Preclinical safety evaluation of an antibiotic-free LAMP-1 plasmid allergy immunotherapy

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Introduction

Nature Technology Corporation (NTC) has developed the regulatory agency compliant, minimal, antibiotic-free (AF) mammalian expression vectors which incorporate and express a 150 bp RNA-OUT antisense RNA . This represses expression of a host strain chromosome-encoded counter-selectable marker (*Sac*B), which is toxic in the presence of sucrose (**Fig. 1**). These sucrose-selectable vectors combine antibiotic-free selection with highly productive fermentation (>1 g/L plasmid DNA yields). NTC AF vectors (**Fig. 2)** incorporate the HTLV-I R region mRNA translational enhancer that dramatically increases plasmid-mediated transgene expression (and adaptive immune response) *in vivo* compared to conventional plasmid vectors such as pVAX1 (**Fig. 3**) .

Immunomic Therapeutics, Inc. (ITI) utilize an NTC AF vector encoding Lysosomal Associated Membrane Protein ("LAMP-1") DNA sequence, containing an antigen DNA sequence inserted into the luminal LAMP-1 DNA sequence in the DNA vaccine vector (LAMP-Vax™ Technology). The transfection of a LAMP-Vax™ plasmid results in the expression of the LAMP-1 polypeptide linked to the antigen (LAMP-1-antigen fusion protein). This protein molecule is transported through the lysosome membrane by the LAMP-1 portion of the fusion protein and is internally linked to the internal lysosomal membrane (**Fig. 4**). LAMP-1 vectors induce primarily IgG immune responses through the MHC-II pathway, which is located in the lysosome. Studies indicate that none of the LAMP-1-antigen fusion protein is released into the peripheral circulation; thus, allowing therapeutic immunization of patients with existing atopic (allergic) responses to the antigen fused to LAMP-1. This intracellular sequestration of the LAMP-1-antigen, together with the dramatic increase in IgG antibody levels (titers) against antigens/allergens achieved in animal models (**Fig. 5**) forms the rationale for the therapeutic treatment of allergic patients.

ITI performed GLP compliant pre-clinical Biodistribution and 85-Day Toxicology safety studies using an investigational NTC AF Japanese Red Cedar pollen allergy immunotherapy (JRC- LAMP-vax™). The 85-Day Toxicology in New Zealand white rabbits used a 5 dose (4 mg per dose) intramuscular regimen (days 1, 14, 28, 42 & 56) with termination at day 85, observing no alterations of the routine clinical pathology, body weights, food consumption, temperature, ophthalmology, dermal irritation and the histopathology of any tissue examined microscopically from these five (5) vaccine doses. In biodistribution and elimination studies of a single dose of JRC- LAMP-vax™ (2.064 or 4.128 mg) assessed at 3, 30 and 60 days, PCR analysis indicated tissue samples were below the Limit of Quantitation (LOQ – 20 copies per PCR reaction), except for very few exceptions (**Fig. 6**). Definitive clearance was evident at 3, 30 and 60 days in both dosing groups of both sexes. In both animal studies, there were minor variations, but no abnormal safety issues were evident. Overall, JRC- LAMP-vax™ caused no abnormal safety issues in bio-distribution and toxicology studies in animals. These safety and additional efficacy studies resulted in FDA approval of an ongoing ITI sponsored clinical trial for JRC-LAMP-vax™ in subjects sensitive to Japanese Red Cedar pollen.

