AACR 2015
Abstract 2477

Engineered Toxin Bodies: A next-generation immunotoxin scaffold with novel immuno-oncology functionality

Erin K. Willert, Garrett L. Robinson, Sangeetha Rajagopalan, Brigitte Brieschke, Jennifer Erdman, Jane Neill, Rodney Flores, Julia Foree, William Null, Jensing Liu, Jack P. Higgins
Molecular Templates, Inc. Georgetown, Texas

BACKGROUND

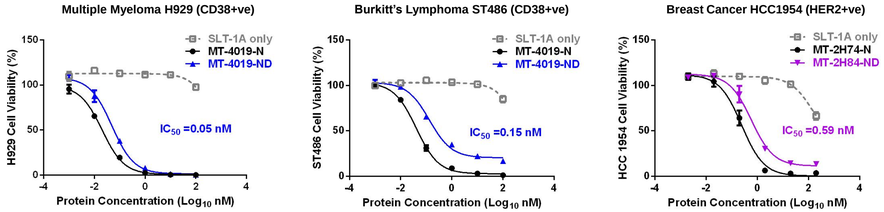
The potential of immunotoxins in oncology has been limited due to immunogenicity as well as a restricted number of appropriate targets. Molecular Templates has developed an engineered toxin body (ETB) platform, a next-generation recombinant immunotoxin scaffold based on the Shiga-like toxin A subunit (SLT-1A) specifically directed to cancer cells via antibody fragment binding domains. Proprietary engineering technology replaces immunogenic B and CD4+ T cell epitopes with potential MHC-I, CD8+ T cell epitopes (class switching technology) to reduce the anti-drug response after repeat ETB administration. ETBs have been selected for specificity and potency; also, the ETB scaffold is engineered to force the internalization of receptors that typically do not internalize or internalize poorly. Molecular Templates' lead compound, MT-3724, is the first immunotoxin targeting CD20 to enter the clinic and is currently in a phase I study for refractory non-Hodgkin's Lymphoma (NHL, NCT02361346).

Additionally, we have taken advantage of the immunotoxin's localization to the cytosol to engineer in a novel immuno-oncology mechanism of action. Molecular Templates' Antigen Seeding Technology involves genetically fusing MHC class I antigens derived from human cytomegalovirus (HCMV) to the immunotoxin scaffold in order to mark these cells as targets for cytotoxic T lymphocyte (CTL) mediated cell lysis. Because antigen presentation does not require de novo protein synthesis, this mechanism of action is complementary to the inactivation of ribosomal function by the SLT-1 A domain of the ETBs. Since a large portion of the populace has a robust population of high-affinity effector T-cells targeting HCMV, the presentation of these antigens on ETB-intoxicated cancer cells may recruit a pre-existing CTL response to tumor cells. The recruitment of a CTL response has the potential to act additively or synergistically to the direct cell kill activity of the ETB and may expand the efficacy of these immunotoxins.

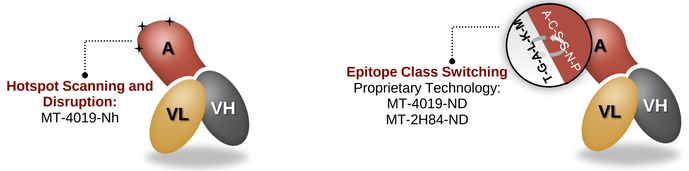
Disease	Target	Lead	Pre-clinical	Phase I
Non-Hodgkin's Lymphoma	CD20	MT-3724		
Multiple Myeloma	CD38	MT-4019-ND		
		MT-4019-N-AS		
Breast Cancer	HER2	MT-2H84-ND		

PROPRIETARY DE-IMMUNIZATION OF ETBS

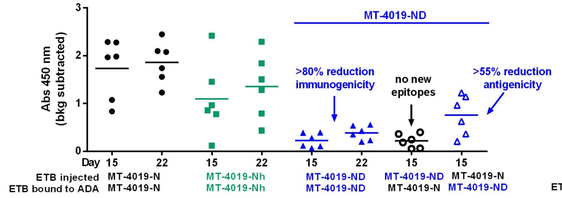
De-immunized ETBs retain specific potency



