Design and Characterization of Bispecific Engineered Toxin Bodies for Targeted Cancer Therapy

Aimee Iberg, Caleigh Howard, Garrett Cornelison, Paul Amador, Steven Rivera, Michael Jamaleddine, Garrett L. Robinson, Erin K. Willert
Molecular Templates Inc., Austin, TX.

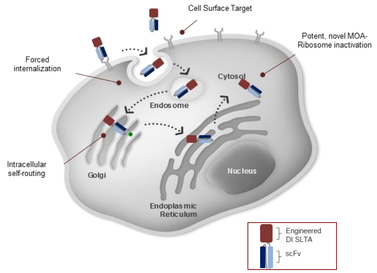
Abstract 2984
AACR 2019

MOLECULAR TEMPLATES

Background

Engineered Toxin Bodies (ETBs) are a distinct class of targeted immunotoxins in development as anti-cancer therapeutics by Molecular Templates. ETBs drive a potent and targeted response mediated by antibody-like binding, induced internalization, and enzymatic ribosomal inhibition via the delivery of a Shiga-like toxin subunit A (SLTA) that has been proprietarily modified to avoid innate and adaptive immune recognition.

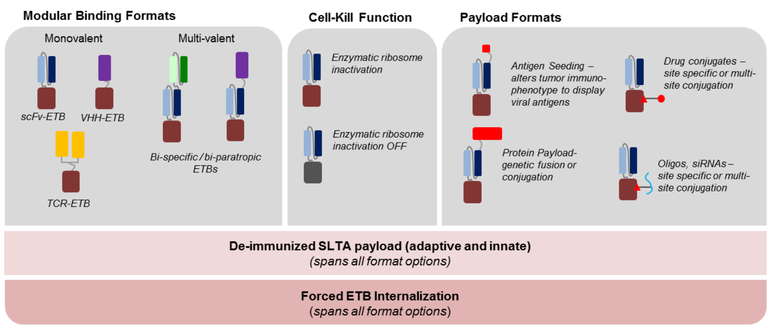
The novel MOA inherent to ETB therapeutics allows for activity in relapsed/refractory settings, as previously acquired resistance mechanisms should not hinder efficacy. MTEM has a pipeline of scFv targeted ETBs, including its clinical lead MT-3724. MT-3724 targets CD20 for treatment of B-cell lymphoma and has shown promising signs of activity in heavily pretreated patients.



MTEM is characterizing ETBs that are targeted through multiple binding domains. ETBs that target two epitopes on the same receptor, or two distinct cell surface molecules both expressed on cancer cells, may allow for enhanced activity profiles**. Data presented here support the use of bispecific and biparatopic ETBs for the following: (i) activity in the presence of a competitive binding protein (ii) synergistic binding events to increase overall potency, and (iii) delivery of a protein for intrabody applications**

The ETB Scaffold is Modular and Versatile

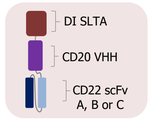
- Evolution of ETB scaffold engineering
  - Pairing the A-subunit with a scFv for targeted delivery
  - Deimmunization of the A-subunit
    - Combinations of single domain antibodies and scFvs for dual targeting
    - Addition of payload delivery for MOA expansion

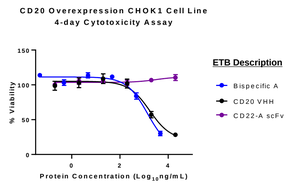


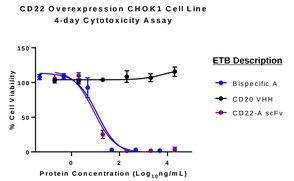
- Secondary binding arm modules include:
  - Targeting the same receptor via bivalent engagement (**bivalent double**)
  - Targeting the same receptor in 2 different locations (**biparatopic**)
  - Targeting two different proteins (**bispecific**)
    - Two cell surface targets (external) for greater breadth and/or specificity
    - Combination of external targeting to cell surface and intracellular targeting after internalization: intrabody delivery

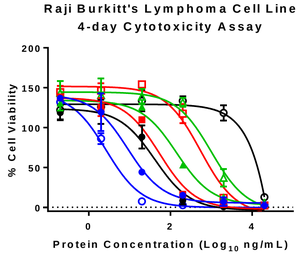
Bispecific and Bivalent Targeting in Lymphoma

- Bispecific Targeting of CD22 and CD20 in lymphoma
  - Malignant B cells often express both CD20 and CD22
  - Bispecific ETBs directly kill cells expressing CD20, CD22 or both receptors
    - Different CD22 targeted scFvs combined with a consistent CD20 VHH can tune the activity of the ETB
  - CD22xCD20 bispecific ETBs can act in the presence of excess Rituximab, demonstrating how bispecific ETBs can overcome competition for a single receptor.

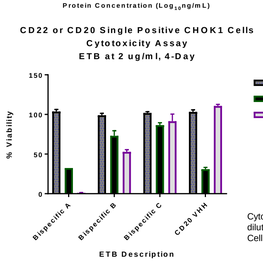








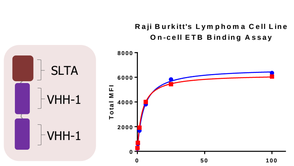
Competition Assay: Cells are plated into the assay plate with or without Rituxan at 100 ug/ml. 1h after plating, cells are treated with a dilution series of ETB and incubated at 37C/5% CO2. Viability is measured after 4 days (Promega Cell Titer-Glo).

