C-KIT/CD117 targeted ETBs for cancer therapy and HSC transplant conditioning

Caleigh Howard, Shu Wiley, Wenzhao Dong, Andrea Mendiola, Veronica Partridge, Antonio Luz, Sara LeMar, Paul Amador, Amit Kumar Chaudhary, Joseph D. Dekker, Jay Zhao, Ross Durland, Aimee Iberg
Molecular Templates Inc., Austin, TX.

AACR Annual Meeting 2022
Permanent Abstract Number 335

Engineered Toxin Bodies (ETBs)

Engineered Toxin Bodies are fusion proteins consisting of an antibody fragment genetically fused to a proprietary de-immunized form of the Shiga-like toxin A subunit (SLTA). When the antibody fragment portion of the ETB binds its target, the SLTA portion of the ETB induces internalization into the cell, routing to the cytosol, and cell cytotoxicity through enzymatic depurination of 28S rRNA and irreversible ribosomal inactivation.

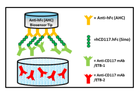
CD117 (c-KIT) is a type III receptor tyrosine kinase that regulates apoptosis, cell differentiation, proliferation, chemotaxis, and cell adhesion upon activation by its ligand, the stem cell factor (SCF). CD117 is overexpressed in a high percentage of certain cancers including gastrointestinal stromal tumor (GIST), small cell lung cancer (SCLC), and acute myeloid leukemia (AML). While tyrosine kinase inhibitors such as imatinib are effective therapies for CD117-mutant GIST, resistance often occurs by the development of secondary mutations in the intracellular signaling domain and CD117 gene amplification. **ETBs provide a new strategy to overcome drug resistance by targeting the extracellular domain of CD117 to deliver SLTA into the cells and induce cell apoptosis independent of CD117 mutational status.** Thus, ETBs offer a novel mechanism of action compared to existing treatments for relapsed or treatment-refractory CD117+ cancers.

CD117 is also a well-known marker of hematopoietic stem and progenitor cells (HSPCs). Antibody-based therapy targeting CD117 has shown effective depletion of host HSPCs to enable donor hematopoietic stem cell engraftment in preclinical models. Importantly, CD117 targeting depletes the progenitor pool while leaving intact the mature immune populations, thus sparing patients from temporary immunosuppression. ETBs present advantages over antibody approaches due to a relatively short half-life that enables rapid transplant post conditioning and exquisite target specificity while utilizing a non-genotoxic payload.

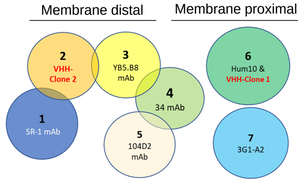
ETB Designed For CD117 Targeting

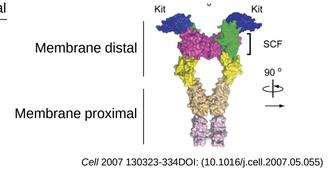
Two novel llama heavy chain antibody fragments (VHHs) bin to distinct CD117 epitopes

Supplier	Molecule Type	Description	CD117 KD (M)	Unique Bin	AB#	Reponse of Competing Molecule (nm shift)								
						1	2	3	4	5	6	7	8	9
Caprico Bio	mAb	SR-1 (BA7.3C.9)	1.29E-09	1	1	0.02	0.03	0.39	0.39	0.36	0.16	0.27	0.21	0.02
MTEM	ETB	VHH-Clone2	1.29E-09	2	2	0.15	0.19	0.15	0.25	0.24	0.01	0.18	0.12	-0.03
ThermoFisher Sci	mAb	YB5.B8	1.66E-07	3	3	0.38	-0.07	0.04	0.07	0.33	0.13	0.24	0.19	0.00
Sino Biological	mAb	34	1.42E-08	4	4	0.36	0.18	0.02	0.03	0.02	0.14	0.26	0.20	0.01
Biolegend	mAb	104D2	3.10E-09	5	5	0.41	0.20	0.42	0.03	0.02	0.15	0.27	0.21	0.02
MTEM	scFv	Hum10	7.28E-10	6	6	0.28	0.20	0.31	0.28	0.27	0.02	0.08	0.11	-0.03
MTEM	ETB	VHH-Clone1	1.32E-09		7	0.24	0.16	0.27	0.25	0.23	0.00	0.04	0.13	-0.03
MTEM	scFv	3G1-A2	3.70E-12	7	8	0.31	0.33	0.35	0.31	0.31	0.05	0.22	0.02	0.01
MTEM	Off Target ETB	Frp5	NB	N/A	9	0.37	0.19	0.31	0.23	0.27	0.15	0.25	0.18	0.02



