Targeted Antigen Delivery to the Liver Induces Antigen-Specific Immune Tolerance and Modulates Pathology in Preclinical Models of Autoimmunity

Stephan Kontos[1], Kristen M. Lorentz[1], Thomas B. Thornley[1], Francisco Maldonado-Arocho[1], D. Scott Wilson[2], Jeffrey A. Hubbell[1,2]

[1]Anokion US, Inc., [2]University of Chicago, USA

ABSTRACT

Current therapies for multiple sclerosis (MS) aim to inhibit function of T and B cells with the goal of reducing pathogenic T cell infiltration into the central nervous system (CNS). While effective at reducing symptoms, these products may carry risks of serious infection and other complications associated with broad immunosuppression. Addressing the fundamental mechanism of MS pathology with a targeted next-generation approach remains a key unmet need in the treatment armamentarium. The induction of antigen-specific immune tolerance may be a potentially transformative and targeted approach for the treatment of autoimmune disease. In MS, several autoantigens in the CNS are the target of inflammatory attack by self-reactive T cells that drive neurodegeneration resulting in disease pathology.

We have developed a preclinical stage novel therapeutic approach for MS that induces antigen-specific tolerance to CNS autoantigens and ameliorates neuroinflammation. Our antigen-based, novel synthetic glycosylation platform harnesses natural tolerogenic pathways in the liver to drive liver-specific antigen processing and presentation. In preclinical models we demonstrate how our liver-targeted antigens prime antigen-specific T cells towards tolerogenic phenotypes, including expression of inhibitory and exhaustion markers, clonal T cell deletion, and the induction of regulatory T cell responses. In addition, we demonstrate how these mechanisms translate to the non-human primate using a novel model of antigen-specific T cell tolerance in a cynomolgus macaque, which supports the translational potential of our platform.

In a preclinical setting, the tolerogenic T cell mechanisms induced by our liver-targeting technology were effective at preventing or reducing disease pathology, including mouse models of type-1 diabetes and MS. Mice treated with liver-targeted islet antigen (chromogranin-A) were completely protected from hyperglycemia in the BDC2.5 model of type-1 diabetes. In the EAE mouse model of MS, liver-targeted MOG antigen induced pathogenic T cell anergy and deletion, resulting in complete protection from disease onset and therapeutic remission when tolerized during active disease.

Our liver-targeting immune tolerance platform drives potent tolerogenic T cell responses capable of inducing therapeutic efficacy in various preclinical models of autoimmunity, and its translational mechanism of action represents a promising potential strategy for the treatment of MS.

METHODS

Liver-targeted (LT) antigen synthesis: Antigens were synthesized by standard solid-phase peptide synthesis and purified by RP-HPLC. Chemical conjugation of antigen to glycosylation groups was conducted via standard bioconjugate techniques under sterile aqueous conditions, followed by purification via liquid chromatography. Purity was confirmed by HPLC, and sterility was confirmed by a LAL assay.

EAE Mouse Model: EAE was induced by adoptive transfer of MOG-reactive encephalitogenic T cells into host C57BL6 mice. In brief, C57BL6 mice were vaccinated with MOG_{35-55} in CFA, euthanized 10 days later, and splenocytes were activated in vitro with MOG_{35-55} for 4 days before being adoptively transferred i.v. into naïve C57BL6 host mice. Test articles were administered at pre-determined time points via i.v. injection into the tail vein. Mice were monitored daily for EAE disease score by trained technicians blinded to group identities. Cells in spleen, cervical LN, and spinal cord were isolated by mechanical and enzymatic separation of tissue and immunologically phenotyped via flow cytometry following extra- and intra-cellular antibody staining.

T1D Mouse Model: T1D was induced by adoptive transfer of p31/chromogranin-A-reactive T cells into host NOD/SCID mice. In brief, splenocytes from naïve BDC2.5 mice were activated in vitro with p31 peptide for 4 days before being adoptively transferred i.v. into naïve NOD/SCID mice. Test articles were administered on day 0 and day 4 via i.v. injection into the tail vein. Blood glucose levels of host mice were measured daily starting on day 4 with an Accu-Check glucometer (Roche).