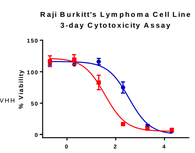


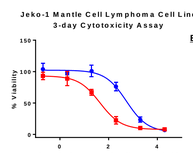
In Vitro potency on B lymphoma cell lines: IC50 (ng/ml)				
	Raji		ST486	
ETB	No mAb	+ Rituxan	No mAb	+ Rituxan
Bispecific A	9	2.7	1.8	5.4
Bispecific B	54	549	27	1869
Bispecific C	141	1006	23	984
MT-3724	42	>2000	53	>2000
CD20 VHH ETB	452	>2000	118	>2000
Cell viability is relative to cells not treated with ETB, +/- Rituxan				

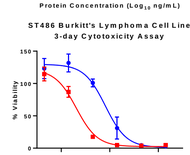
Cytotoxicity Assays: Cells are incubated at 37C/5% CO2 with a dilution series of ETB. Viability is measured after 4 days (Promega Cell Titer-Glo).

- CD20 Bivalent Double VHH ETB
  - Binding and cytotoxicity of single vs. double VHH ETBs on lymphoma cells. The bivalent and single VHH ETBs demonstrate similar on-cell binding affinities, however the double VHH is more potent









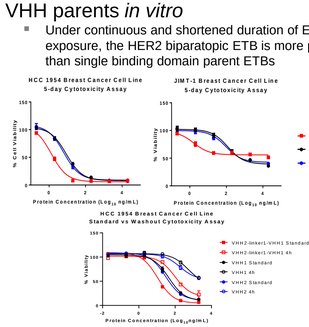
ETB binding assay performed on live cells: Cells are incubated with ETB on ice for 1h prior to wash and detection by an α-ETB mAb-FITC conjugate

Cytotoxicity Assays: Cells are incubated at 37C/5% CO2 with a dilution series of ETB. Viability is measured after 3 days (Promega Cell Titer-Glo).

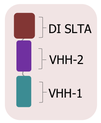
In vitro potency IC50 (ng/ml)			
ETB	Raji	Jeko-1	ST486
Single VHH	347	540	72
Double VHH	36	44	6.7

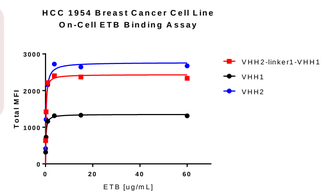
Biparatopic VHH ETB Targeting HER2

- Biparatopic is more potent than single VHH parents *in vitro*
  - Under continuous and shortened duration of ETB exposure, the HER2 biparatopic ETB is more potent than single binding domain parent ETBs



- Increased potency not correlated to affinity





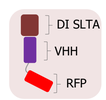
Cytotoxicity Assays: Cells are incubated at 37C/5% CO2 with a dilution series of ETB. Viability is measured after 4 days (Promega Cell Titer-Glo). For washout experiments, ETB is removed after 4h.

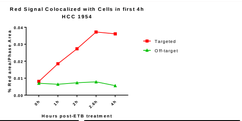
ETB binding assay performed on live cells: Cells are incubated with ETB on ice for 1h prior to wash and detection by an α-ETB mAb-FITC conjugate

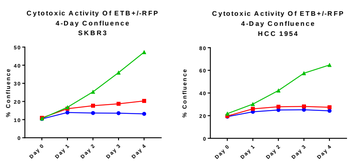
In vitro potency on various breast cancer cell lines: IC50 (ng/ml)				
ETB	HCC 1954 ($HER2^{High}$)	SKBR3 ($HER2^{High}$)	JIMT-1 ($HER2^{Mid}$)	MDA-MB-468 ($HER2^{Neg}$)
VHH 1	8.7	21.7	118.9	>2000
VHH 2	6.4	46.9	92.2	>2000
VHH 1 and VHH 2	1.3	3.8	1.6	>2000
Standard 5-Day Cytotoxicity Assay				

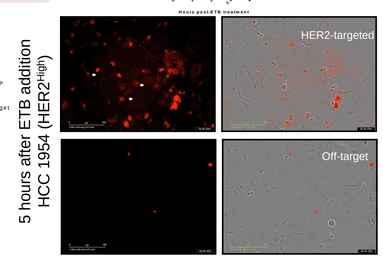
ETBs for Targeted Intracellular Protein Delivery

- MOA1 remains intact and cells glow red when RFP is appended to the ETB
  - Red signal showing distinct intracellular patterns observed only with on-target ETB









Confluence Assays: Cells are incubated at 37C/5% CO2 with a fixed concentration (5 ug/ml) of ETB, and live cell imaging is used to visualize cell confluence per well over 4 days. Confluence masks are determined with IncuCyte software to quantify % confluence.

CONCLUSIONS

- **The ETB scaffold is modular and allows for combinations of multiple binding domains and protein delivery**
- **ETBs with two binding domains have demonstrated increased potency when compared to the single domain parents *in vitro***
- **Biparatopic and bispecific binding domains allow for potent, specific ETBs with increased target breadth**
- **The ETB scaffold can be modified for targeted intracellular protein delivery (including intrabodies)**

Contact: aimee.iberg@mtem.com

mtem MOLECULAR TEMPLATES