TriTACs are novel T cell-engaging therapeutic proteins optimized for treatment of solid tumors and long serum half-life

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ABSTRACT

T cell engagers are antibody-derived therapeutics that transiently tether T cells via the T cell receptor complex (TCR) to surface antigens on tumor cells. This leads to activation of T cells and redirected lysis of the attached target cell. The therapeutic potential of this modality was demonstrated by Blincyto (blinatumomab), a CD19/CD3-bispecific T cell engager approved for the treatment of adult patients with relapsed/refractory acute lymphoblastic leukemia. Despite success of this T cell-engaging therapy in a hematological malignancy, clinical studies in solid tumors with other T cell engagers have been less encouraging so far.

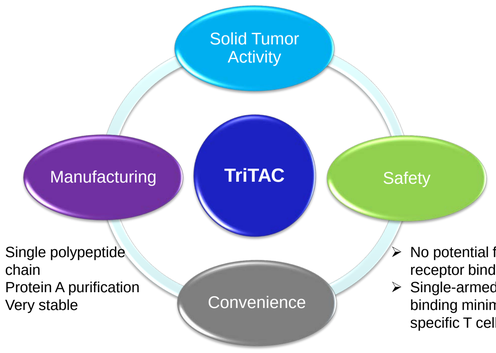
The TriTAC (Tri-specific T cell Activating Construct) platform was developed to address shortcomings of existing T cell engagers, including short serum half-life, limited tissue penetration, and suboptimal activity. TriTAC constructs are made of a single polypeptide designed to bind to a cancer surface antigen, the CD3 epsilon subunit of the TCR, and to human serum albumin. CD3 is bound by a single-chain variable fragment (scFv) while both tumor-targeting and albumin-binding is achieved by single domain antibodies. The latter allow TriTACs to be very small, stable, and easily produced and purified. Non-covalent binding to serum albumin has been validated as an effective way to extend the serum half-life of other proteins up to several weeks. Even though TriTACs have three binding domains, their overall size is only ~50 kDa, one third of the size of a monoclonal antibody. This is expected to allow for faster diffusion into human tumor tissues than is possible with antibodies given the high interstitial pressure and dense extracellular matrix in solid tumors.

TriTACs can induce T cell to kill tumor cells in vitro at single-digit picomolar to femtomolar concentrations with concomitant induction of inflammatory cytokine release and T cell proliferation. TriTACs can diffuse much faster across an extracellular matrix than antibodies, and eradicate tumors in mouse xenograft models supplemented with human T cells. In non-human primates, TriTAC molecules have serum half-lives of approximately 4 days, and appear well tolerated.

BACKGROUND

Harpoon's next generation T cell engager platform is optimized for the treatment of solid tumors

- Small size - Best for diffusion-controlled solid tumor penetration
- Optimized CD3 binding to address T cell-mediated clearance



- Single polypeptide chain
- Protein A purification
- Very stable
- No potential for Fc receptor binding
- Single-armed CD3 binding minimizes non-specific T cell activation
- Extended serum half-life

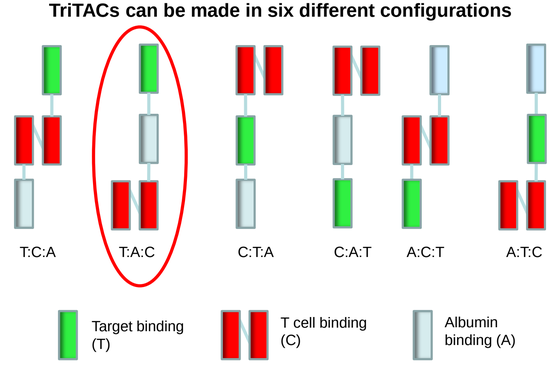
PLATFORM ENGINEERING

TriTACs incorporate single domain antibodies and use albumin binding for half-life extension

Single domain antibodies (sdAbs):
- Derived from camelid immunizations or synthetic library screening
- Small size (12.5 kDa)
- Exceptional thermal stability

Albumin binding:
- Enables FcRn-mediated serum half-life extension
- Non-covalent binding to albumin enables antibody-like half-life without the need to incorporate the constant regions of antibodies
  - Enables small, flexible molecules
  - Avoids low affinity Fc Receptor binding