SIV-Nef NHP Tolerance Model: Cynomolgus macaques were vaccinated against SIV-Nef using GTU®-DNA vaccine administered intradermally on weeks 0, 6, and 12. Test articles were administered at pre-determined time points via i.v. injection into the saphenous vein. Nef-specific T cell responses were assessed via IFNγ ELISPOT following in vitro stimulation of PBMCs with Nef antigen. Regulatory T cells ($CD4^+$, $CD25^+$, $CD45RA^-$, $CD127^-$, $FoxP3^+$) were characterized by flow cytometry following extra- and intra-cellular antibody staining.

All animal experimentation (mouse and cynomolgus macaque) was conducted in accordance with standardized protocols that were pre-approved by local veterinary and ethical authorities.

RESULTS & CONCLUSIONS

I. Antigen Functionalization with Engineered Synthetic Glycosylation Induces Liver-Specific Targeting & Antigen-Specific Tolerogenic T Cell Responses: Liver-targeted ovalbumin (LT-OVA) effectively localized to liver 3 hours following i.v. administration in mice, without detectable localization to other organs. Liver-resident antigen presenting cells prime antigen-specific T cells towards tolerogenic phenotypes, including the induction of regulatory T cells, anergy, exhaustion, & deletion.

II. LT-MOG Prevents EAE by Tolerizing Encephalitogenic T Cells & Protecting CNS from Immune Attack: Mice administered with LT-MOG were protected from EAE over the course of the study, which correlated with a decrease in MOG-reactive T cells in the spleen, cervical LN, and spinal cord. Immunological phenotyping of remaining MOG-reactive T cells illustrated a tolerogenic response to LT-MOG treatment, as indicated by upregulation of exhaustive markers (PD-1, LAG-3, ICOS) and downregulation of inflammatory cytokine expression (TNFα, IFNγ, IL-2, and IL-17a). Mice treated with an equivalent dose of soluble, non-liver targeted MOG peptide were not protected from disease, thus illustrating the potency of the liver-targeting technology in modulating autoimmune pathology by leveraging tolerogenic pathways in the liver.

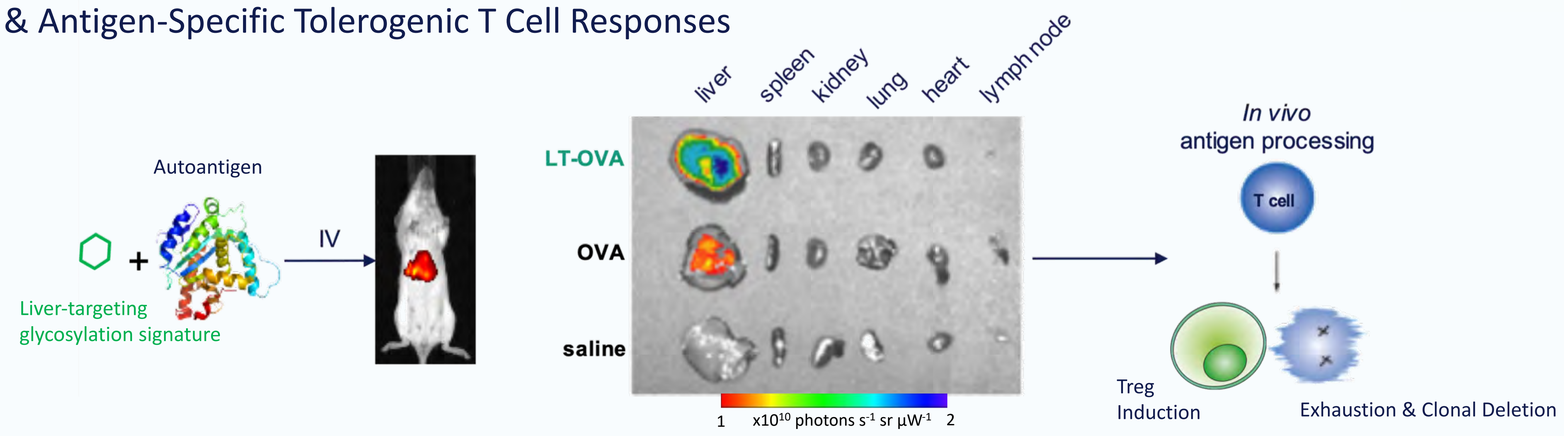
III. LT-MOG Induces Disease Remission in a Mouse EAE Model: LT-MOG was administered to mice with established EAE to determine if LT-antigen was capable of tolerizing pathogenic T cells that are actively mediating a neuroinflammatory response. Mice treated with LT-MOG upon first observation of EAE symptoms (blue squares) exhibited an arrest of disease development that completely resolved by day 10. Mice treated with LT-MOG after establishment of robust disease (pink squares) exhibited an arrest of disease severity followed by partial disease remission during the study. These results illustrate the potency of liver-targeted tolerance mechanisms in tolerizing highly activated pathogenic T cells, which is relevant in human autoimmune diseases such as MS.