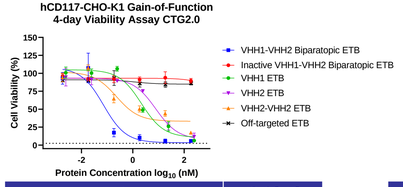
Key
Bidirectional Blocking
Unidirectional Blocking
No Binding
Isotype Control
Self-Self Competition

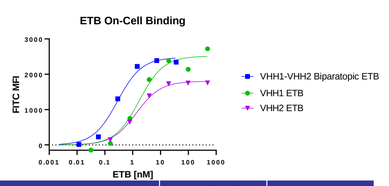




Cell 2007 130323-334DOI: (10.1016/j.cell.2007.05.055)

Biparatopic ETB demonstrates improved binding affinity and potency compared to related single VHH ETBs





ETB	IC50 [nM]
VHH1-VHH2 Biparatopic ETB	0.072
Inactive VHH1-VHH2 Biparatopic ETB	---
VHH1 ETB	2.2
VHH2 ETB	7.9
VHH2-VHH2 ETB	0.23
Off-targeted ETB	---

ETB	KD [nM]	Bmax
VHH1-VHH2 Biparatopic ETB	0.29	2477
VHH1 ETB	1.7	2514
VHH2 ETB	1.4	1802

ETB binding to hCD117-CHO-K1 cells was measured by flow cytometry using an ETB detection antibody. Inactive ETBs contain two mutations that abolish SLTA enzymatic activity.

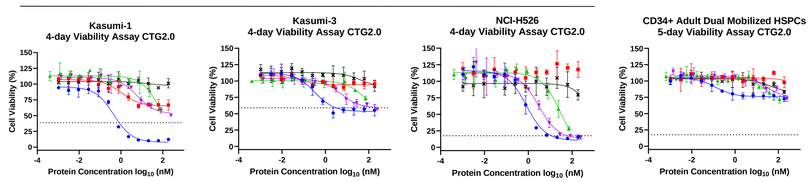
ETB Potency on Cancer Cell Lines and Primary CD34+ HSPCs

Cancer cells express more CD117 receptors per cell compared to HSPCs

Cell Line	Origin	CD117 Genotype	Approximate # Receptors Per Cell
Kasumi-1	AML	Het mutant	25,000
Kasumi-3	AML	WT	31,000
NCI-H652	SCLC	WT	11,000
CD34+ HSPCs	Normal adult	WT	3,000

CD117 expression on various cell lines was measured by flow cytometry. Saturating levels of CD117 PE-antibody were bound to live cells at 4°C. BD Quantibrite Beads are run during the acquisition to convert gMFI to receptors per cell.

Biparatopic ETB displays picomolar potency on cancer cell lines and HSPCs, but with reduced efficacy (depth of kill) on HSPCs

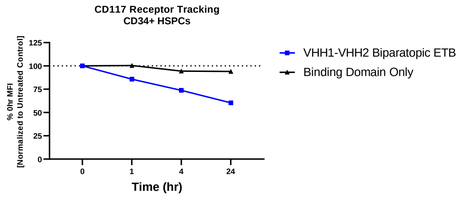


Cell Line	Biparatopic ETB IC50 [nM]	%Viability at Max Dose
Kasumi-1	0.45	12
Kasumi-3	0.33	57
NCI-H652	0.82	13
CD34+ HSPCs	0.13	73

Cell viability was measured 96-120 hours after ETB addition using Cell Titer-Glo®(Promega). CD34+ HSPCs were cultured in the absence of SCF. The dashed line on each graph represents the RLU value of cells at time 0h.

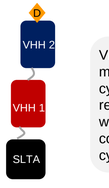
ETB Drug Conjugation Improves Overall Efficacy

The SLTA domain of the ETB induces more CD117 internalization vs. binding domain alone in CD34+ HSPCs

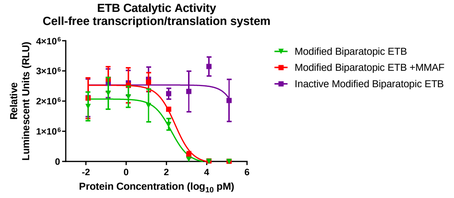


ETB or the equivalent VHH1-VHH2 binding domain only (without SLTA) was bound to live CD34+ HSPCs at 4°C, then warmed to 37°C. At various times, the cells were harvested and fixed. CD117 remaining on the cell surface was detected with a non-competitive CD117 antibody and measured by flow cytometry.

