Novel engineered toxin bodies targeting SLAMF7 (CS1)

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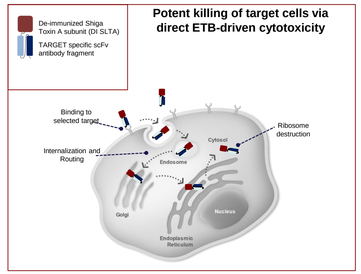
SLAMF7 Targeted ETBs for the Treatment of Multiple Myeloma

Engineered Toxin Bodies (ETBs) are fusion proteins consisting of an antibody fragment genetically fused to a proprietarily de-immunized (DI) form of the Shiga-like toxin A subunit (SLTA). Once 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 death through enzymatic and irreversible ribosomal destruction.

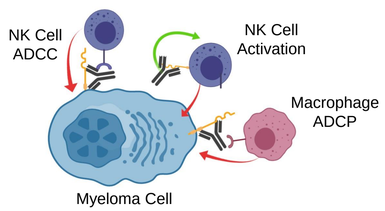
SLAMF7-targeted ETBs are designed to deplete *SLAMF7* positive multiple myeloma cells through:

- **Specificity**: SLAMF7-specific targeting domains (scFv or VHH) allow target-specific engagement and selective depletion of SLAMF7 over-expressing myeloma cells
- **Potency**: Direct cell-kill via enzymatic and irreversible inactivation of ribosomes mediated by DI SLTA



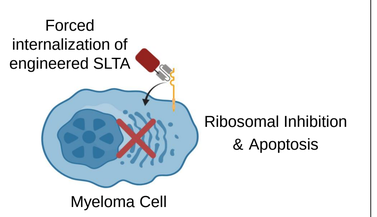
Rationale for Employing ETB Technology to Target SLAMF7

Anti-SLAMF7 mAb Elotuzumab[1,2]



- Indirect cell kill via immune effector function
- No efficacy as a monotherapy; requires combination with IMiDs for efficacy
- Approved only in the relapse/refractory setting

Engineered Toxin Body



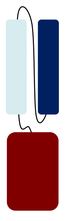
- Direct cell kill through permanent enzymatic ribosomal inactivation
- Does not rely on immune effector function
- Novel MoA with potential to be combined with standard of care

ETB Design & Evaluation

ETBs designed to target SLAMF7 epitopes distinct from Elotuzumab

- Investigated ETBs targeting SLAMF7 in a manner non-competitive with Elotuzumab, which may persist in pre-treated patients
- Designed and tested ETBs in monomer and dimer formats
- Evaluated ETBs for enzymatic activity, caspase induction, cytotoxicity, on-cell binding, and activity in combination with Elotuzumab and standard of care chemotherapeutics

Monomer

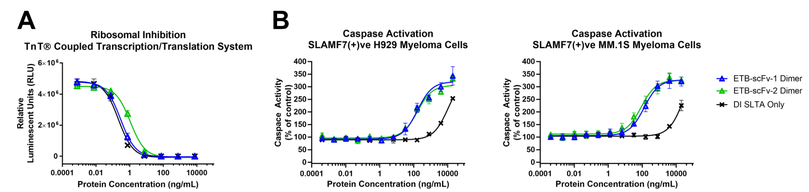


SLTA-V1-long linker-V2

Dimer (diabody)

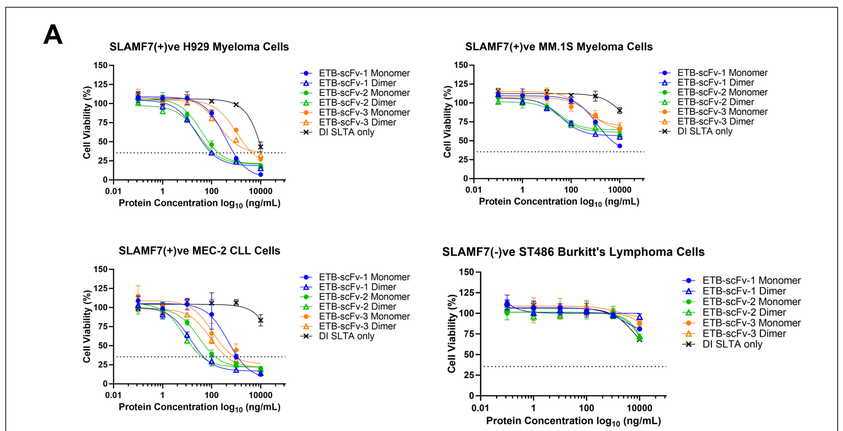
SLTA-V1-short linker-V2

SLAMF7 ETB – Mechanism of Action



- **(A) ETBs retain the catalytic activity of the DI SLTA subunit:** Protein synthesis inhibition by SLAMF7-targeted ETBs measured with a TnT® T7 Quick transcription/ translation system (Promega) *in vitro* luciferase assay
- **(B) SLAMF7-targeted ETBs induce significantly greater caspase activation than the DI SLTA subunit in SLAMF7(+)ve cell lines:** Concentration-dependent caspase activation was measured after 48 hour ETB treatment using Caspase-Glo ® 3/7 (Promega)