IV. LT-Islet Antigen Protects Mice from T1D in a BDC2.5 Mouse Model: Mice administered with LT-p31 were protected against T1D during the course of the study; comparatively, mice treated with soluble p31 were not protected from disease, which again illustrates the potency of liver-targeted antigens in tolerizing highly activated pathogenic T cells and highlights the potentially broad applicability of our liver-targeting platform technology in multiple autoimmune settings.

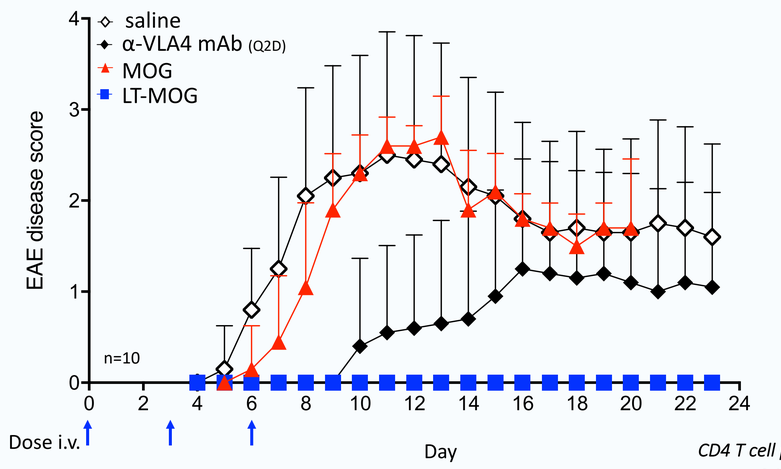
V. LT-Nef Reduces Established Nef-Specific Immune Responses in a SIV Vaccination Model in the NHP: After an 18-week course of vaccination to generate Nef-specific T cell responses, cynomolgus macaques were treated with LT-Nef or saline and immune tolerance was monitored by measuring Nef-specific immune responses in PBMCs via ELISPOT. NHPs treated with LT-Nef exhibited a 39% decrease in Nef-specific IFNγ-expressing cells and a corresponding increase in effector regulatory T cells 1 week after treatment. In contrast, saline-treated animals exhibited a 109% increase in Nef-specific IFNγ-expressing cells and no changes in regulatory T cells 1 week after treatment. These results further illustrate the potency of our liver-targeting technology in tolerizing activated, endogenously-generated T cells. Importantly, the functional and mechanistic findings of this NHP study were consistent with findings from mouse models, and thus further supports the translational potential of our liver-targeting technology in treating human autoimmune diseases such as MS.

