

ONT-10, a liposomal vaccine targeting hypoglycosylated MUC1, induces a potent cellular and humoral response and suppresses the growth of MUC1 expressing tumors

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Background

MUC1 as a Cancer Vaccine Target

- Highly expressed in multiple tumor histologies
- Hypoglycosylated relative to normal tissue, with prematurely terminated carbohydrate modifications including α -N-acetyl-D-galactosamine (Tn)
- Unique tumor glycosylation state of MUC1 makes this antigen immunologically distinct and an attractive vaccine target.
- Incorporation of Tn moieties in MUC1 vaccine design may increase the likelihood of breaking immunological tolerance and enhance the generation of T cells and antibodies that are directed to the hypoglycosylated MUC1 tumor antigen

ONT-10 Composition

- Glycolipopeptide antigen comprising two 20 amino glycosylated VNTR repeats from human MUC1A including six glycosylated sites modified by Tn
- Fully synthetic PET Lipid A (penta erythritol lipid A) adjuvant
- Microparticle liposome formulation composed of carrier lipids cholesterol, DPPC, and DMPG

M40Tn6: ONT-10 Peptide Antigen

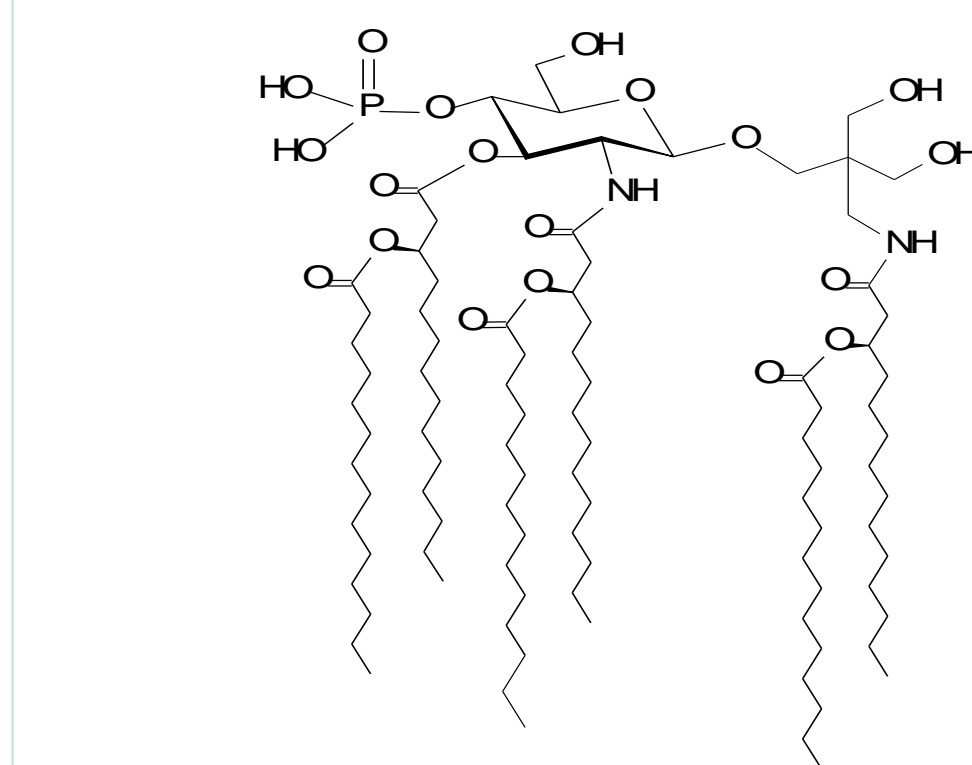
NH₂-TSAPDT(Tn)RPAPGS(Tn)T(Tn)APPAHGV-TSAPDT(Tn)RPAPGS(Tn)T(Tn)APPAHGV*S*L-COOH

