Poster #1003 CMV gB/pp65 eVLPs Formulated with GM-CSF as a Therapeutic Vaccine Against GBM

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ABSTRACT

Glioblastoma multiformae (GBM) is an incurable brain tumor with 75% of patients dead two years after diagnosis. A limitation of past immunotherapeutic vaccines against GBM has been the difficulty in inducing a potent tumor-specific response, due in part to the poor immunogenicity of tumor-associated antigens, the means of formulation/delivery of the vaccine, or a combination of both.

Human cytomegalovirus (CMV) is a ubiquitous, generally asymptomatic virus that is present in over 90% of GBM tumors. Memory CD4$^+$ and CD8$^+$ T cells are most frequently directed against the gB and pp65 antigens, respectively. Thus, CMV gB and pp65 represent attractive, highly immunogenic "foreign" antigen components of a vaccine against GBM. A recent phase I clinical trial based on vaccination against CMV pp65 demonstrated significant improvement in overall survival, and identified chemokine CCL3 as a correlate of efficacy.

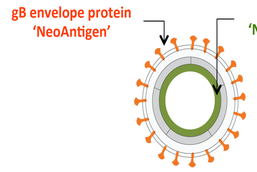
Enveloped virus-like particles (eVLPs) are produced after transfection of HEK 293 cells with plasmid encoding murine leukemia virus Gag plasmid fused in-frame with CMV pp65 antigen, which gives rise to particles. Co-transfected CMV gB plasmid enables particles budding from the cell surface to incorporate the gB protein into the lipid bilayer. Surface expression of gB and internal expression of pp65 have been confirmed by CryoEM and immunogold labelling. Pilot (10L) scale production and purification at a GMP-compliant manufacturer is underway.

eVLPs restimulate IFN--secreting CD4$^+$ and CD8$^+$ T cells in PBMCs from healthy subjects (n=8) at mean frequencies of 0.27% and 1.28%, respectively. eVLP formulation with GM-CSF augments IFN-γ and CCL3 secretion in stimulated PBMCs from healthy subjects (n=4) and GBM patients (n=4) at comparable levels between these groups. This vaccine candidate also induces *de novo* CD4$^+$ and CD8$^+$ responses in mice.

A pre-IND meeting with FDA is planned for H1 2016.

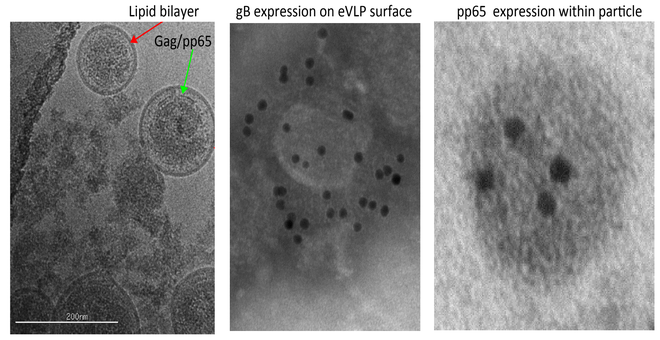
Design of GBM CMV eVLP Vaccine Candidate

Rationale for vaccine components/mechanisms of action



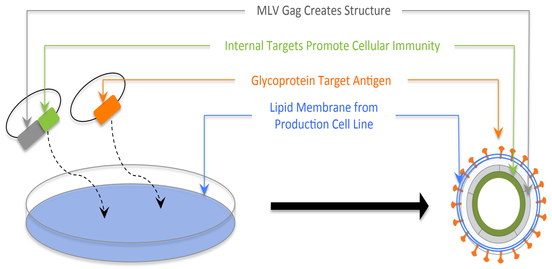
Vaccine Component	Immune Response	Scientific Support
CMV gB	Antibody response against gB expressed on surface of tumor cells	• Prevent gB activation of PDGFR-AKT signaling in tumor cells *(Cobbs C et al, 2014)* • Antibody-dependent cell cytotoxicity (ADCC)-mediated tumor cell destruction/immune activation
CMV pp65	Polyvalent CD4$^+$ T helper cell & CD8$^+$ CTL responses	• CMV pp65 vaccination in concert with dendritic cell activation prolongs overall survival of GBM patients *(Mitchell DA et al, 2015)* • Responses against multiple epitopes and antigens (gB & pp65) avoid immunoselection/tumor escape
eVLP formulation with GM-CSF	Augment tumor-specific IFN-γ and CCL3 responses	• Clinical data demonstrate IFN-γ and CCL3 as key biomarkers of efficacious tumor immunity *(Galon J et al, 2006; Mitchell DA et al, 2015)*

Cryo-EM & Immunogold Labeling Demonstrates Location of CMV Antigens in Bivalent gB/pp65 eVLPs



