Improving immunotoxin-based therapeutics for cancer with de-immunized Engineered Toxin Bodies

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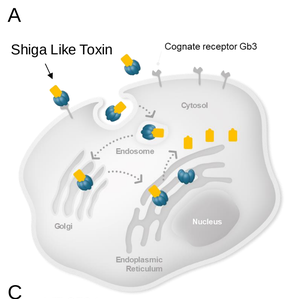
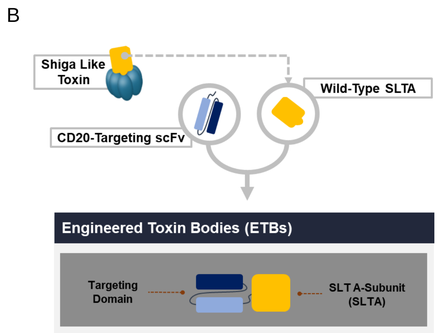
Background: ETB Mechanism of Action

Engineered Toxin Bodies (ETBs): Targeted cell kill by ribosomal destruction





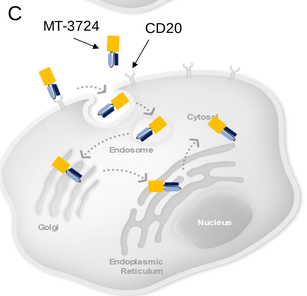


Figure 1: Featuring an antibody-based targeting domain fused with the cytotoxic, ribosome inactivating Shiga-like toxin A subunit (SLTA), ETBs enable killing of target expressing cells.

(A) Shiga-Like Toxin mechanism of direct cell kill via internalization, routing, and ribosomal inactivation

(B) Structure of MT-3724, a 1st generation CD20-targeting ETB

(C) MT-3724 mechanism of action illustrating direct, targeted cell kill of CD20 expressing cells

MT-3724 in relapsed/refractory non-Hodgkin lymphoma patients

Figure 2: Despite showing efficacy in heavily pre-treated non-Hodgkin lymphoma patients, MT-3724 was discontinued due to manufacturing/stability issues

(A) Summary table for MT-3724 multi-center phase 1 clinical trial in non-Hodgkin lymphoma patients

A

Patients evaluable for efficacy in phase I (n=25)
DLBCL or Mixed DLBCL/FL (n=19)
Low serum Rituximab (RTX) levels (n=13)
2 Complete Responses (CR)
1 Complete Metabolic Response (CMR)
2 Partial Responses (PR)
3 Stable Disease (SD) (49%, 47% tumor reduction)
5 Progressive Disease (PD)
38% Objective Response Rate (ORR)[a]
60% ORR at MTD: 2 CRs, 1 PR, 2 PD

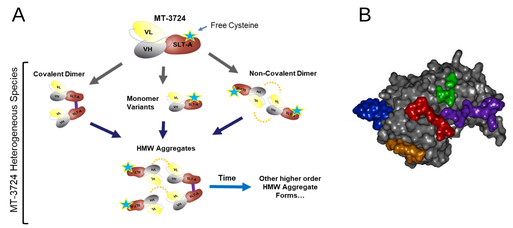
Grade 5 CLS (Capillary leak syndrome) observed in one patient treated with MT-3724 that had a high proportion of aggregated species

a) Median number of prior therapies in responders was 3; responses seen in R-CHOP refractory pts. Includes 2 stable disease patients who had 49% and 47% tumor reductions

ETB improvements and de-immunization of SLTA

Aggregation and immunogenicity in MT-3724

Figure 3: First generation ETB MT-3724 featured immunogenic WT SLTA scaffold and exhibited propensity to form aggregates

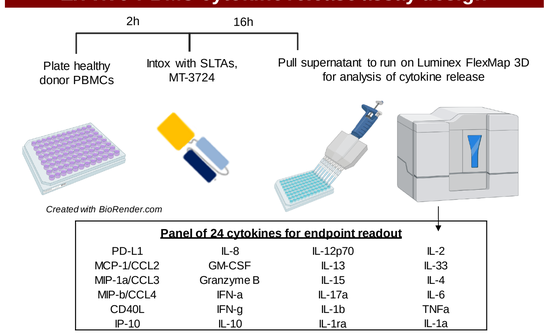


(A) Mechanism of aggregate formation in 1st gen ETB scaffold

(B) Predicted immunogenic epitopes of WT SLTA mapped to surface structure

(C) De-immunization of WT SLTA to genetically engineered de-immunized SLTA (DI-SLTA) scrubbed of predicted immunogenic epitopes for next generation ETBs

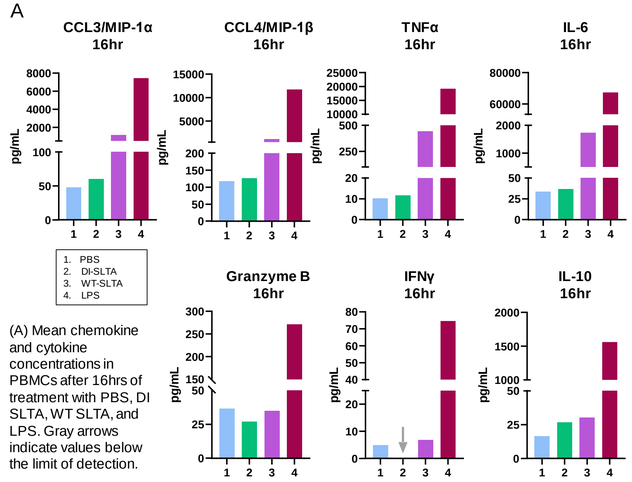
Ex vivo PBMC cytokine release assay design