M25: ONT-25 Peptide Antigen

NH₂-STAPPAHGVTSAPDTRPAPGSTAPPAK*G-COOH

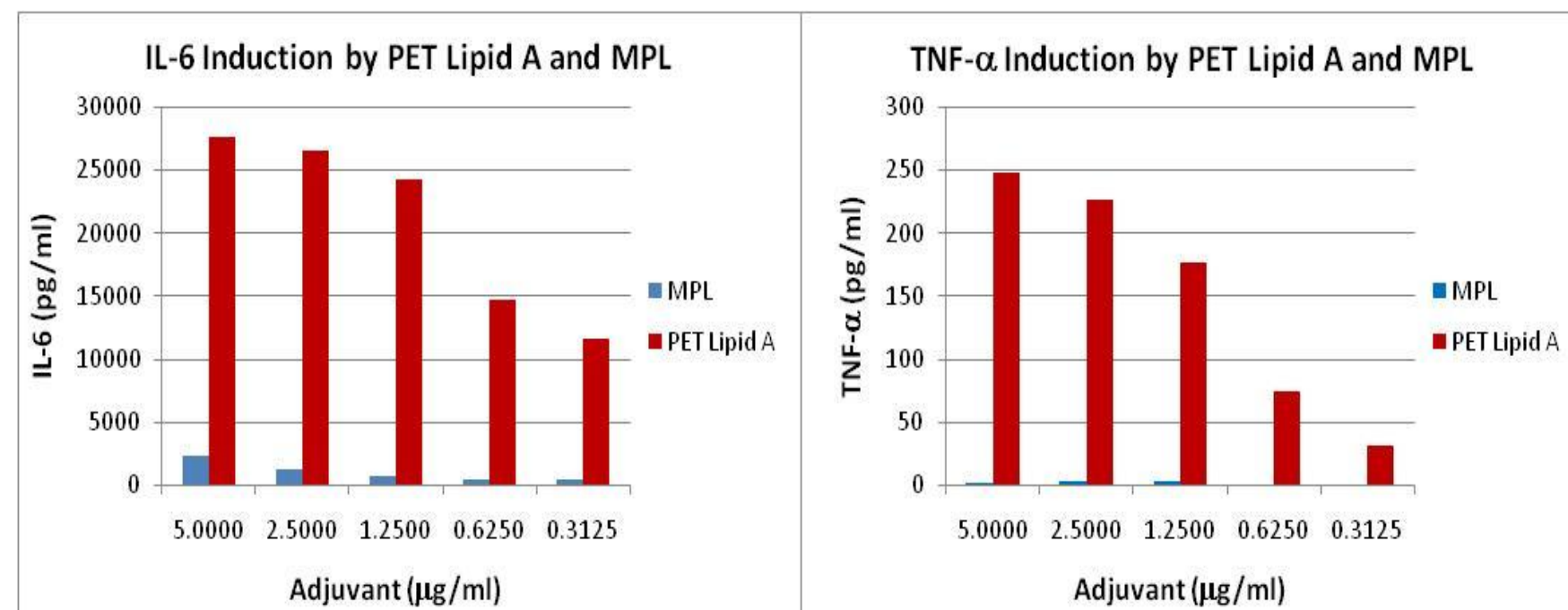
- Lipid chains (*) attached to carboxy-terminal amino acid residues to anchor peptide in liposome