SLAMF7 ETB - Potency

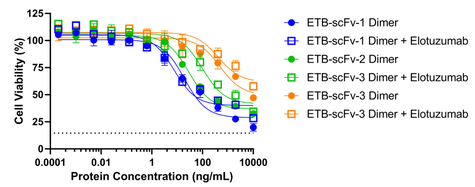


- **(A) Target-dependent Cytotoxicity:** Concentration-dependent cytotoxicity measured after 4-Day ETB incubation with cells (CellTiter-Glo® 2.0 - Promega)
- **(B) Activity/Affinity Relationship:** Cytotoxicity trends with ETB affinity & target engagement as measured by flow cytometry
- Dimer ETBs were generally more potent with IC50 values ~10 – 1,000 ng/ml

SLAMF7 ETBs are Active Alone and in the Presence of Elotuzumab

Cytotoxicity in the presence of Elotuzumab

- Elotuzumab can persist between ~ 10 - 100 ug/ml 2 months after the last dose of a 4-dose 10mg/kg q.wk. cycle[2]
- SLAMF7 ETBs maintain activity in the presence of 100 μg/ml Elotuzumab
- Epitopes distinct from Elotuzumab are options for ETB engagement, and would allow activity in patients previously treated with Elotuzumab

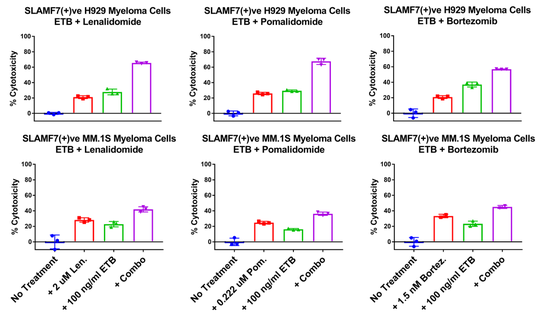


ETB	IC50 (ng/ml) - Elo	IC50 (ng/ml) + Elo
ETB-scFv-1 Dimer	19.3	7.6
ETB-scFv-2 Dimer	28.7	99.0
ETB-scFv-3 Dimer	486.2	418.0

SLAMF7 ETBs are Active Alone and in Combination with IMiDs & Bortezomib

ETB Activity in the Presence of Standard of Care Chemotherapeutics

- A submaximally-effective concentration of ETB was incubated +/- IMiD or Bortezomib for 4 days and cytotoxicity was assessed via CellTiter-Glo® 2.0 (Promega)
- Combination of ETB with IMiDs or Bortezomib resulted in increased cytotoxocity compared to either agent alone



CONCLUSIONS

- The direct cell-kill mechanism of SLAMF7 ETBs is distinct from antibody therapy targeting SLAMF7, which relies on indirect cell-kill through the engagement of effector cells
- SLAMF7-targeted ETBs have the potential to be an effective monotherapy for MM; Elotuzumab has not shown activity as a monotherapy in the clinic for MM[1,2]
- SLAMF7-targeted ETBs bind and kill various multiple myeloma cell lines at concentrations expected to be achievable *in vivo*[3]
- Epitopes distinct from Elotuzumab are options for ETB engagement, allowing activity in the presence of Elotuzumab
- SLAMF7 ETBs combine with IMiDs & Bortezomib in a positive manner *in vitro*
- Animal studies and lead selection are planned for 2020

References:
1. Campbell *et al.*, 2017. Mechanisms of NK cell activation and clinical activity of the therapeutic SLAMF7 antibody, elotuzumab in multiple myeloma. *Front. Immunol.* 9:2551. doi: 10.3389/fimmu.2018.02551
2. Zonder *et al.*, 2012. A phase 1, multicenter, open-label dose escalation study of elotuzumab in patients with advanced multiple myeloma. *Blood.* 120(3):552-559. doi: 10.1182/blood-2011-06-360552.
3. Fanale *et al.*, 2017. Phase I/Ib study of the novel CD20-targeted immunotoxin MT-3724 in relapsed/refractory non-Hodgkin's B-cell lymphoma. *2017 AACR Annual Meeting.* Poster CT049.

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