.1	4520	5450	75.4	0.0555	6.06
1,000	2	846	35600	61200	147	0.0493	10.4

PHARMACODYNAMICS

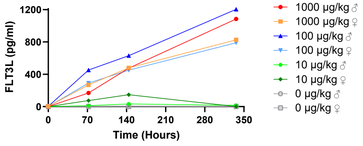
Single doses of 100 and 1000 µg/kg of a FLT3 TriTAC eliminate FLT3 cells cynomolgus monkeys and transiently induce cytokines

Elimination of FLT3-expressing cells from bone marrow



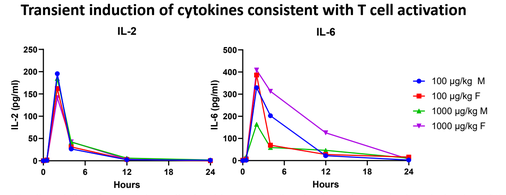
RNA was isolated from bone marrow samples collected at different timepoints after TriTAC dosing. RT-qPCR was used to quantify FLT3 RNA levels. TriTAC treatment dramatical reduced FLT3 RNA expression as expected with directed T cell killing of FLT3-expressing cells.

Increase in Soluble FLT3-Ligand owing to depletion of FLT3-expressing cells



Soluble FLT3-Ligand (FLT3) is induced when FLT3 signaling is eliminated. Observe modest increase in FLT3L in 1 of 2 subjects dosed at 10 µg/kg and robust induction of FLT3L at 100 and 1000 µg/kg

Transient induction of cytokines consistent with T cell activation



Directing T cells to kill FLT3 expressing cells should cause T cell activation; observed robust induction of IL-2 and IL-6 in response to FLT3 TriTAC dosing

FLT3 TriTACs SUMMARY

- Have robust biophysical properties with high monomer content
- Bind human and cynomolgus FLT3, CD3ε, and albumin
- Redirect T cells to kill FLT3 expressing cells in vitro
- Eliminate FLT3 expressing cells in cynomolgus monkeys and are well tolerated after a single dose
- FLT3 TriTACs represent promising drug candidates for treating AML, a disease with significant unmet medical need

Abstract 2643 AACR Annual Meeting 2021