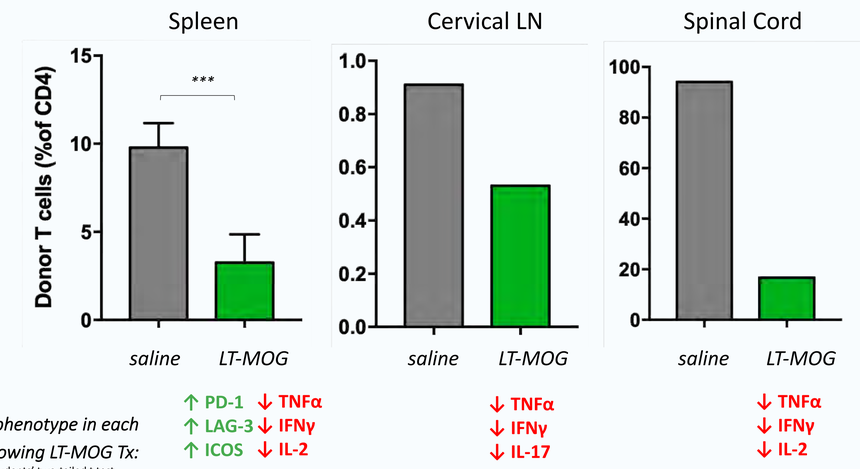
Taken together, these results illustrate the promising potential of our liver-targeting technology in inducing antigen-specific tolerance to treat autoimmune diseases, and provide strong translational support for our clinical candidate ANK-780, which will be investigated in an MS clinical trial in 2020.

I. Antigen Functionalization with Engineered Synthetic Glycosylation Induces Liver-Specific Targeting & Antigen-Specific Tolerogenic T Cell Responses



II. LT-MOG Prevents EAE by Tolerizing Encephalitogenic T Cells & Protecting CNS from Immune Attack

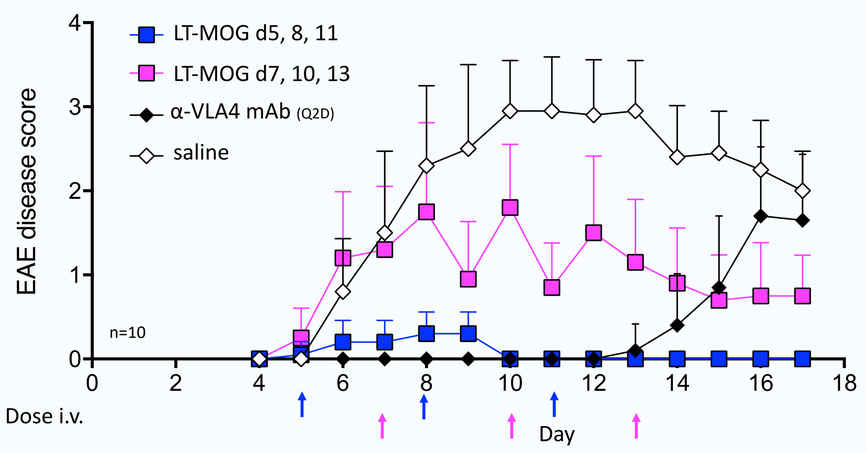




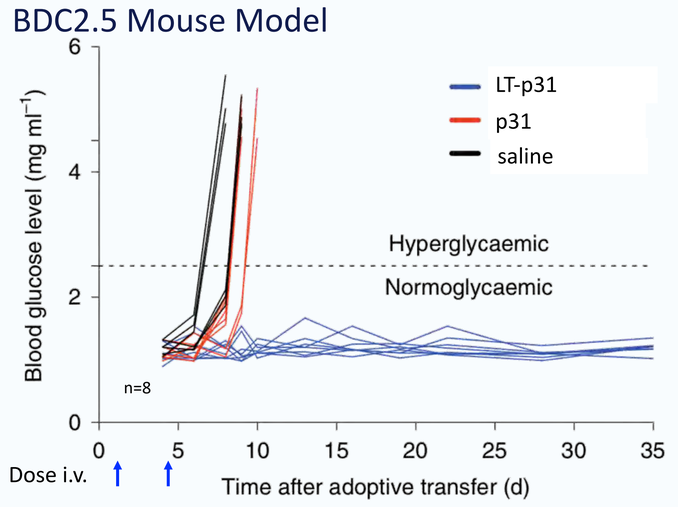
CD4 T cell phenotype in each organ following LT-MOG Tx:

***p<0.001, Students' two-tailed t test

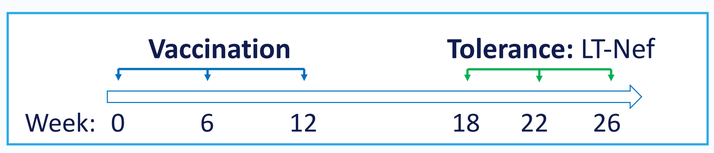
III. LT-MOG Induces Disease Remission in a Mouse EAE Model



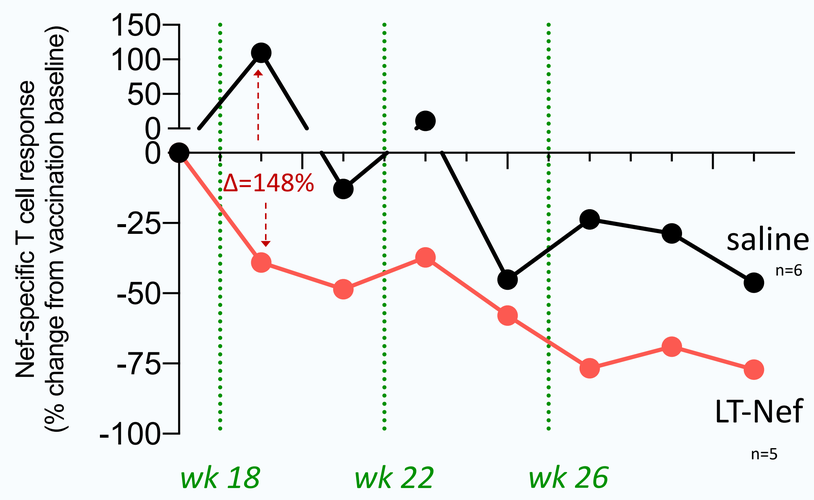
IV. LT-Islet Antigen Protects Mice from T1D in a BDC2.5 Mouse Model



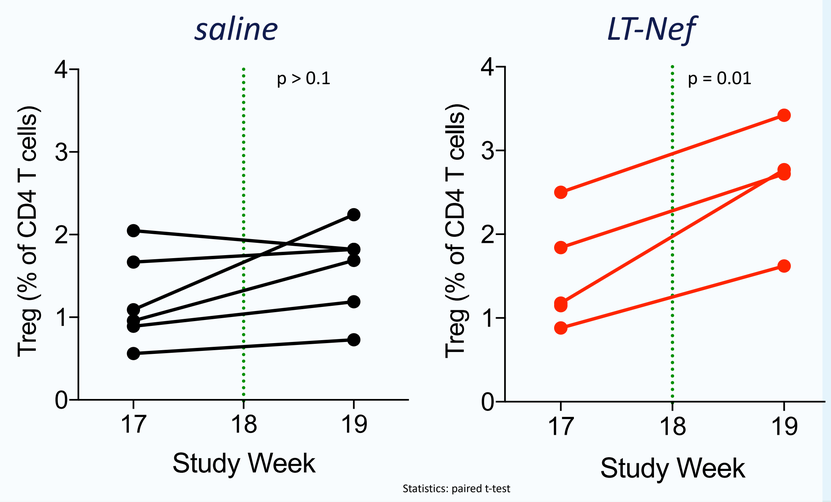
V. LT-Nef Reduces Established Nef-Specific Immune Responses in a SIV Vaccination Model in the NHP



Nef-Specific T cell Response Following Tolerization (ELISPOT)



LT-Nef Induces Regulatory T Cells in NHP



Statistics: paired t-test