Ex vivo cytokine release in SLTA and ETB-treated PBMCs

Reduced release of cytokines with de-immunized (DI) SLTA

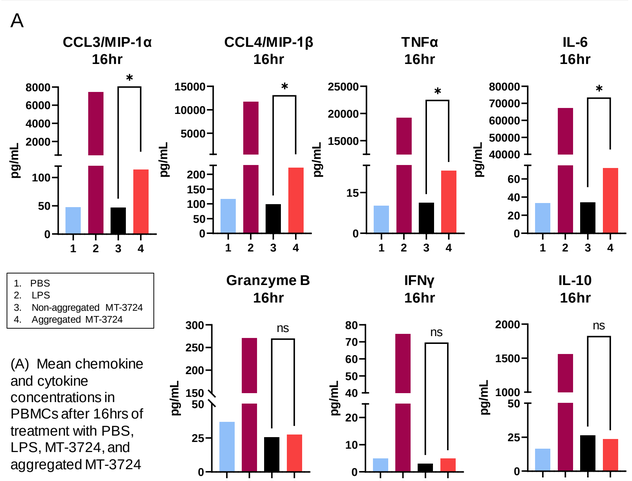
Figure 4: Non-targeted DI SLTA-treated PBMCs exhibited baseline cytokine levels while non-targeted WT SLTA induced release of CCL3, CCL4, TNFα, and IL-6 in PBMCs.



(A) Mean chemokine and cytokine concentrations in PBMCs after 16hrs of treatment with PBS, DI SLTA, WT SLTA, and LPS. Gray arrows indicate values below the limit of detection.

Increased cytokine release with aggregated MT-3724

Figure 5: Aggregated MT-3724 induced moderate increases in TNFα, IL-6, CCL3, and CCL4 relative to a non-aggregated MT-3724



(A) Mean chemokine and cytokine concentrations in PBMCs after 16hrs of treatment with PBS, LPS, MT-3724, and aggregated MT-3724

B cell independent *ex-vivo* cytokine release

B cell depletion *ex vivo* cytokine release assay

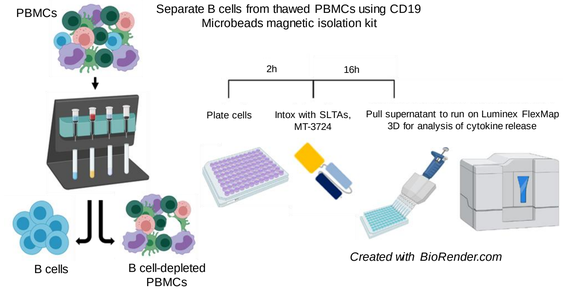
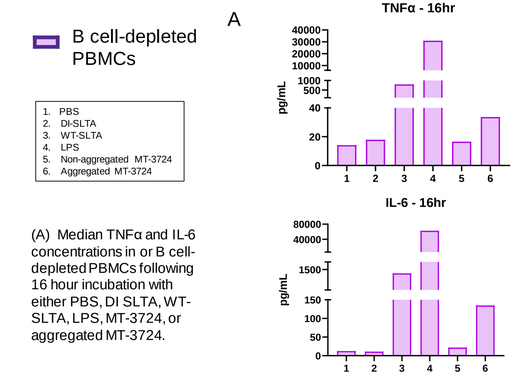


Figure 6: Removal of B cells from PBMCs did not alter cytokine release patterns of LPS, WT SLTA, or aggregated MT-3724.



(A) Median TNFα and IL-6 concentrations in or B cell-depleted PBMCs following 16 hour incubation with either PBS, DI SLTA, WT-SLTA, LPS, MT-3724, or aggregated MT-3724.

Conclusions

- Non-targeted WT SLTA displayed upregulation of CCL3, CCL4, TNFα and IL-6, indicating a similar, but not identical, pattern of cytokine release relative to the positive control, LPS.
- De-immunized SLTA did **NOT** activate cytokine or chemokine release, indicating that scrubbing WT SLTA of immunogenic components can improve the safety profile of our 2nd gen ETBs.
- Targeted WT-SLTA (MT-3724) shows cytokine release similar to untargeted DI-SLTA , likely through scFv-mediated steric inhibition of WT-SLTA immune recognition; however, aggregates in MT-3724 induced moderate increases in TNFα, IL-6, CCL3, and CCL4 relative to non-aggregated MT-3724
- Removal of B cells from PBMCs did not alter cytokine release patterns of LPS, WT SLTA, or aggregated MT-3724
- These data suggest that cytokines were released in response to WT SLTA and protein aggregates in an off-target manner.
- This assay will be used as a triage for ETB safety by testing ETBs for cytokine release prior to selection as candidates for clinical trials.

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