Checkpoint modification of BTLA-HVEM-LIGHT signaling by HSV-1 glycoprotein D (gD) improves vaccine-induced CD8+ T cell responses in pre-clinical cancer models

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BACKGROUND

- Checkpoint inhibition by mAbs against PD-1/PD-L1, CTLA-4 and other immuno-inhibitors has revolutionized cancer treatment
- Current checkpoint inhibitors target activated T cells that are differentiating towards exhaustion[1]
  - they fail to rescue fully exhausted T cells
- Immunotherapies that can alter CD8+ T cell activation have the potential to enhance and broaden T cell responses to various cancers[1–3]
- There remains a need to produce novel cancer treatments, alone, or in combination that:
  - have better safety and tolerability
  - provide more potent and prolonged T cell responses
- **Here we present data from several preclinical cancer models investigating a novel immunotherapy platform that uses gD, a genetically encoded checkpoint modifier of early T cell activation[2–9]**

METHODS

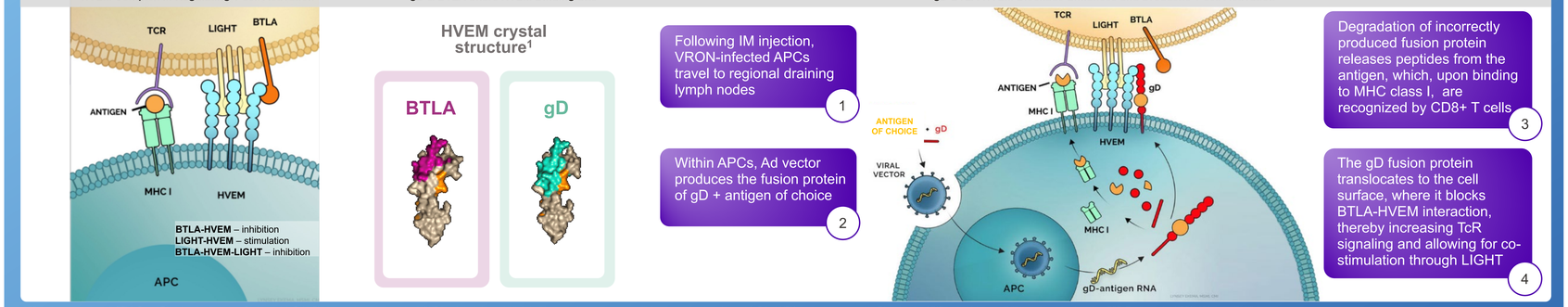
The effects of gD on immunogenicity and efficacy of antigens were assessed in a series of preclinical studies in mice:*

- HPV-16 associated cancers using early oncoprotein vaccines against transplantable TC-1 tumors in a transgenic adenocarcinoma mouse model[2,4]
- Melanoma model using an epitope vaccine called Melapoly against transplantable B16F10 tumors[5]
- Antigens were fused into gD and expressed by adenoviral vectors
  - Prime only[2,4,5] and Prime/Boost[6,7] vaccinations using heterologous vectors were explored in various studies
  - Control vaccinations used either gD alone,[7] a mutated gD that does not bind to HVEM with the antigen[2] or the antigen without gD[2,4–7]
  - Vaccination with gD-Melapoly vaccine was investigated in combination with anti-PD1 mAbs[8]
- The frequencies of antigen-specific CD8+ T cells were determined by ICS at various time points after vaccination[2–9]
- Frequencies and phenotypes of antigen-specific CD8+ T cells were tested for by staining with an MHC I tetramer and antibodies to various markers[2–9]
- Anti-tumor activity was evaluated by tumor progression and survival, which were monitored over time[2,4,7] and through histology[6]

*The full methods have been previously described[2–9]

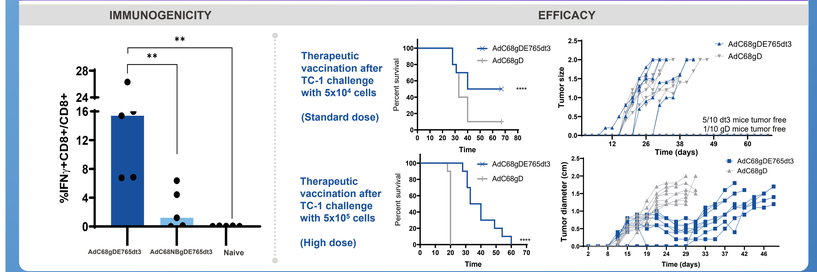
RESULTS

Herpes Simplex Virus Glycoprotein D - The Genetically Encoded Checkpoint Modifier Adjuvant[2,3,9]



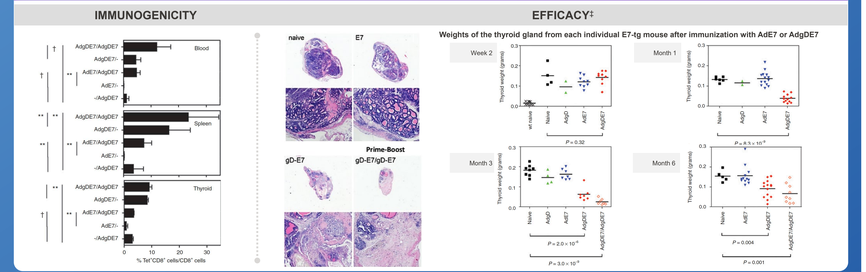
APC, antigen presenting cell; BTLA, B- and T-lymphocyte attenuator; gD, glycoprotein D; HVEM, herpes virus entry mediator; LIGHT, lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed by T lymphocytes; MHC, major histocompatibility complex; pol, polymerase; TCR, T cell receptor; VRON, Virion specific I/O therapy.

