

# Safety and Immunogenicity of an Alphavirus Replicon Vaccine for Cytomegalovirus (CMV) in Healthy Adults

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## Introduction

Cytomegalovirus (CMV) is a  $\beta$ -herpesvirus that causes a chronic, life-long infection that can result in significant morbidity and mortality in immunocompromised individuals. CMV is also the most common congenital infection infecting 0.5-2% of newborns. Existing drugs for treatment or prevention of CMV disease are only partially effective, have a variety of side effects, and may fail because of drug-resistance. An effective CMV vaccine would provide great medical benefit and would also result in multi-billion dollar annual net savings in the cost of caring for persons with CMV disease.

Protective immunity to CMV involves both humoral and cellular immune mechanisms. The principal target of CMV neutralizing antibody is the major CMV surface glycoprotein, gB, while the principal targets for CTL activity are a phosphoprotein (pp65) and an immediate-early protein (IE1). Protection in animal models of congenital CMV have been found with both gB and pp65 vaccines.

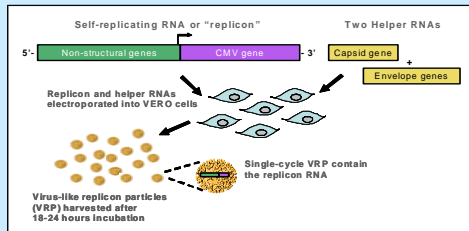
We have developed an alphavirus replicon vector system for use as a platform vaccine technology for the development of prophylactic and therapeutic vaccines for infectious diseases and cancer. Use of virus-like replicon particles (VRP) based on an attenuated strain of Venezuelan equine encephalitis (VEE) virus is especially attractive because VRP are propagation-defective, single cycle vectors that express heterologous proteins to high levels and target expression to dendritic cells. VRP vaccines have been shown to elicit both humoral and cellular immune responses to the vectored gene products that have conferred protection against challenge in many animal disease models.

We have utilized this system to produce AVX601, a bivalent VRP vaccine expressing human CMV pp65, IE1 or gB proteins (Reap et al., Vaccine 25, 2007). Described below is the Phase I Safety and Immunogenicity evaluation of AVX601 in healthy, CMV seronegative volunteers.

## Vaccine Design

AVX601 is a bivalent alphavirus replicon vaccine expressing the HCMV proteins gB, pp65 and IE1. The two separately formulated VRP components that comprise AVX601 include: gB VRP expressing a soluble form of CMV gB (Towne strain) and pp65/IE1 VRP expressing a fusion protein of the phosphoprotein (pp65) and the immediate-early protein (IE1) from HCMV strain AD169 (Reap et al., Vaccine 25, 2007). Each component of AVX601 contains a self-amplifying RNA (replicon) in which the structural protein genes of an attenuated strain of VEE virus, V3014, are replaced by a CMV gene. The vaccine was produced by purification of VRP from Vero cells co-transfected with a self amplifying RNA (replicon) transcript containing a CMV gene and two additional RNA transcripts encoding the structural protein genes of V3014 (Fig 1). The use of two separate helper RNAs greatly reduces the chance of an intact genome being assembled, by RNA-RNA recombination events, which might generate replication competent virus (RCV). Each batch of VRP vaccine is tested to confirm the absence of detectable RCV. After VRP enter a cell, VEE virus non-structural proteins are synthesized, the replicon RNA is amplified, and the CMV protein is expressed to a high level. Since the packaged replicon RNAs do not encode the VEE virus structural protein genes, the VRP are genetically restricted to a single round of replication and are not capable of producing progeny VRP in the infected cell.

Figure 1. Production of AVX601



## Clinical Trial Description

**Subjects:** Healthy, CMV seronegative males and females, 18-45 yrs old.  
**Objectives:** Primary: To evaluate the safety and immunogenicity of AVX601 in healthy volunteers  
**Study Design:** Randomized, double-blind, placebo-controlled dose escalation study of 40 participants enrolled into two groups of 20 each. Participants were randomized to receive the active vaccine by IM injection (N = 8) or SC injection (N = 8) or placebo saline by IM injection (N = 2) or SC injection (N = 2) at 2 dosage levels (Low or High Dose,  $10^7$  or  $10^8$  infectious units (IU) of each VRP component of AVX601, respectively). Participants received 2 injections at each visit at three time points (Weeks 0, 8 and 24), and were followed for 12 months after the first immunization. Participants received 0.5 mL of gB VRP in one arm and 0.5 mL pp65/IE1 VRP in the other arm, or two 0.5 mL injections of placebo. After review of safety data from Group 1 (LD cohort), Group 2 (HD cohort) was enrolled.  
**Safety** was monitored by evaluation of local and systemic reactivity, adverse events (AEs), and hematology and clinical safety chemistry parameters.  
**Immunogenicity** was assessed by measuring cellular immune responses to CMV by a gamma interferon (IFN- $\gamma$ ) ELISPOT assay and Intracellular Cytokine Staining (ICS). Antibodies to CMV were measured by CMV neutralization (50% neutralization; Towne strain).

