Poster #71

# Glycoprotein D, a Checkpoint Inhibitor of Early T cell Activation, Improves Immunogenicity and Efficacy of an HPV-16 Vaccine in Preclinical Studies

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## Introduction

- An effective therapeutic vaccine for the treatment of HPV-induced cancers has remained elusive.
- HPV E6 and E7 oncoproteins are expressed in premalignant and malignant cells thereby making them ideal therapeutic vaccine targets.
- The E5 protein augments E6 and E7 oncogenicity by increasing cell surface expression of epidermal growth factor (EGFR). E5 is expressed in premalignant lesions and in early stage cancers.
- Following vaccination, HPV specific CD8$^+$ T cell activity is blunted by T cell exhaustion resulting in loss of functions and eventual T cell death.
- VRON-0100 is an investigational therapeutic vaccine being evaluated for HPV-16-associated cancers and persistent, precancerous infections. VRON-0100 delivers a detoxified E7-E6-E5 antigenic insert of HPV-16 fused into HSV-1 glycoprotein D (gD) and inserted into a chimpanzee adenoviral vector (AdC68-gDE765dt3).
  - Humans generally lack pre-existing neutralizing antibodies to chimpanzee adenoviral vectors, which would otherwise blunt their immunogenicity.
  - gD is a herpes virus protein that acts as a checkpoint inhibitor of the immunosuppressive BTLA-HVEM pathway which impacts early T cell activation (Fig 1).
  - Replication-defective viral vectors only allow for expression of gD at the injection site and regional draining lymph nodes thereby minimizing the risk for serious "off target" side effects.

## Methods

**Vector constructs**

- An E765 fusion gene was synthesized, containing key mutations in HPV-16 E7 and E6 which reduce binding and therefore degradation of the E7 and E6 target proteins, the retinoblastoma gene product or p53, respectively.
- The detoxified E765 fusion gene was then cloned into the HSV-1 gD sequence and subsequently inserted into a E1-deleted, partial E3-deleted chimpanzee adenoviral vector (AdC68).

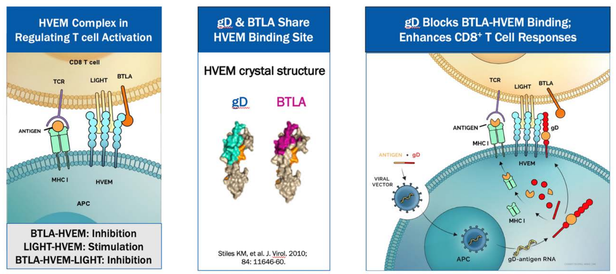
**Immunogenicity**

- C57Bl/6 mice (n=5 per group) were evaluated in all studies.
- Mice were intramuscularly (IM) injected with 5x10$^{10}$ virus particles (vp) comprising: 1) AdC68-gD (negative control); 2) AdC68 with wildtype E765 sequence inserted into gD (AdC68-gDE765wt), 3) AdC68 with detoxified E765 sequence inserted into gD (AdC68-gDE765dt3) or 4) AdC68 with detoxified E765 sequence inserted into a non-binding gD construct (AdC68-NBgDE765dt3)
- E7-specific CD8$^+$ T cell responses were tested from blood 14 days post vaccination by stains for T cell markers and an E7-specific MHC class I tetramer followed by flow cytometry.
- Vector immunogenicity was also assessed by vector dose (1x10$^9$, 2x10$^9$ and 5x10$^9$ vp) comparing frequencies of E7-specific CD8$^+$ T cells from blood of mice immunized 14 days earlier with AdC68-gDE765wt or AdC68-gDE765dt3.
- CD8$^+$ T cell function was assessed from splenocytes of mice vaccinated with AdC68gDE765wt and AdCgDE765dt3 by intracellular staining for granzyme B, interferon-γ, interleukin (IL)-2, and tumor necrosis factor (TNF)-α using peptides representing the E7 sequence.

