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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Abstract No: 143

ESMO TAT Targeted Anticancer Therapies ONLINE 7-8 MARCH 2022

ESMO-TAT Meeting
Paris France. March 7–8, 2022

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:*

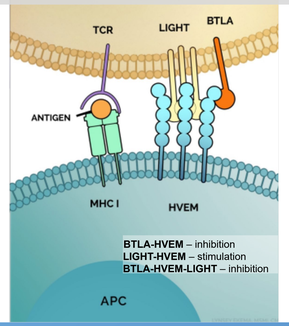
- HPV-16 associated cancers using early oncoprotein vaccines against transplantable TC-1 tumors in a transgenic adenocarcinoma mouse model[2,4]
- Melanoma model using an epitope vaccine called Melapoly against transplantable B16F10 tumors[5]
- Antigens were fused into gD and expressed by adenoviral vectors
  - 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]

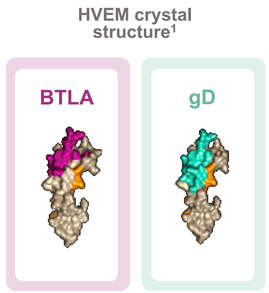
RESULTS

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

HVEM Complex in Regulating T cell Activation



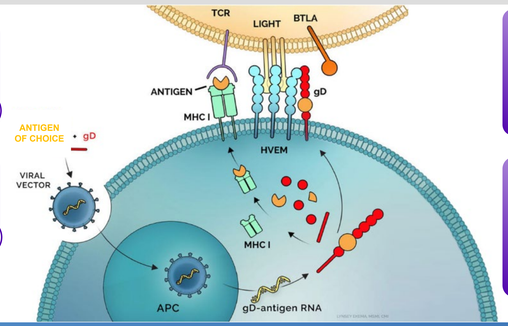
gD & BTLA Share HVEM Binding Site



Following IM injection, VRON-infected APCs travel to regional draining lymph nodes (1)

Within APCs, Ad vector produces the fusion protein of gD + antigen of choice (2)

gD BTLA-HVEM Blockade Enhances and Broadens T cell Activation[2,3]



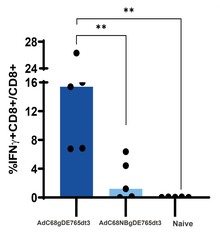
Degradation of incorrectly produced fusion protein releases peptides from the antigen, which, upon binding to MHC class I, are recognized by CD8+ T cells (3)

The gD fusion protein translocates to the cell surface, where it blocks BTLA-HVEM interaction, thereby increasing TcR signaling and allowing for co-stimulation through LIGHT (4)

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]

IMMUNOGENICITY



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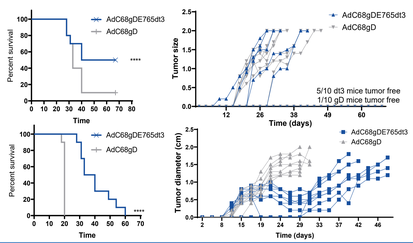

*Analysis via one-way ANOVA; **p-value between 0.001–0.01; ***p-value between 0.0001-0.001; ****p>0.0001 via multiple t tests; survival via Log-rank test. NBgD has a deletion to gD eliminating the herpes virus entry mediator binding site. AdC68 – chimpanzee adenovirus serotype 68.*

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

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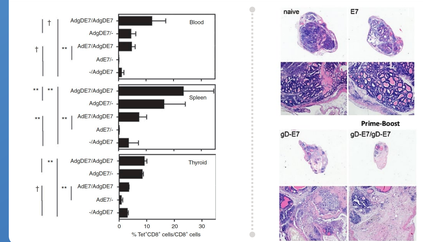

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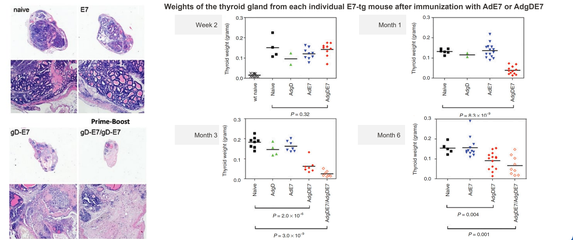

**The transgenic mouse model gradually develops adenocarcinomas due to expression of HPV-16 E7 within their thyroid. ‡The histology and weights are from the thyroid glands. Graphs show mean values +/- SD; Tp<0.05, **p<0.01. HPV, human papillomavirus; gD, glycoprotein D; E7, HPV E7 oncoprotein; treatment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

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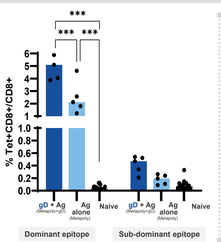
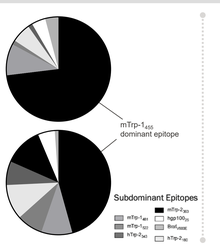


BREADTH OF RESPONSE*



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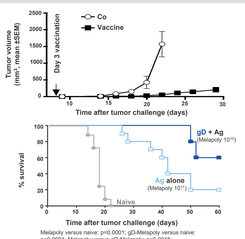
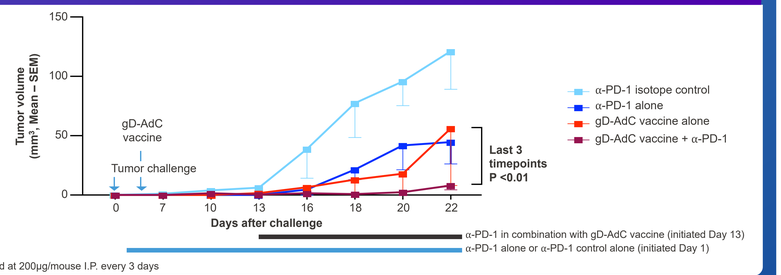


**Blood collected at different time points after vaccination and measured for immune responses to individual MAA epitopes by ICS for production of interferon-γ, tumor necrosis factor-α, and interleukin-2. Analysis via two ANOVA with Sidak correction. ***p-value >0.0001; Melapoly, melanoma antigens (Trp-1, 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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ACKNOWLEDGEMENTS

We would like to thank The Ertl Laboratory at the The Wistar Institute. All authors contributed to and approved the poster.

Medical writing assistance was provided by Sara Shaw PhD of BOLDSCIENCE Inc. and was funded by Virion Therapeutics.

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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