Antibiotic-free selection

E. coli **NTC4862** DH5α att$_\lambda$::P$_{5/6\ 6/6}$-RNA-IN- SacB, catR
E. coli DH5α: F- Φ 80d*lac*ZΔM15 Δ(*lac*ZYA -*arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r$_K$-, m$_K$+) *pho*A *sup*E44 λ- *thi*-1 *gyr*A96 *rel*A1;

NTC8382 contains a 150 bp RNA-based selectable marker (RNA-OUT) that confers sucrose resistance onto NTC4862 by suppression of SacB translation. This allows antibiotic free plasmid selection in sucrose containing media (**Fig. 1**) and high yield fermentation (>1 g/L plasmid). (Carnes *et al.,* 2010; Williams *et al.,* 2009)

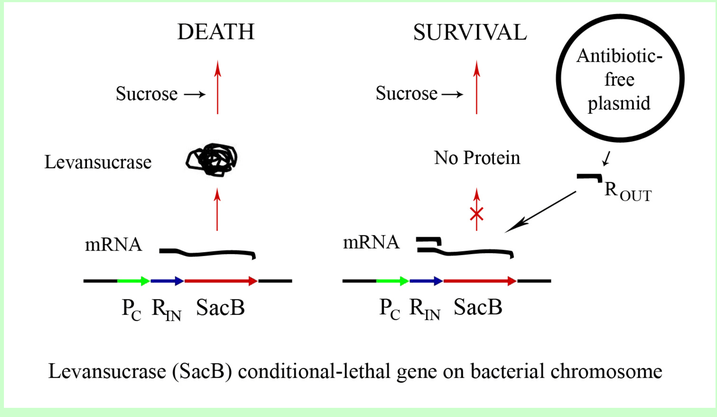

Fig. 1: Antibiotic Free (AF) RNA-OUT selection

Results

NTC8382 backbone

The NTC8382 vector backbone (**Fig. 2**) is utilized in the LAMP-VAX™ technology vectors. This vector utilizes a RNA-based antibiotic free selection (RNA-OUT; **Fig 1**) and further encodes the HTLV-R region (incorporated as part of Exon 1 and Intron 1 downstream of the CMV promoter) and Adenoviral VA1 RNAI that improves DNA vaccine expression (**Fig. 3**) and immunogenicity (Luke *et al.,* 2009, 2011).

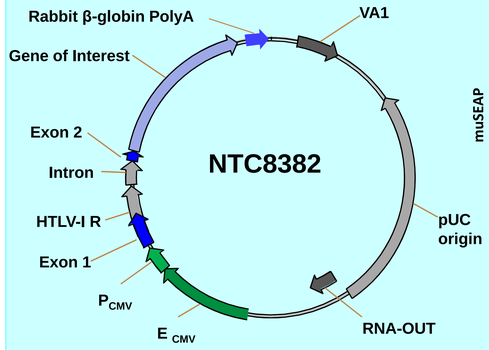

Fig. 2: NTC8385-VA1 vector with HTLV-I R and VA1 expression enhancers

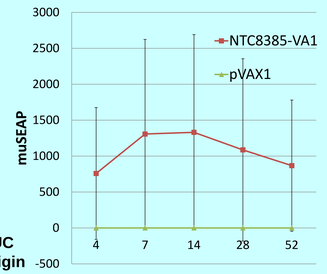

Fig. 3: 5 µg muSEAP plasmids delivered by IM EP to 5 BALB/C mice/group on day 0, muSEAP determined on days 4, 7, 14, 28, & 56. pVAX1 muSEAP was below limit of detection at all timepoints.