**Efficacy**

- TC-1 cells: a tumor cell line from primary lung epithelial cells of C57Bl/6 mice that were transformed with HPV-16 E6, E7 and V-Ha-*ras* oncogene.
- Prevention Model: C57Bl/6 mice (n=5 per group) were immunized with a single IM dose of 2x10$^{10}$, 5x10$^9$ or 1x10$^9$ vp of AdC68-gDE765wt or AdC68-gDE765dt3 vectors. Two weeks later, vaccinated mice (as well as 5 naïve control mice) were injected with 5x10$^5$ TC-1 cells subcutaneously into the left flank. Tumor progression was monitored over time.
- Treatment Model: C57Bl/6 mice (n=10 per group) were challenged with 5x10$^5$ (high challenge dose) or 5x10$^4$ (standard challenge dose) TC-1 cells injected subcutaneously into the left flank. Three days later, mice received a single IM injection of 5x10$^{10}$ vp of the AdC68-gDE765wt, AdC68-gDE765dt3 or AdC68-gD (control) vector. Tumor progression and survival were monitored over time.

***Figure 1 - Glycoprotein D Mechanism of Action***

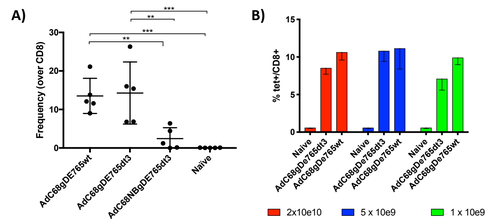



**Left** - HVEM on APC interacts with naïve T cells through a number of ligands that control CD8$^+$ T cell activation - BTLA results in inhibition; LIGHT binding, which occurs at a different domain on HVEM that that of BTLA, produces stimulation and when both ligands are bound to HVEM in a tri-molar complex, BTLA's inhibitory signaling predominates.
**Middle** - HSV gD and BTLA bind to the same domain on HVEM, however gD binds with higher affinity and thereby blocks the HVEM-BTLA interaction.
**Right** - Upon vaccination the gD protein is expressed on the APC's surface where it can bind to HVEM and block its interaction with BTLA without reducing LIGHT binding. Part of the gD-antigen fusion protein is degraded and enters the antigen presentation pathways so that the same cells that express gD-HVEM-LIGHT complexes also express antigenic peptides displayed on major histocompatibility antigens. Concomitant display of gD and the antigen blocks the BTLA-HVEM interactions, thereby removing BTLA's inhibitory signal and allowing LIGHT's co-stimulatory signaling to predominate – CD8$^+$ T cell responses to the vaccine antigen at the time of T cell stimulation are more potent and sustained and they are broadened to subdominant epitopes, which renders them more resistant to immune exhaustion within an immunosuppressive microenvironment.

## Results

- The addition of gD significantly enhances E7 immunogenicity to greater than 10%.
- The detoxifying E7 mutations have only minor effects on protein immunogenicity (Fig 2A and B).
- Splenocytes obtained from mice vaccinated with wild-type or E765dt3 antigens fused into gD show similar cytokine production profiles (Fig 3).
- The AdC68-gDE765dt3 vector is 100% protective in the TC-1 prevention model whereas untreated mice show rapid tumor growth (Fig 4).
- The AdC68-gDE765dt3 vector shows similar protection to AdC68-gDE765wt vector in the treatment model (Fig 5) and improves survival and delays tumor growth as compared to control mice upon high or standard dose TC-1 cell challenge (Fig 6A and B). In the cohorts of animals receiving standard doses of TC-1 cells, 50% of the detoxified construct vaccinated animals remained alive (Fig 6A) and tumor-free (Fig 6C).

***Figure 2 - E7 Specific CD8$^+$ T cells 14 Days After Vaccination***




(**) p = 0.001-0.01, (***) p = 0.0001 – 0.001; via 2-way Anova

***Figure 3 - CD8$^+$ T cell Function in Splenocytes***

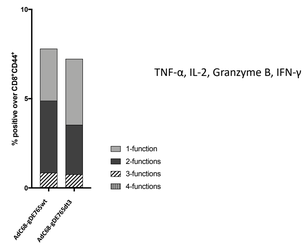
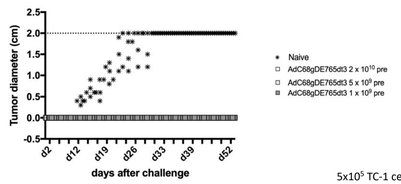



