Glycoprotein D, a Checkpoint Inhibitor of Early T Cell Activation 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 receptor (EGFR). E5 is expressed in premalignant lesions and in early stage cancers.
- Following vaccination, the specific CD8+ T cell response can be abrogated by T cell exhaustion resulting in loss of functions and eventual T cell death.

Methods

Vector constructs

- An E7/65 fusion gene was synthesized, containing key mutations in HPV-16 E7 and E6 which reduce their immunogenicity.
- The detoxifying E7 mutations have only minor effects on protein immunogenicity (Fig 2A and B).

Immunogenicity

- Mice were intramuscularly (IM) injected with 5x10^10 virus particles (vp) comprising: 1) AdC68-gD (control) vector
- The AdC68-gDE765dt3 vector shows similar protection to AdC68-gDE765wt vector in the treatment model (Fig 5) and improves survival and delays tumor growth compared to removing BTLA high on standard dose TC-1 and challenge (Fig 6A and B).

Efficacy

- TC-1 cells: a tumor cell line from primary lung epithelial cells of C57Bl/6 mice that were transformed with HPV-16 E6 and E7 oncogene.
- Prevention Model: C57Bl/6 mice (n=5 per group) were challenged with 5x10^3 (high challenge dose) or 5x10^5 (standard challenge dose) TC-1 cells injected subcutaneously into the left flank. Three days later, mice received a single IM injection of 5x10^6 of the AdC68-gDE765wt, AdC68-gDE765dt3 or AdC68-gD (control) vector. Tumor progression and survival were monitored over time.

Results

- The addition of gD significantly enhances E7 immunogenicity to greater than 100%.
- The detoxifying E7 mutations have only minor effects on protein immunogenicity (Fig 2A and B).
- The AdC68-gD vector contains the wildtype E765 sequence inserted into gD (AdC68-gDE765wt), 3) a AdC68-gD vector containing the wild type E765 sequence inserted into gD (AdC68-gDE765wt), 4) a AdC68-gD vector containing the wild type E765 sequence inserted into gD (AdC68-gDE765wt).
- 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, the vaccine 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 Ad vector in this model of oncolytic 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 using a live growing HPV-16 induced thyroid adenocarcinoma model (8) thereby supporting this strategy.

Conclusion

- The addition of gD to a detoxified HPV-16 E765 antigen improves both immunogenicity and efficacy.
- VRON-0100 (AdC68-gDE765wt) and VRON-0101 (AdC68-gDE765dt3) are being developed for HPV-16 induced cancers and persistently infected precancerous lesions – Phase Ib study is scheduled for Q2 2021.

Figure 1 - Glycoprotein D Mechanism of Action

Figure 2 - E7 Specific CD8+ T Cells 14 Days After Vaccination

Figure 3 - CD8 T Cell Function in Splenocytes

Figure 4 - TC-1 Prevention Model

Figure 5 - TC-1 Treatment Model

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

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. HPV-16 gD (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 would otherwise abrogate their immunogenicity.

Poster #71

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Reference

Lasaro MO, et al. Mol Ther. 2011