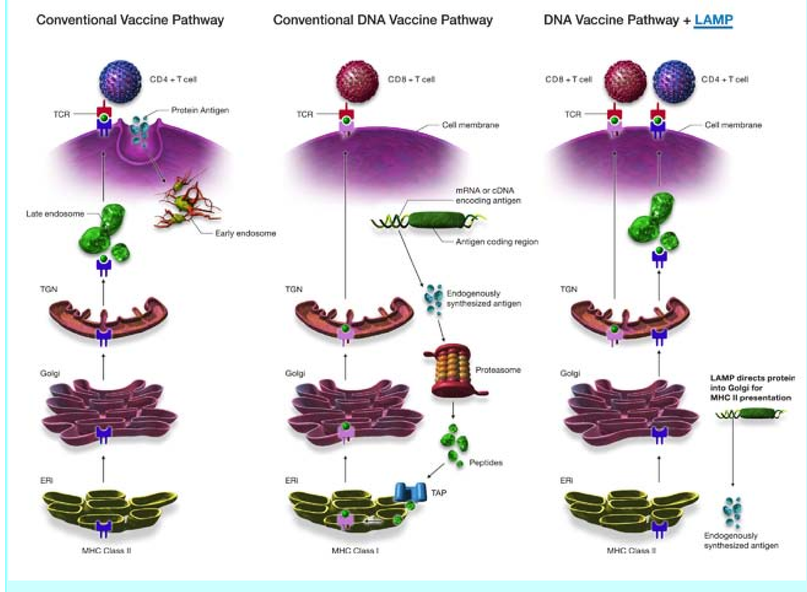

Fig. 4: LAMP-VAX™ technology MHC-II Targeting Strategy

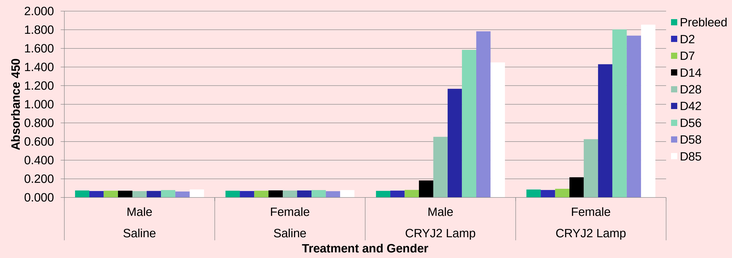

Fig. 5: JRC- LAMP-VAX™ 85-Day Toxicology safety study: Anti- Japanese Red Cedar antigen IgG response

Time Point: Day 30 / Group: High Dose / Gender: Female	Sample Type	Animal# 1181		Animal# 1182		Animal# 1183		Animal# 1184		Animal# 1185	
		Sample Value (copies / µg)									
	Adrenal Gland	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Bone Marrow	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Brain	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	0.9 µg
	Heart	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Injection site: Muscle	2.99E+02	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	2.26E+02	1.0 µg	BQL	1.0 µg
	Injection site: Sub-cutis	4.60E+03	0.3 µg	BQL	0.2 µg	BQL	0.5 µg	4.82E+03	0.5 µg	5.39E+03	0.4 µg
	Kidney	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	1.87.E+03	1.0 µg
	Liver	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Left Popliteal Lymph Node	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	0.3 µg
	Lungs	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Mesenteric Lymph Node	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Right Popliteal Lymph Node	BQL	1.0 µg	BQL	0.3 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Spleen	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Testes/Ovaries	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
	Thymus	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg	BQL	1.0 µg
		Sample Value (copies / 10µl PCR Reaction)									
	Blood	BQL	10 µl	BQL	10 µl	BQL	10 µl	BQL	10 µl	BQL	10 µl

Fig. 6: JRC- LAMP-VAX™ Biodistribution safety study: plasmid clearance from high dose group at 30 days

Summary

- JRC- LAMP-vax™ caused no abnormal safety issues in bio-distribution and toxicology studies in animals.
- ITI sponsored clinical trial for JRC-LAMP-vax™ in subjects sensitive to Japanese Red Cedar pollen is ongoing.

References

Carnes AE, Luke J, Vincent JM, Anderson S, Schukar A, Hodgson CP. and Williams JA. (2010) Critical design criteria for minimal antibiotic-free plasmid vectors necessary to combine robust RNA Pol II and Pol III-mediated eukaryotic expression with high bacterial production yields. *J. Gene Med.* 10: 818-831.

Immunomic Therapeutics, Inc. LAMP Brochure: **http://www.immunomix.com/pdf/Competitve-Advantages-of-LAMP.pdf**

Immunomic Therapeutics, Inc Press release April 11th, 2013; Immunomic Therapeutics Announces Interim Data Analysis of Phase I Study to Treat Japanese Red Cedar Allergy: **http://www.immunomix.com/Phasel_prelim_PR**

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