## Results

**Safety:** AVX601 was well tolerated. Local reactivity (injection site pain, tenderness, erythema, swelling) was reported as none or mild to moderate and for pain and tenderness appeared to be dose dependent (Fig. 2A). Systemic reactivity events (fever, fatigue, malaise, headache, nausea and vomiting) were generally none or few (fever, nausea, vomiting) or mild to moderate (fatigue, malaise, headache) and equally distributed among placebo and vaccine recipients (Fig. 2B). There was no evidence for increasing reactivity with repeated dosing (data not shown). No serious adverse events (SAEs) related to AVX601 occurred.

Figure 2A. Local Reactogenicity\*

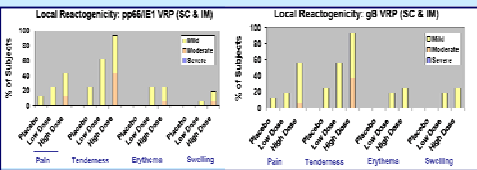
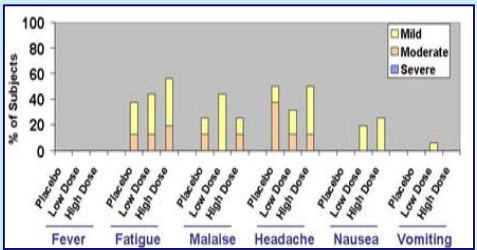


Figure 2B. Systemic Reactogenicity\*



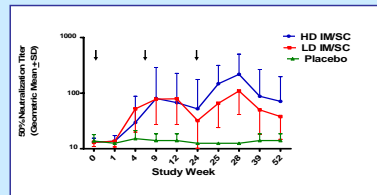
\*Maximum Symptom Toxicity Grade across all days and all injections

## Results-continued

### Immunogenicity:

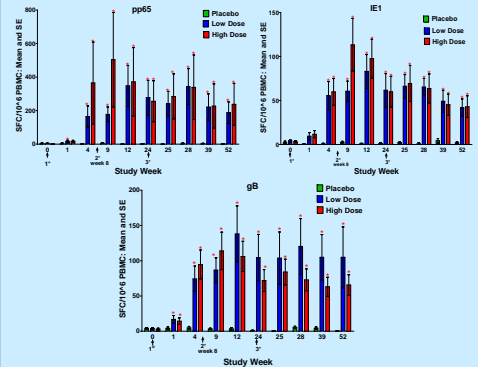
**CMV Neutralizing Antibody (NAb) Responses to AVX601:** Shown in Fig. 3 are the 50% neutralization titers (GMTs for each cohort) across all study time points. At 4 weeks post-prime, 75% and 56% of the LD and HD vaccine recipients, respectively, had measurable CMV serum NAb. After the 2<sup>nd</sup> dose, a small increase in GMT was measured in both cohorts and by study week 52, ~90% of all LD and HD subjects had measurable NAb. (A reciprocal 50% neutralization titer of  $\geq 25$  was considered a positive response.) Following the 3<sup>rd</sup> dose at SW24, titers were boosted as measured at SW 25 and 28 and CMV NAb was detected in all subjects in both the LD group (GMT= 110, range: 25-800, fold increase from SW 0 = 8.4) and HD group ( GMT= 218, range: 25-800, fold increase from SW 0= 16.7). At 6 months after the 3<sup>rd</sup> dose, 73% of LD subjects and 94% of HD subjects maintained NAb to CMV. The NAb titer for Placebo subjects was 12.5-25. (A reciprocal 50% neutralization titer of <25 was given a value of 12.5.)

Figure 3. CMV Neutralizing Antibody Responses to AVX601



**IFN- $\gamma$  ELISPOT:** The pp65-, IE1- and gB-specific T cell responses, as measured by IFN- $\gamma$  ELISPOT, are shown in Fig. 4 and Table 1. Following overnight stimulation of PBMC with pp65, IE1 or gB peptides, antigen-specific IFN- $\gamma$  secreting spot-forming cells (SFC) were detected in 93-100% of LD and HD subjects at one or more time points. The maximal mean SFC per  $10^6$  PBMC was 504 for pp65, 113 for IE1 and 138 for gB. IFN- $\gamma$  T cell responses to all three immunogens were detected in the majority of LD and HD subjects by 4 weeks post-prime. A slight boost was observed within 1 week after the 2<sup>nd</sup> dose. A booster effect was not observed following the 3<sup>rd</sup> dose at SW 25 and 28, however these responses were sustained in the majority of subjects at 3 (SW 39) and 6 months (SW 52) after the final dose (Fig. 4, Table 1).