PET Lipid A (penta erythritol lipid A) Adjuvant



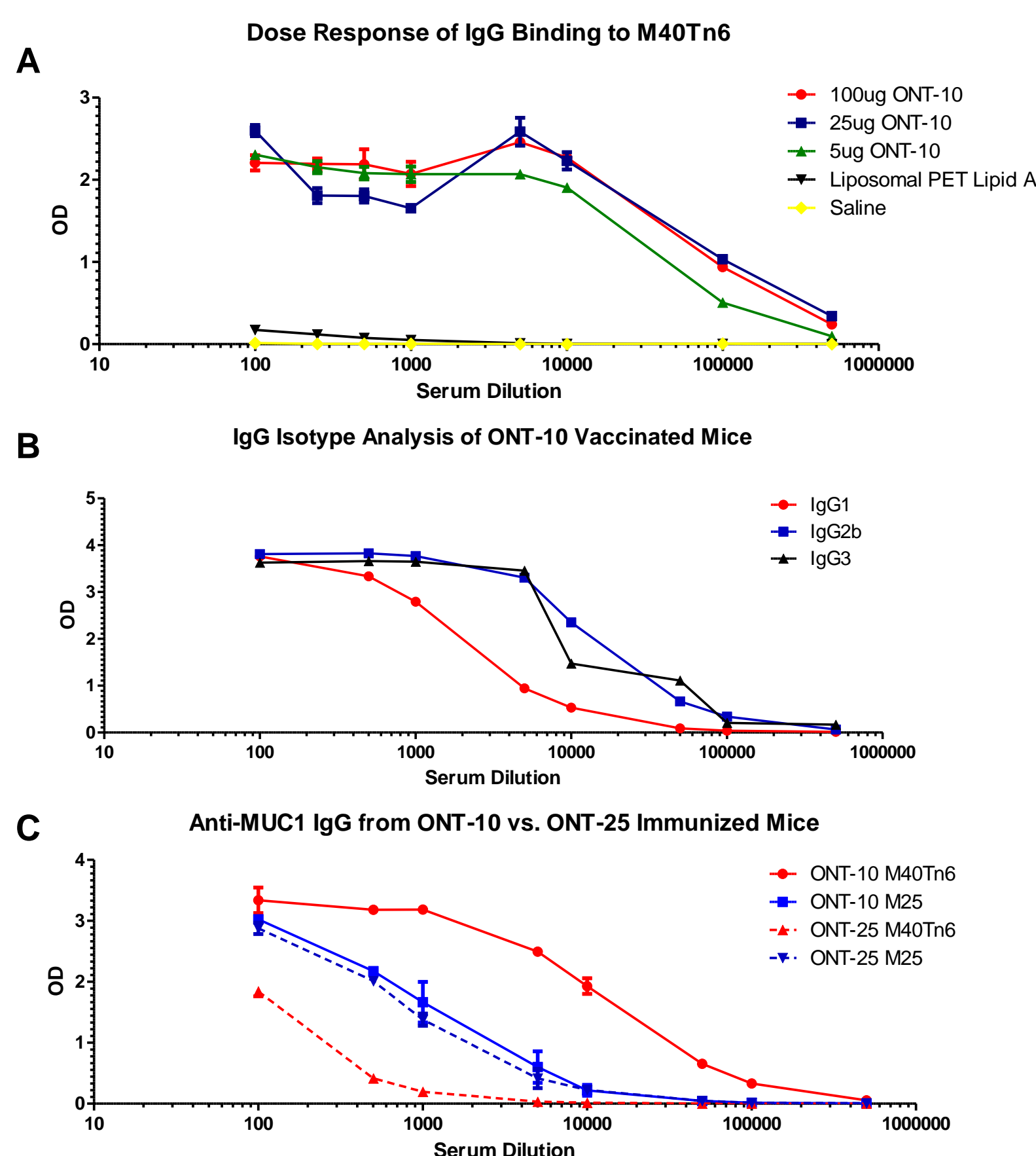
- Fully synthetic "detoxified" form of lipid A
- Interacts with TLR4 to produce a "Danger Signal"
- More potent than MPL at inducing inflammatory cytokine signal in human PBMCs

