CD45 targeted engineered toxin bodies deplete hematopoietic and malignant cells

Aimee Iberg, Garrett L. Robinson, Sara LeMar, Joseph D. Dekker, Jay Zhao, Hilario J. Ramos, Melissa M. Singh, Erin K. Willert - Molecular Templates Inc., Austin, TX.

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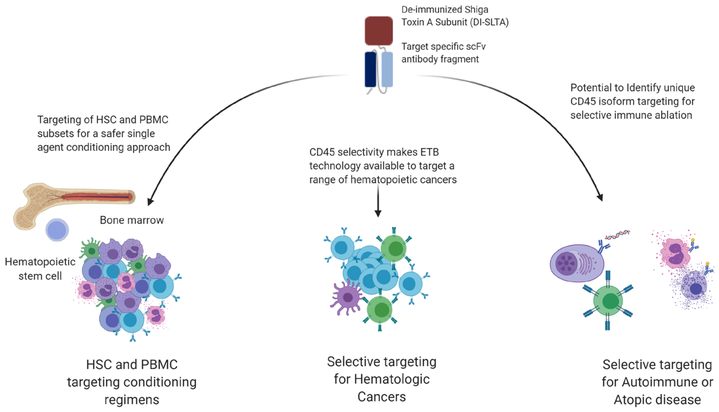
CD45 targeted ETBs are designed for multiple hematologic relevant indications

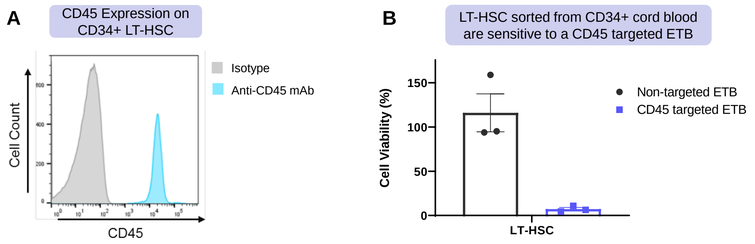


CD45 targeted Engineered toxin bodies (ETB) - designed to deplete *CD45* positive cells across indications

- Unique Shiga-like toxin payload forces internalization to deliver intracellular cytotoxicity via ribosomal inhibition
- Single fused molecule - de-immunized with short half-life to promote enhance activity and limited *in vivo* toxicity
- CD45 ETBs can be employed therapeutically across a range of indications:
  - ETBs are designed to eliminate genotoxic effects of standard conditioning regimens to improve patient safety
  - Modular scaffold allows flexible design and potential for selective targeting of CD45 sub-populations
  - Opportunity for benefit across hematologic disease indications

Specificity: Targeting via antibody fragment domain; potential for broad or specific CD45 isoform coverage
Potency: Direct cell-kill via enzymatic and irreversible inactivation of ribosomes mediated by de-immunized SLTA
Modular scaffold: Bivalent targeting offers potent cytotoxicity – options for biparatopic and VHH
Small size: Unique PK profile and tissue penetration for a safe single agent option

CD45 targeted ETBs deplete primary human HSC *ex vivo*



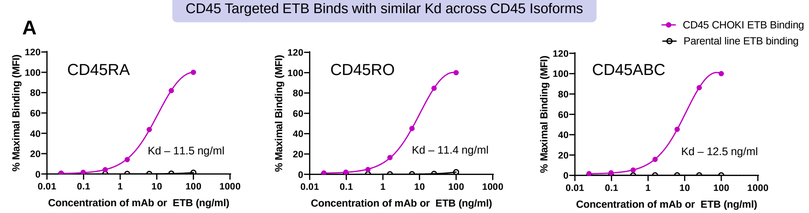
CD34+ LT-HSC were sorted from pooled donor cord blood samples and surface CD45 expression was determined by flow cytometry **(A)**

Sorted LT-HSC were expanded in stem cell factor and ETB potency was evaluated at 96 hours after addition of the CD45 targeted or non-targeted ETB control to cells using Cell Titer-Glo® viability assay (Promega) (**B**).

CD45 targeted ETB displays potent cytotoxicity against human HSC populations in an in vitro assay

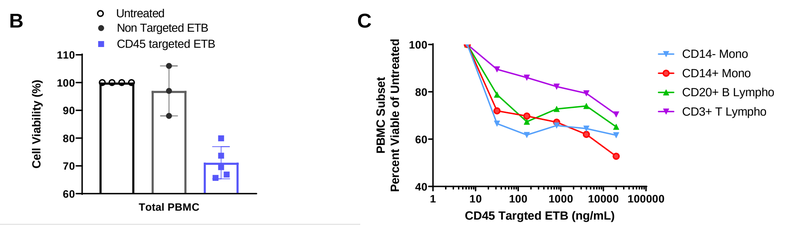
CD45 targeted ETBs kill human PBMC subsets *ex vivo*

CD45 Targeted ETB Binds with similar Kd across CD45 Isoforms



CD45 isoforms were expressed on engineered cell lines (RA, RO, ABC). To evaluate the specificity of the CD45 targeted ETB to CD45 isoforms, ETB binding to engineered lines was evaluated by flow cytometry and Kd curves were calculated for comparison (A).

