

# Novel Toxin Library For the Discovery of Oncology Therapeutics

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## Abstract

The A subunit of Shiga-like Toxin 1 (SLT1A) is a ribosome-inactivating protein that works by catalyzing the depurination of the 28 S rRNA. The A subunit lacks the ability to bind and internalize into cells, and is normally dependent on the B subunit of SLT1 for this function. A toxin variant library has been created by inserting random amino acid stretches into an exposed loop structure of the A subunit. The inserts can confer binding and internalization properties to the SLT1A variants but do not inhibit the A subunit's ability to intoxicate. This toxin library has been expressed in a phage display system that can be rapidly screened for variants with specific cell kill activity and predictable PK/PD properties. The library is being used to develop therapeutics for oncology.

## Background

A prototype toxin variant library was previously created by inserting a random stretch of seven amino acids into the protease-sensitive loop of the SLT1A subunit. A small subset of this library (9,400 variants) was expressed and screened as His-tagged recombinant proteins. One variant in particular (SAM3) showed excellent and specific cell-kill activity in a screen against melanoma cells but was inactive against a variety of other cell types<sup>1</sup>. The difficulty in screening a recombinant library led to the creation of a second-generation toxin library.

The second-generation toxin library was created using twelve amino acid inserts to increase diversity. The library was expressed in a T7 phage display system to improve the screening of the library. Data are presented demonstrating that the 12-mer insert library has excellent diversity and functionality and that variants expressed in a T7 phage system retain the biological properties of recombinantly-expressed variants. A novel library of molecules with direct-cell kill properties, highly predictable PK/PD, and reduced immunogenicity has been created making them well-suited for therapeutic development.

## 1. Binding Domain Insertion

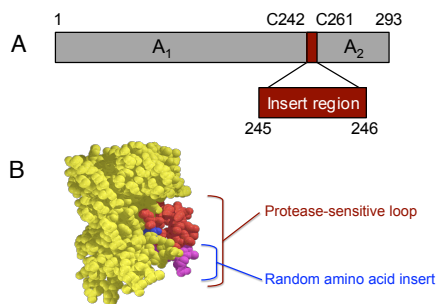


Figure A: A schematic illustration of the SLT1A subunit identifying the insert region used to build the SLT1A variant library.

Figure B: 3-D representation of a SLT1A scaffold with an exposed peptide insert

The A subunit of SLT1 contains an exposed protease-sensitive loop bounded by cysteine residues at positions 242 and 261. Peptides of 12 random amino acids were engineered into the protease-sensitive loop between positions 245 and 246 (Figure A) creating a theoretical library of SLT1A toxin variants numbering  $4 \times 10^{15}$ . The inserted peptides are exposed and can potentiate binding and internalization of the SLT1A scaffold (Figure B).

## 2. Immunogenicity

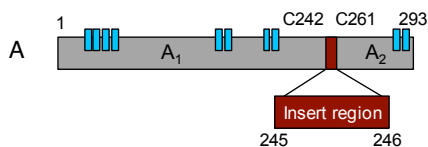


Figure A: Illustration of the immunogenic regions identified using *in-silico* analysis in blue.

Immunogenic areas of the toxin scaffold were identified using ProPred *in-silico* analysis. Of the identified peptides with putative high binding affinity to MHC Class II receptors, the majority fall outside of the catalytic domain and have been removed. The immunologically "scrubbed" A subunit retains the biological properties of the wild-type.

## 3. Genetic Diversity

Genetic Variability of Variant Library	
% without insert	8.7%
% with insert	91.3%
Variability of inserts	100%

N=46

Table A: The SLT1A variant library exhibits a high degree of genetic variability.

Random SLT1A variants were picked (N=46) and their DNA sequenced to determine whether a peptide insert was present and whether peptide insert sequences repeated. The vast majority of SLT1A variants sequenced (91.3%) contained a peptide insert. There was no instance of the same peptide insert being repeated in one or more variants.

## 4. Functional Diversity

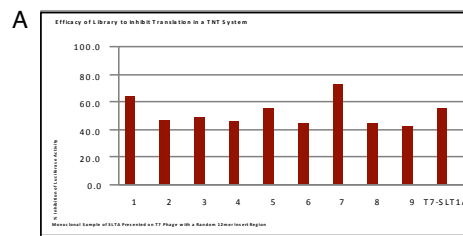


Figure A: Toxin variants picked from the 12-mer library showed equivalent function to wild-type SLT1A.

Randomly sampled SLT1A variants expressed on T7 phage were tested in a rabbit reticulocyte assay for their ability to inhibit protein translation. All variants exhibited ribosome inhibition comparable to that seen with wild-type SLT1A at similar concentrations.

## 5. Cell Viability

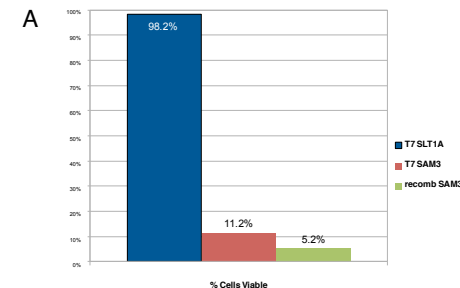


Figure A: T7-SAM3 exhibits high levels of cell kill

Recombinant SAM3 was compared to a T7 phage-expressed version of itself for its ability to kill melanoma cells. The recombinant and phage-expressed version showed similar levels of cell kill at comparable concentrations.

## Conclusion

- A vast ( $4 \times 10^{15}$ ) toxin library was created by inserting random amino acid stretches into an exposed loop in the SLT1A subunit.
- The cytolytic activity of wild-type SLT1A is preserved while unique binding affinities are conferred to the variants.
- The library has been expressed in a phage display system that allows for rapid screening of the variants while retaining their biological properties.
- The library is currently being screened against a variety of targets and disease states for the discovery and development of novel therapeutics.

## References

- M Cheung et al, Molecular Cancer 2010, 9:28