PET Lipid A is a Potent TLR4 Agonist



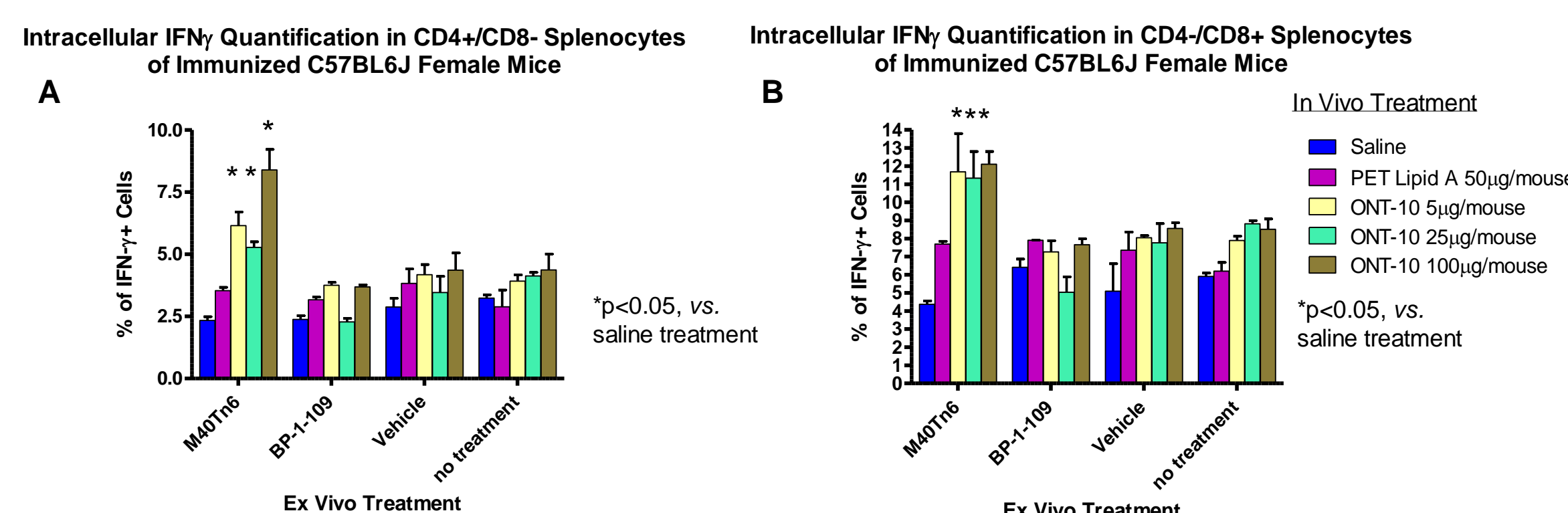
PET lipid A was compared to equal concentrations of MPL using human PBMCs. The induction of IL-6 and TNF- α was measured by ELISA 24 hours after addition of adjuvant.