HPV-16 TC-1 Challenge Study: gD plus a detoxified HPV-16 E765 antigen insert improved immunogenicity and efficacy[2]



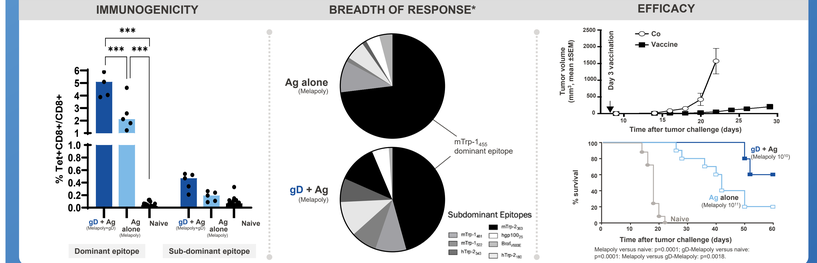
*Analysis via one-way ANOVA; **p-value between 0.001–0.01; ***p-value between 0.0001-0.001; ****p>0.0001 via multiple t tests; survival via Log-rank test. NBgD has a deletion to gD eliminating the herpes virus entry mediator binding site. AdC68 – chimpanzee adenovirus serotype 68.*

HPV-16 E7 Transgenic Mouse Model:* BTLA-HVEM blockade with gD enhanced immunogenicity and improved efficacy[6]



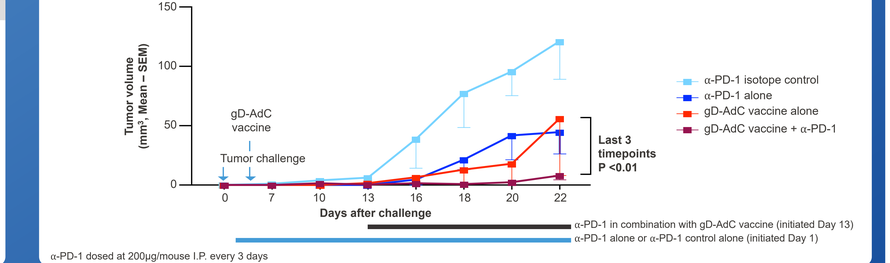
**The transgenic mouse model gradually develops adenocarcinomas due to expression of HPV-16 E7 within their thyroid. ‡The histology and weights are from the thyroid glands. Graphs show mean values +/- SD; Tp<0.05, **p<0.01. HPV, human papillomavirus; gD, glycoprotein D; E7, HPV E7 oncoprotein; treatment groups received vaccination via human adenovirus serotype 5 or chimpanzee adenovirus serotype 68.*

Melanoma (Melapoly): gD induced an Ag-specific CD8+ T cell response with increased efficacy[5,8]



**Blood collected at different time points after vaccination and measured for immune responses to individual MAA epitopes by ICS for production of interferon-γ, tumor necrosis factor-α, and interleukin-2. Analysis via two ANOVA with Sidak correction. ***p-value >0.0001; Melapoly, melanoma antigens (Trp-1, Trp-2, gp100, mutated Braf*v600E); Co, AdC68-gD; tet, tetramer.; gD –Glycoprotein D; all treatment groups vaccinated via chimpanzee adenovirus serotype 68*

Melanoma (Melapoly): anti-PD-1 mAb plus gD-containing combination demonstrated delayed tumor growth[8]



*Melapoly, melanoma antigens (Trp-1, Trp-2, gp100, mutated Braf*v600E); AdC – chimpanzee adenovirus serotype 68.*

CONCLUSIONS

Expressing a tumor-associated antigen as a gD fusion protein in preclinical cancer studies shows:

- Consistently enhanced CD8+ T cell frequencies to the disease-specific antigen(s)[2,4–7]
- Improved clinical outcomes, including survival and delayed tumor growth[2,5–7]
- Broadened CD8+ T cell responses to subdominant epitopes, which are typically not induced by the tumors[7]
- Addition of anti-PD-1 mAb treatment further improved the efficacy of gD-containing immunotherapies[8]

- Checkpoint modification by gD of the BTLA-HVEM-LIGHT pathway lowers the CD8+ T cell activation threshold and enhances, broadens and prolongs T cell responses[2,3]
- Clinical studies to evaluate therapeutic vaccination with gD are planned

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ABBREVIATIONS

AdC, chimpanzee adenovirus serotype 68; Ag, antigen; BTLA, B- and T-lymphocyte attenuator; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; gD, glycoprotein D; HBV, hepatitis B; HPV, human papillomavirus; HVEM, herpes virus entry mediator; ICS, intracellular cytokine staining; LIGHT, lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed by T lymphocytes; mAb, monoclonal antibody; MHC, major histocompatibility complex; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; SD, standard deviation; SEM, standard error of mean.

DECLARATION OF INTERESTS

Dr Ertl has the following disclosures: Co-founder of Virion Therapeutics; Advisor roles with: Freelance, Inc, Takeda, Biogen, Regenxbio, Gamaleya Institute, Ring Therapeutics, and the Canine Rabies Treatment Initiative.

FOR MORE INFORMATION

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