Figure 4. CMV-Specific T Cell Responses as Measured by IFN- $\gamma$  ELISPOT



\* Denotes statistical significance at the 0.05 level (comparison with SW 0 baseline value).

## Conclusions

- AVX601 was well tolerated, with no significant safety issues identified. Local reactions (pain, tenderness) elicited by each component were similar and appeared to be dose dependent. No SAEs related to AVX601 occurred.
- AVX601 induced CMV NAb in the majority of LD and HD subjects after the 1st dose and titers were boosted after the 2nd and 3rd doses. All LD and HD subjects had CMV NAb at 4 weeks after the final dose and measurable levels were maintained in a large majority of LD and HD subjects at 6 months after the 3<sup>rd</sup> dose.
- The IFN- $\gamma$  ELISPOT assay results demonstrated that immunization with AVX601 elicited robust cellular immune responses to all three CMV immunogens in >90% of subjects in both LD and HD cohorts at one or more time points.
- Significant increases in pp65-, IE1- and gB-specific IFN- $\gamma$  secreting T cells were detected after the 1st and 2nd doses of AVX601. The magnitude of the responses measured soon after the 3<sup>rd</sup> dose was sustained in the majority of LD and HD subjects for 6 months after the final dose.
- Initial characterization of the quality of the AVX601 induced CMV-specific T cell responses has shown that the 5 HD subjects evaluated to date developed polyfunctional CD4+ and CD8+ T cells to all 3 CMV immunogens.
- The Phase I results are encouraging and support the further development of AVX601.

## Results-continued

**ICS:** Antigen-specific T cells were analyzed by flow cytometry for their ability to produce IFN- $\gamma$ , TNF- $\alpha$ , or IL-2 either individually or in any combination after stimulation with CMV peptide pools. The frequency of antigen-specific CD4+ or CD8+ T-cells producing one, two or three cytokines was calculated using FlowJo software, formatted in PESTLE and analyzed with SPICE (PESTLE and SPICE software provided by M. Roederer, NIH). Polyfunctional subsets of both CD4+ and CD8+ lineages specific for all three CMV antigens were detectable at multiple time points after vaccination. The magnitude and quality of the T cell responses varied depending on the peptide pool used for stimulation of PBMC. Shown in Fig. 5 are the profiles of 5 subjects' PBMC analyzed to date.

Fig. 5. CMV-Specific Polyfunctional T-Cell Profiles in 5 HD Subjects

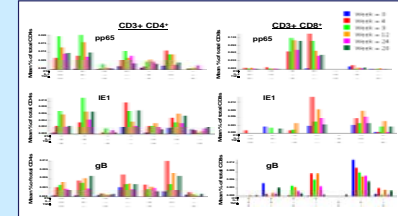


Table 1. Summary of IFN- $\gamma$  ELISPOT Responses in LD and HD Subjects

Study Week	1	4	8	12	24	28	39	52
<b>LD</b>								
Responders (R)	3	14	14	13	13	13	14	11
Total (T)	5	16	16	16	16	16	16	16
R/T	0.19	0.88	0.81	0.87	0.87	0.87	0.93	0.73
<b>HD</b>								
Responders (R)	4	13	16	15	15	15	15	13
Total (T)	16	16	16	16	16	16	16	16
R/T	0.25	0.81	1.00	0.94	0.94	0.94	0.94	0.81
<b>LD High Dose</b>								
Responders (R)	1	13	11	11	11	11	12	9
Total (T)	16	16	16	16	16	16	16	16
R/T	0.06	0.81	0.69	0.69	0.69	0.69	0.75	0.56
<b>HD High Dose</b>								
Responders (R)	3	10	13	13	13	13	14	4
Total (T)	16	16	16	16	16	16	16	16
R/T	0.19	0.63	0.81	0.81	0.81	0.81	0.88	0.25
<b>LD Low Dose</b>								
Responders (R)	3	12	13	13	11	12	13	11
Total (T)	16	16	16	16	16	16	16	16
R/T	0.20	0.75	0.87	0.87	0.73	0.80	0.87	0.73
<b>HD Low Dose</b>								
Responders (R)	3	13	14	13	13	12	13	13
Total (T)	16	16	16	16	16	16	16	16
R/T	0.19	0.81	0.88	0.81	0.81	0.75	0.81	0.81

\* Responder =  $\geq 20$  SFC (after background subtraction) and  $\geq 4$ -fold increase over Study Week 0