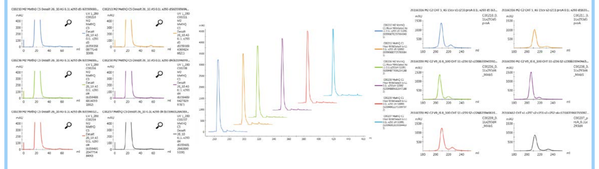
TriTACs can be made in six different configurations



- Domains are connected with G_4SG_3S linkers
- Shorter linkers negatively impact protein yields

TriTACs can be purified in a scalable process in all six configurations

CM → Protein A affinity → Desalt → Multimodal chromatography (CHT Type I) → Analytical HPLC SEC -/+ SDS-PAGE → Fraction pooling → Desalt (formulation)



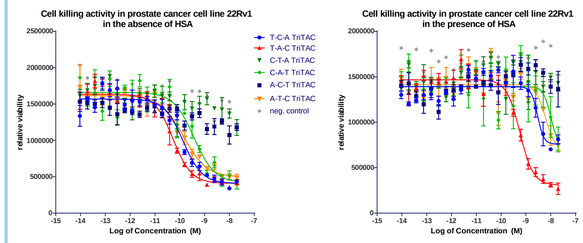
- TriTACs expressed in 293 or CHO cells, purified by Protein A
- Desalt profiles of TriTACs after Protein A purification
- CHT multi-modal chromatography profiles of TriTACs after Protein A and desalting
- CHT main peak pooled, final formulated yields ranging from 130-190 mg/L from transiently transfected 293 cells

T:A:C IS THE PREFERRED TRITAC ORIENTATION

EGFR targeting TriTACs in T:A:C configuration have potent cell killing activity in the presence of HSA

Description	NCI-1563 EC50 Run A [pM]	NCI-1563 EC50 Run B [pM]	NCI-1563 EC50 Run A 15 mg/ml HSA [pM]	NCI-1563 EC50 Run B 15 mg/ml HSA [pM]	NCI-1563 HSA Shift Run A	NCI-1563 HSA Shift Run B	CAPAN2 No HSA [pM]	CAPAN2 15 mg/ml HSA [pM]	CAPAN2 HSA Shift	22Rv1 EC50 [pM]	22Rv1 HSA Shift
EGFR-scFv:αCD3	1.7	1.3	3.4	1.7	2.0	1.3	1.6	1.1	0.7	7.5	1.3
EGFR-scFv:αCD3:αAlb	1.7	1.6	32	13	18.3	7.8	1.7	8.2	4.7	9.2	10.0
G8:αCD3:αAlb	1.3	1.3	45	36	35.4	28	1.3	9.7	7.3	7.2	10.9
G8:αAlb:αCD3	1.4	1.1	17	9.1	12.3	8.7	1.6	7.3	4.7	7.4	5.1
αCD3:G8:αAlb	5.6	3.0	120	44	20.4	14.7	3.2	2.3	7.3	2.1	12.9
αCD3:αAlb:G8	5.5	5.5	200	110	36.2	19.7	9.3	8.6	9.2	3.7	18.7
αAlb:G8:αCD3	6.9	4.9	560	210	81.5	43.5	6.3	71	11.3	43	18.7
αAlb:αCD3:G8	6.1	4.3	280	93	45.6	21.6	5.1	69	13.5	54	11.3

The configuration T:A:C is preferred for PSMA-specific TriTACs



	αPSMA:αCD3:αAlb	αPSMA:αAlb:αCD3	αCD3:αPSMA:αAlb	αCD3:αAlb:αPSMA	αAlb:αPSMA:αCD3	αAlb:αCD3:αPSMA
EC50 (no HSA) [pM]	138	53	inactive	374	140	inactive
EC50 (with HSA) [pM]	2,976	573	inactive	10,002	6,185	inactive
HSA Shift	22x	11x		27x	44x	

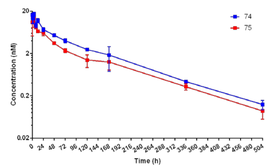
Experiment performed with tool TriTAC of low affinity for PSMA

CD3 affinity is optimized for maximum activity and exposure

TriTAC 75 and 74 differ 5x in their affinity for CD3, resulting in 2x to 10x reduction in cell killing EC50s.