***Figure 4 - TC-1 Prevention Model***




5x10$^5$ TC-1 cells

***Figure 5 - TC-1 Treatment Model***

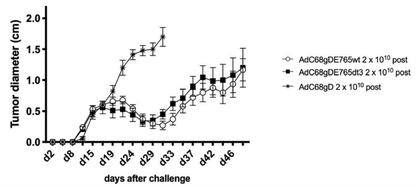
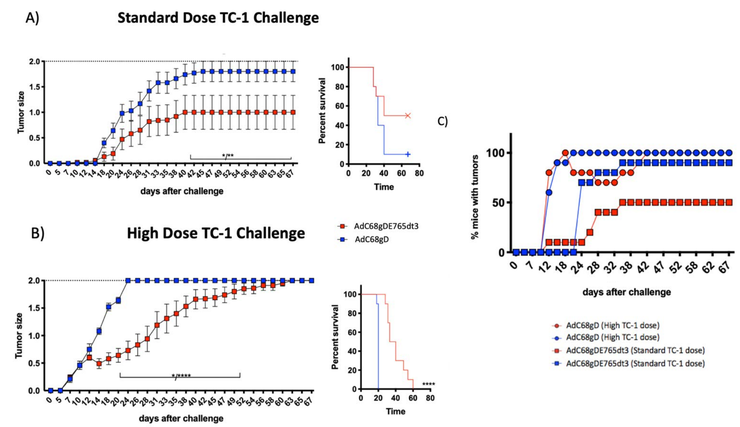



***Figure 6 - TC-1 Treatment Model Efficacy Following Single IM Injection***




(*) p = 0.05; (**) p = 0.01-0.05; (**) p = 0.001-0.01; (***) p = 0.0001 – 0.001; (****) p<0.0001 via multiple t-tests; Survival via Log-rank test

## Discussion

Therapeutic vaccines for HPV-associated cancers have been limited by a number of factors including low antigen immunogenicity and T cell exhaustion within the tumor microenvironment. HSV-1 glycoprotein D (gD), when genetically expressed as a fusion protein with tumor antigens, serves as a checkpoint inhibitor of the B and T cell attenuator (BTLA)-herpes virus entry mediator (HVEM) pathway, which acts early during T cell activation – the resultant antigen-driven CD8$^+$ T cell responses are more potent and durable and broadened to include sub-dominant epitopes which renders them more resistant to exhaustion.

Following a single IM injection, the addition of gD to a detoxified HPV-16 E765 sequence significantly enhances E7 immunogenicity and provides complete and 50% protection in the TC-1 prevention and treatment models, respectively. In the treatment model, half the vaccinated animals did not develop tumors and remained alive at study end. Both findings (enhanced immunogenicity and improved treatment responses) are highly favorable when compared with other investigational programs that use combination multi-modal therapies, multiple injections and/or prime and boost strategies (1-7) and support the mechanism of gD-induced BTLA-HVEM blockade. Boosting with a heterologous AdC vector in this model of rapid tumor growth did not improve responses (data not shown) and may be a result of gD-induced hyper-stimulation of T cells requiring a longer interval prior to boost – we did, however, see improved anti-tumor responses following prime and boost in a slow growing HPV-16 induced thyroid adenocarcinoma model (8) thereby supporting this strategy.

## Conclusions

- The addition of gD to a detoxified HPV-16 E765 antigen insert improved both immunogenicity and efficacy.
- VRON-0100 (AdC68gDE765dt3) and VRON-0101 (AdC6gDE765dt3) are being developed for HPV-16 induced cancers and persistently infected precancerous lesions – a Phase 1b study is scheduled for Q1 2021.

(1) US Patent Application 15/580964; (2) US Patent Application 16/058411; (3) US Patent Application 15/70182; (4) Khan S, et al. J Cancer. 2017; (5) Girgis N, et al. Keystone Symposium Conf Cancer Immunother. 2019; (6) Peng S, et al. Cell Biosci. 2016; (7) Chandra J, et al. J Immunother. 2017; (8) Lasaro MO, et al. Mol Ther. 2011

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