ONT-10 Produces a Robust IgG Response



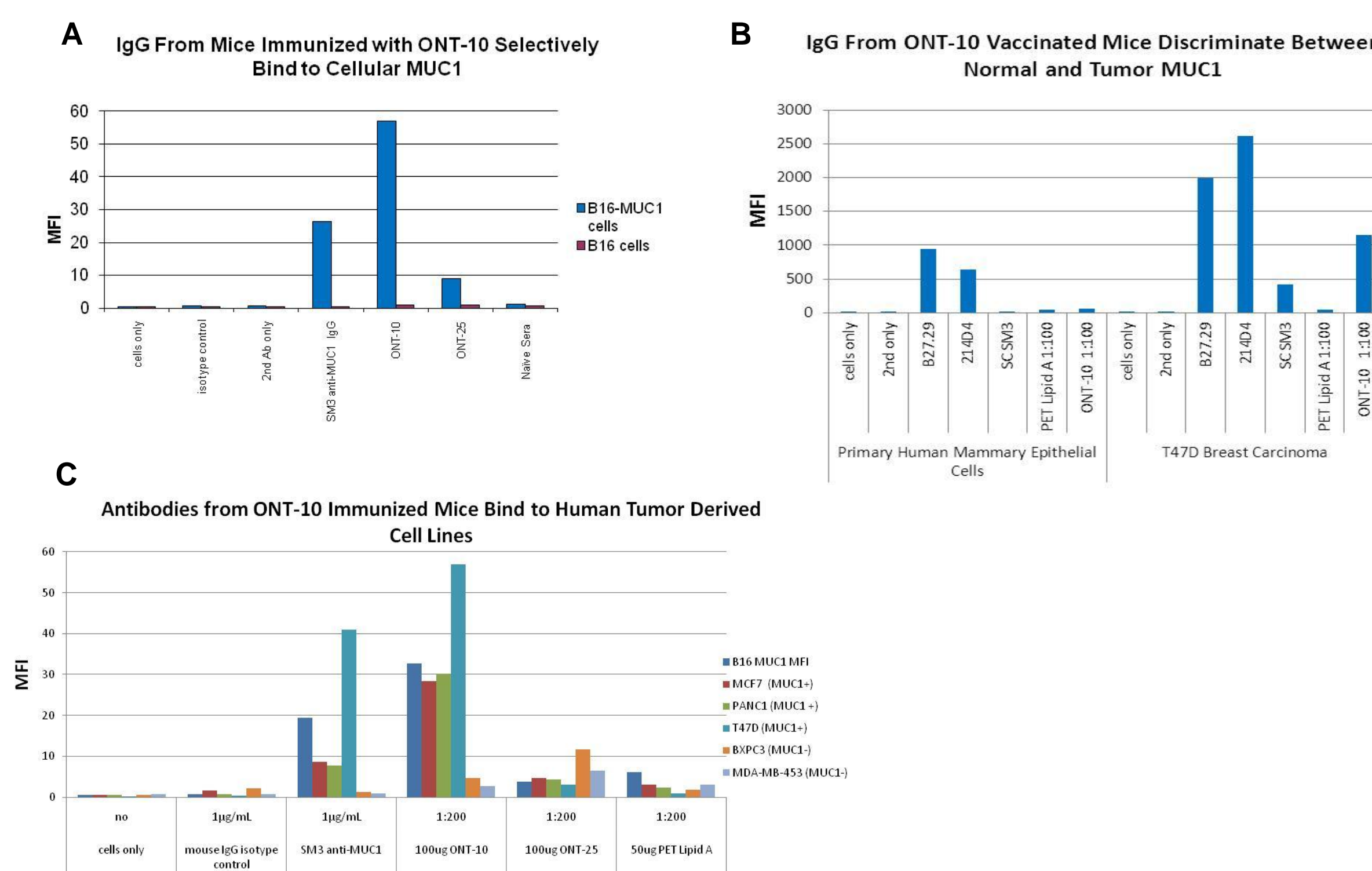
ELISA analysis of anti-MUC1 IgG levels in serum from ONT-10 vaccinated mice. **A)** Analysis of total IgG from mice vaccinated with ONT-10 (as indicated) or treated with liposomal PET lipid A or saline. **B)** Isotype analysis of serum from mice vaccinated with 100 μ g of ONT-10. **C)** Analysis of IgG binding to M40Tn6 and non-glycosylated MUC1 VNTR peptide M25 using serum from mice vaccinated with 100 μ g ONT-10 or 100 μ g of the non-glycosylated vaccine ONT-25 (as indicated).