Human PBMCs are targets for CD45 ETB depletion



Human donor PBMCs were treated with non-targeted ETB or CD45 targeted ETB and total CD45+ (**B**) or specific immune cell subsets (**C**) were monitored for depletion at 48 hrs. in a flow cytometry-based cell kill assay.

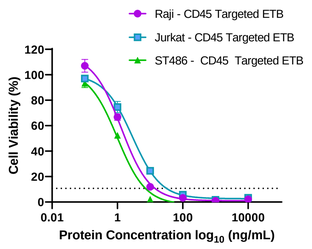
CD45 targeted ETBs bind to major CD45 isoforms and deplete human PBMC immune subsets from human donors ex vivo

CD45 Targeted ETBs kill hematologic tumor cell lines *in vitro*

		IC50 (ng/mL)	CD45 Flow Cytometry	
Indication	**Cell Line**	**CD45 Targeted ETB**	**% Pos**	**MFI Positive Cells**
High Percentage and High signal for CD45 expression				
B-cell lymphoma (Burkitt's)	ST486	2.2	99.8	17,223
B-cell lymphoma (Burkitt's)	Daudi	58.2	99.8	47,052
B-cell lymphoma (Burkitt's)	Raji	38.9	99.9	40,291
B-CLL	MEC-2	41.2	100	41,570
Hodgkin lymphoma (B-cell)	L1236	2.6	93.3	20,293
T-cell lymphoma	Jurkat	12.6	99.9	31,065
Myeloma	MC/CAR	52.9	100	30,693
AML (eosinophilic)	EOL-1	102.9	99.7	15,431
Low Percentage or Low signal for CD45 expression				
AML	OCI-AML-5	>20,000	22.7	22,097
AML	MOLM-14	>20,000	22.9	19,749
myeloma	H929	>20,000	65.3	1,069
epithelial	HEK-293	>20,000	2.3	153

Cytotoxicity of a CD45-targeted ETB was measured 96 hours after addition to cells using Cell Titer-Glo®(Promega) and compared to surface expression of CD45 by flow cytometry

CD45 targeted ETB shows potency across hematologic tumor lines

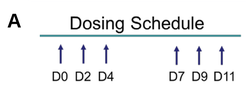


Cytotoxicity of various CD45-targeted ETBs was measured 96 hours after addition to cells using Cell Titer-Glo®(Promega)

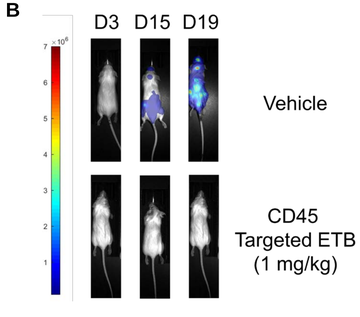
CD45 ETB selectively targets a range of hematopoietic cancer cells with high surface expression of CD45

CD45 Targeted ETBs deplete hematologic cancers *in vivo*

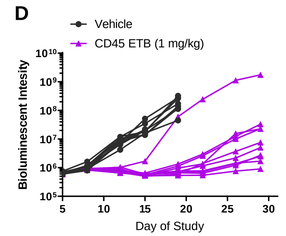
CD45 targeted ETB administration schedule at 1 mg/kg over two cycles

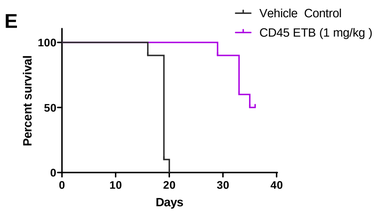


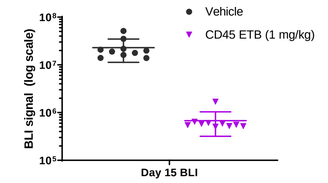
CD45 targeted ETB controls tumor progression



CD45 targeted ETB controls tumor progression prolongs survival over time







CB.17 SCID Mice were challenged with a Raji-luc lymphoma cell line in a 3-week disseminated tumor model. Mice were administered a CD45 targeted ETB at 1 mg/kg dosed every 3 days for two consecutive weeks (**A**). Tumor bearing mice were evaluated for tumor growth over time via bioluminescence imaging (**B-D**) and survival after treatment with a CD45 targeted ETB (**E**).

A CD45 targeted ETB can control systemic growth and increase survival in an in vivo lymphoma xenograft model

CONCLUSIONS

A CD45 targeted Engineered Toxin Body approach for depletion of immune subsets

- CD45 targeted ETBs are a unique therapeutic approach designed to eliminate hematopoietic derived cells across an array of disease indications
  - CD45 targeted ETBs can deplete LT-HSCs *ex vivo*
  - Cells from different cancer indications are efficiently killed *in vitro* by a CD45 ETB that is capable of recognition of all CD45 isoforms
  - CD45 targeted ETB can deplete human peripheral blood mononuclear cell subsets
  - CD45 targeted ETBs demonstrate good efficacy against hematologic cancer *in vivo*
- Antibody discovery campaigns have the potential to direct ETBs to specific isoforms of CD45 for refinement of indications including various cancers and autoimmune diseases

Website: www.mtem.com; Contact: bd@mtem.com; Hilario.ramos@mtem.com

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