		TriTAC 75	TriTAC 74
Affinity measurements (M)	Kd hu tumor target	5.9E-10	6.0E-10
	Kd cy tumor target	3.2E-09	
	Kd huCD3E	1.0E-08	5.2E-08
	Kd huALB	5.9E-09	6.5E-09
Binding in 50% Cyno Serum (M): measured by MSD (ELISA-like) assay	no serum	1.4E-08	2.1E-08
	50% cyno serum	5.3E-09	3.0E-08
Cyno Serum Stability / HSA Shift (M): measured using SKOV3 TDCC	untreated	2.3E-12	2.0E-11
	untreated + huALB	1.3E-11	9.5E-11
	Pre-treated 2 days 37 cyno serum	2.1E-12	6.8E-12
Serum stability and activity in cyno serum (M): measured by OVCAR TDCC, assay run +/- 20% cyno serym	untreated	1.4E-12	1.6E-12
	untreated, assay with cyno serum	4.1E-12	3.2E-11
	Pre-treated 2 days 37 cyno serum, assay with cyno serum	2.7E-12	1.9E-11

5x reduction in CD3 affinity leads to approximately 30% to 50% increases in AUC.

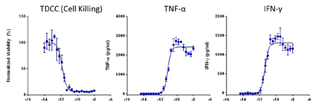


TriTAC	Terminal t1/2 (h)	AUC, 0-last (h*nM)	AUC, 0-inf (h*nM)	Clearance (ml/h/kg)	Vss (ml/kg)
74	84.9	1030	1050	0.367	36.8
75	89.4	715	727	0.522	54.2

- Dosed 0.02 mg/kg IV bolus
- Serum levels measured using MSD assay with anti-idiotype antibodies (mean, n=2)

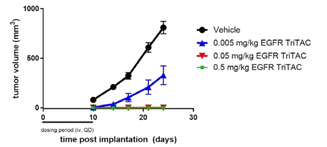
TRITACS ARE POTENT AND EFFICACIOUS

EGFR targeting TriTACs can engage and activate T cells to kill EGFR expressing cells with single digit pM EC50s *in vitro*.



	TDCC EC50 (pM)	TNFα EC50 (pM)	IFNγ EC50 (pM)
EGFR TriTAC	1.3	8.0	4.4

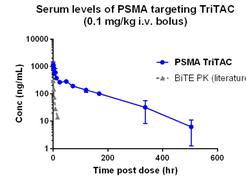
EGFR targeting TriTACs elicit anti tumor activity *in vivo at* doses as low as 5 µg/kg.



HCT116 human colorectal cancer xenograft study in irradiated NOD/SCID mice reconstituted with resting, primary human PBMC mixed at 1:1 ratio with cancer cells

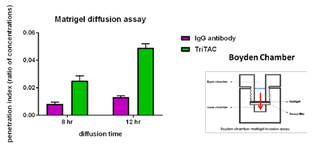
PHARMACOKINETICS

TriTACs have half-lives of 80 to 90 h in cynomolgus monkeys



- Albumin binding domain appears well suited to prolong serum half-life of TriTACs in NHPs
- Terminal serum half-life at 0.1 mg/kg TriTAC dose is 85-95 h (likely consistent with weekly dosing interval in humans)
- Half-life of Blincyto in humans is only 2 hours*

TriTACs have PK properties similar to antibodies, but can migrate through extracellular matrixes much more efficiently.



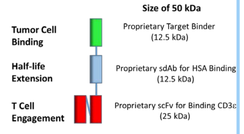
* Journal of Clinical Oncology. 2013 ASCO Annual Meeting Abstracts. Vol 31, No 15_suppl (May 20 Supplement), 2013: 3048

SUMMARY

TriTACs are the smallest, half-life extended T cell engager format in the industry, optimized for the treatment of solid malignancies

- Very potent and efficacious *in vitro* and *in vivo*
- Combine the advantages of IgG based approaches (long half-life, manufacturability and stability) with the hallmarks of the pioneering BiTE molecules (small size, good tissue penetration and high degree of flexibility)

Two TriTAC programs are expected to enter the clinic within the next 12 months (see AACR posters 1773 and 1781).



Abstract 3814 AACR Annual Meeting 2018