Induction of IFN γ in CD4+ and CD8+ T Lymphocytes by ONT-10



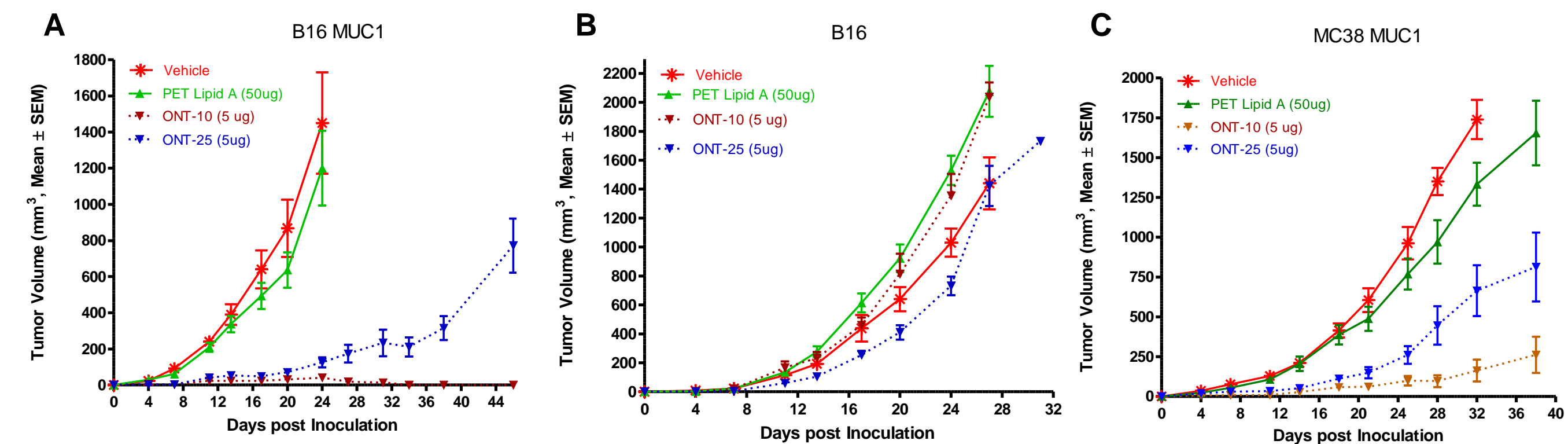
FACS analysis of IFN γ in CD4+ (**A**) or CD8+ (**B**) T cells derived from spleens of C57BL/6 mice vaccinated with ONT-10 (as indicated), treated with liposomal PET Lipid A adjuvant or saline. Splenocytes were stimulated ex vivo for 48 hrs with M40Tn6 (MUC1) peptide, BP1-109 negative control peptide, saline or no treatment. Data presented as % positive IFN γ cells relative to total CD4+ or CD8+ cell population.