MMAF payload conjugation does not impair SLTA-mediated ribosomal inactivation in cell-free assay



VHH1-VHH2 Biparatopic ETB was modified to include a single free cysteine at the C-terminus. The resulting Modified Biparatopic ETB was conjugated to MMAF through a covalent maleimide linkage to that cysteine

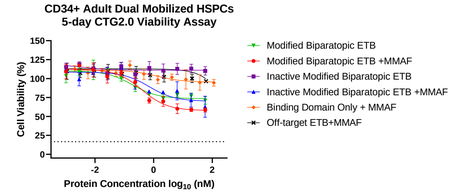


Cell-free transcription and translation assay was performed with Promega rabbit reticulocyte TnT reagent, which measures transcription and translation of a luciferase T7 plasmid. ETB was added to the mixture and incubated for 60-90 min. Luciferase Assay Reagent (E1483 Promega, Madison, WI, U.S.A.) was added to all test samples and the amount of luciferase protein translation was measured by luminescence according to the manufacturer's instructions.

CD117-Targeted ETBs Demonstrate Exquisite Target Specificity

MMAF conjugation to ETB results in increased cytotoxicity due to the combined activity of SLTA and payload

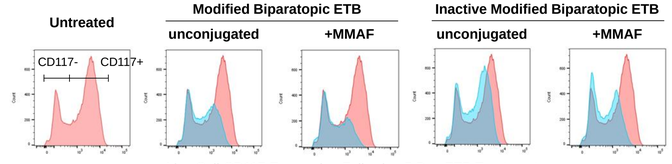
Inactive ETB +MMAF is more effective than binding domain only +MMAF, showing that SLTA improves delivery



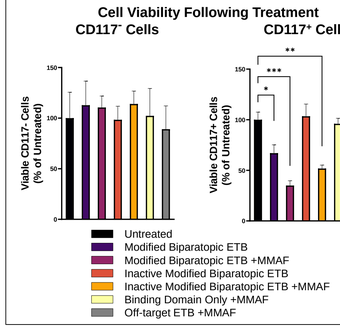
Treatment	% Viability at Max Dose
Modified Biparatopic ETB	77
Modified Biparatopic ETB +MMAF	**59**
Inactive Modified Biparatopic ETB	113
Inactive Modified Biparatopic ETB +MMAF	71
Binding Domain Only + MMAF	95
Off-target ETB+MMAF	100

Viability was measured 120 hours after ETB addition using Cell Titer-Glo®(Promega). CD34+ HSPCs were cultured in the absence of SCF. The dashed line on the graph represents the RLU value of cells at time 0h

ETB drug conjugates demonstrate exquisite specificity and kill ~65% of CD117+ HSPCs ex-vivo



ETB treatment histograms (blue) superimposed on untreated histogram (pink)



TMRE and Annexin V staining of cells followed by flow cytometry was used to measure cell viability and apoptosis, respectively. Following a 5-day treatment with 1 µg/mL ETB or ETB-MMAF, cells were stained with a non-competitive CD117 antibody to mark the CD117 phenotype of cells that remain viable (TMRE+). A CD117 negative cell population appears in the CD34+ HSPC culture. Experiment 1 (top): Treatment with active ETB-MMAF eliminates the CD117 high population. Active ETB alone and Inactive ETB +MMAF have less effect on the CD117 high population, while Inactive ETB alone has no effect. The CD117 low population is not affected by any treatment, showing the specificity of ETB and ETB-MMAF treatment. Experiment 2 (bottom): Viable CD117- cells (left) or CD117+ cells (right) are graphed, normalized to untreated cells. Technical triplicates were used to calculate significance by ordinary one-way ANOVA. *p<0.1, **p<0.01, ***p<0.001. Viable CD117- cells were not significantly different between treatment groups (p>0.05, one-way ANOVA).

Conclusions

- CD117 targeting by ETBs represents a therapeutic opportunity for both oncology and stem cell transplant unmet medical needs
- A novel biparatopic ETB was discovered that engages CD117 at both membrane distal and membrane proximal sites. The biparatopic nature of the ETB enhances CD117 binding and increases potency compared to ETBs comprising either domain alone
- CD117 is expressed at higher levels on the cancer cell lines sampled compared to primary human CD34+ HSPCs. The biparatopic ETB demonstrates picomolar activity in all cases. Efficacy in vitro appears to be tied to target expression levels.
- ETBs induce CD117 internalization to a greater degree than does the matching binding domain only on CD34+ HSPCs, consistent with the inherent internalization activity of SLTA
- ETB conjugation to MMAF results in combined activity by SLTA and the MMAF payload, improving overall efficacy on CD34+ HSPCs. ETBs also improve payload delivery compared to binding domain only
- Flow-based cytotoxicity methods further demonstrate the exquisite specificity of ETB-DC for CD117+ HSPCs

 This poster was sponsored and prepared by Molecular Templates. All authors are employees of Molecular Templates.