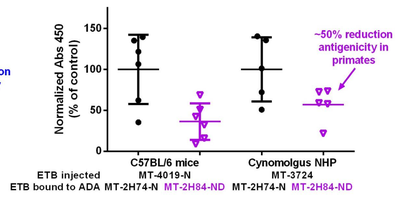
Proprietary de-immunization technology dramatically reduces ADA response



Reduced Immunogenicity of MT-4019-ND in C57BL/6 mice

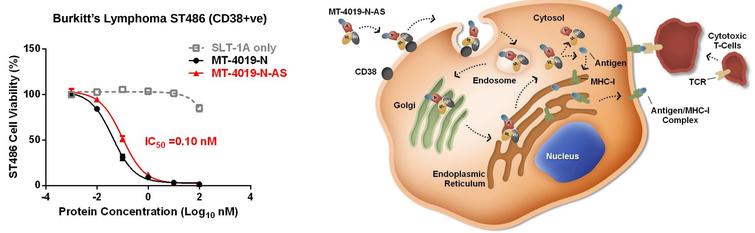


Reduced Antigenicity of MT-2H84-ND

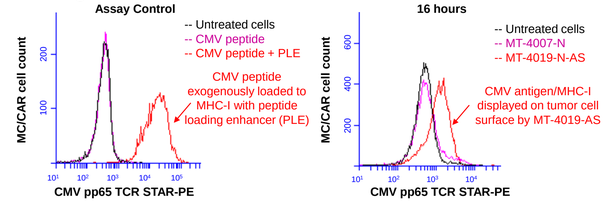


ETB MEDIATED ANTIGEN SEEDING

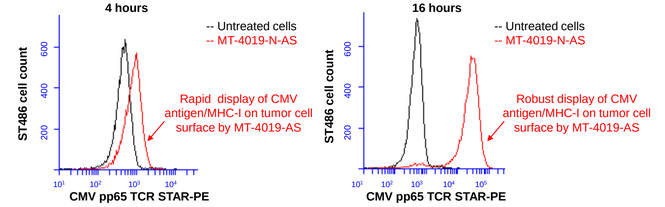
Potent ETB with added Antigen Seeding Technology



Viral Antigen Seeding: MC/CAR cells with MT-4019-N-AS for 16 hours



Viral Antigen Seeding: ST486 cells with MT-4019-N-AS for 4 to 16 hours



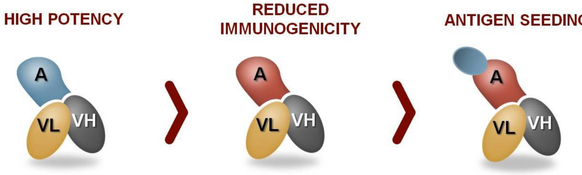
METHODS

Cytotoxicity Assay: Serially diluted proteins were combined with cells and incubated for 72 hours, then cell viability was measured with Cell Titer GLO (Promega). Non-linear regression was performed with Graphpad Prism software.

Relative Anti-drug Antibody (ADA) ELISA: Animals were injected with six doses of ETB at 0.25 mg/kg (C57BL/6 mice) or 0.15 mg/kg (cynomolgus non-human primate, NHP) over two weeks; serum was collected and ADA was measured by ELISA. Immunogenicity is the ability of an agent to induce an adaptive immune response (ie ADA) and antigenicity is the capacity of an agent to bind to the products of the immune response (ie antibodies).

Flow Cytometry: ST486 or MC/CAR (CD38+/HLA-A2+) cancer cells were incubated for 4 to 16 hours with MT-4019-N-AS or controls, then washed and labeled with a T cell receptor reagent (STAR™ Multimer, TCR CMV-pp65-PE, Altor) specific for recognition of HLA A2/CMV-pp65$_{aa495-503}$ antigen complexes. Surface presentation of the complex was detected on an Accuri C6 flow cytometer. Controls: untreated cells, MT-4007-N control (no antigen) ETB, or CMV-pp65$_{aa495-503}$ peptide antigen alone or with peptide loading enhancer (PLE, Altor).

ENGINEERED TOXIN BODIES PLATFORM TECHNOLOGY



- The ETB platform can target non- and poorly internalizing receptors
- These potent and specific next generation immunotoxins have:
  - Dramatically reduced immunogenicity
  - Antigen seeding: a novel mechanism of immuno-oncology

Website: www.moleculartemplates.com ; contact: Erin.Willert@moleculartemplates.com