Antibody Response to ONT-10 is Specific for Human Tumor MUC1

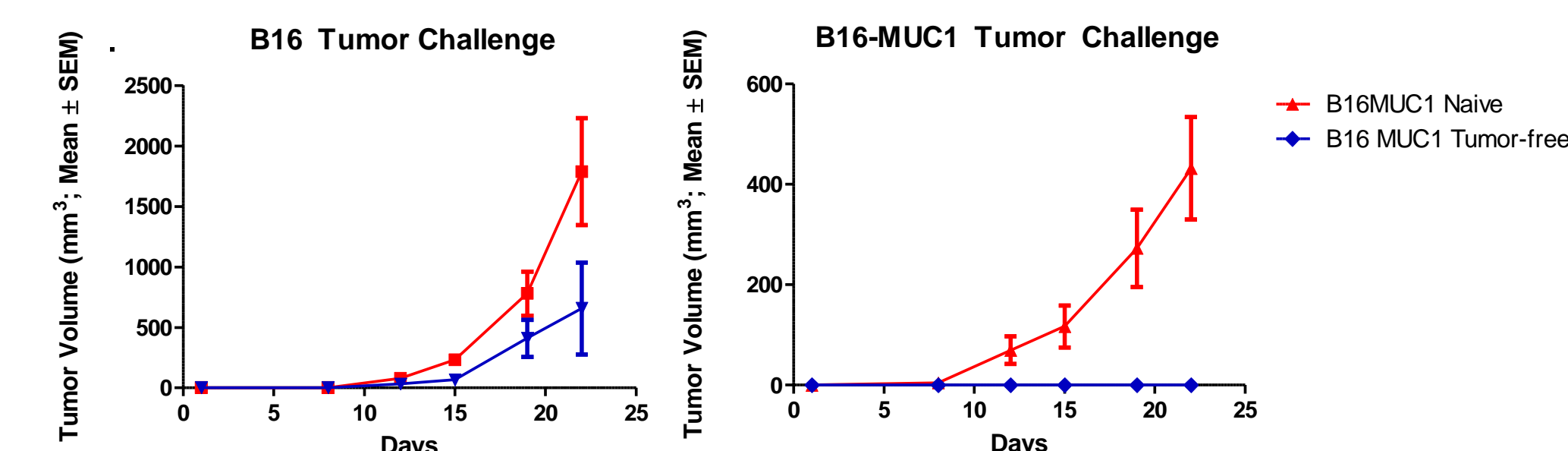


A) B16 and B16-MUC1 cells were analyzed by FACS using serum from mice vaccinated with 100 μ g ONT-10 or 100 μ g of the non-glycosylated vaccine ONT-25 (as indicated). **B)** Human primary breast epithelial cells and T47D breast carcinoma cells were analyzed by FACS using serum from mice vaccinated with 100 μ g ONT-10 or treated with 50 μ g liposomal PET Lipid A. **C)** Human tumor derived cell lines (as indicated) were analyzed by FACS for antibody binding after incubation with serum from mice vaccinated with 100 μ g ONT-10, 100 μ g of the non-glycosylated vaccine ONT-25 (as indicated) or treated with 50 μ g liposomal PET Lipid A. Anti-MUC1 antibody SM3 (SCBT SC-53381) was used as a positive control for hypoglycosylated MUC1 in all FACS studies. In panel B MUC1 monoclonal antibodies B27.29 and 214D4 (Stem Cell Technologies) were used as positive controls for fully glycosylated normal MUC1.

ONT-10 Blocks the Growth of Human MUC1 Expressing Tumors



C57BL/6 mice were treated using a bi-weekly schedule with saline vehicle, 50 μ g Liposomal PET Lipid A, ONT-10, or non-glycosylated MUC1 vaccine ONT-25 (as indicated) starting on day -42. On Day 0, mice were challenged with 2×10^6 B16-MUC1 (**A**), 0.5×10^6 B16 (**B**), or 2×10^6 MC38-MUC1 (**C**) tumor cells followed by two additional vaccinations on days +3 and +17. Tumor growth was recorded twice weekly. In the B16-MUC1 model, 9/12 animals were tumor free in the ONT-10 group and 1/12 animals were tumor free in the ONT-25 group at end of study. In the MC38-MUC1 model, 3/12 animals were tumor free in the ONT-10 group and 1/12 animals were tumor free in the ONT-25 group.



Growth of B16-MUC1 or B16 tumor cells was evaluated in naive mice or tumor free mice previously treated with ONT-10. Mice were challenged with 2×10^6 B16-MUC1 or 0.5×10^6 B16 tumor cells on Day 0, 30 days after the last immunization. Tumor growth was recorded twice weekly.

Summary and Conclusions

ONT-10 is a novel liposomal cancer vaccine that incorporates a MUC1 VNTR glycolipopeptide antigen and the highly potent TLR4 agonist PET Lipid A adjuvant

- Fully synthetic PET Lipid A adjuvant
 - Demonstrates enhanced potency vs. MPL in human PBMC assays
 - Stimulates both cellular and humoral response to antigen
- ONT-10 vaccine demonstrates potent and sustained activation of both humoral and cellular immunity to hypoglycosylated MUC1
 - Th1 biased T cell response with activation of both CD4+ and CD8+ cells
 - High titer IgG2b and IgG3 biased antibody response with specificity for hypoglycosylated tumor MUC1
 - Superior anti-tumor activity in WT mice relative to non-glycosylated MUC1 peptide vaccine (ONT-25) with up to 99% tumor control and high proportion of tumor free animals at end of study