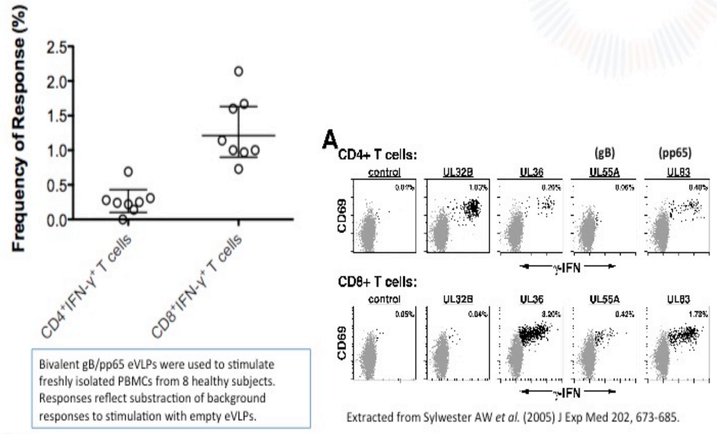
eVLP Production: Multiple Genes can be Expressed on the Surface Within a Membrane and/or Internal to the Particle

Flexible, Customized Antigen Delivery in a Biologically Relevant Construct



- eVLP Manufacturing
  - Two plasmids required for production
    - MLV gag +/- internal antigen creates viral structure
    - Target envelope antigen co-transfected
  - Production cell line contributes lipid membrane
    - Flexibility: Multiple cell lines can be used
    - HEK 293 cells offer human glycosylation patterns (like virus)
  - Stable & transient transfection possible

Bivalent gB/pp65 eVLPs Stimulate Both CD4$^+$ and CD8$^+$ Human T cell Responses *Ex Vivo*

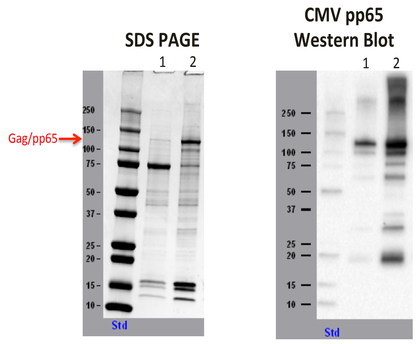


Bivalent gB/pp65 eVLPs were used to stimulate freshly isolated PBMCs from 8 healthy subjects. Responses reflect substraction of background responses to stimulation with empty eVLPs.

Extracted from Sylwester AW *et al.* (2005) J Exp Med 202, 673-685.

Characterization of Purified eVLPs

SDS PAGE & Western Blot analysis confirm good quality of bivalent gB/pp65 eVLP materials

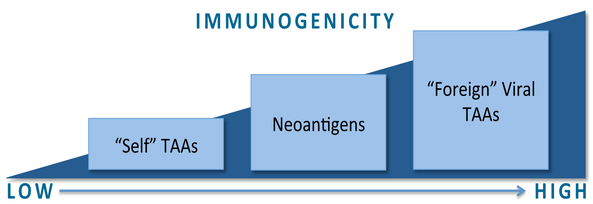


Samples:

Lane 1: VBI bivalent gB/pp65 eVLPs

Lane 2: CMO bivalent gB/pp65 eVLPs

Poor Immunogenicity of Traditional Tumor-Associated Antigens (TAAs) has Limited Past Therapeutic Cancer Vaccines

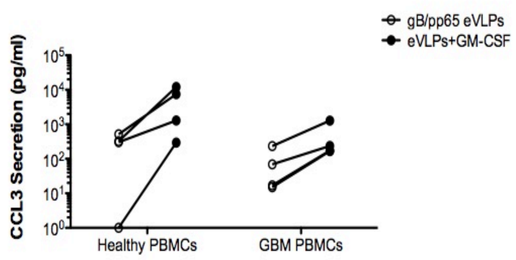


VIRAL CMV ANTIGENS ARE OVER-EXPRESSED (>90%) IN MULTIPLE SOLID TUMORS, INCLUDING:

- Glioblastoma (GBM)[1]
- Medulloblastoma[2,3]
- Meningioma[3]
- Breast cancer[4,5]

Sources: [1]Cobbs CS(2013) Curr Opin Oncol 25, 682; [2]Baryawno N(2011) J Clin Invest 121, 4043-4055; [3]Libard S(2014) PLoS ONE 9, e108861; [4]Taher C(2013) J Clin Virol 54, 240; [5]Harkins LE (2010) Herpesviridae 1, 8

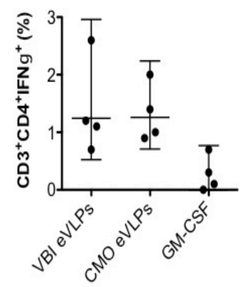
GBM Patient PBMCs Respond to Bivalent CMV eVLP & GM-CSF Stimulation



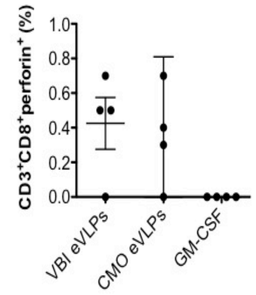
Bivalent gB/pp65 eVLPs were used to stimulate **frozen/ thawed PBMCs** from 4 healthy subjects and 4 primary GBM patients. CCL3 secretion after stimulation with empty eVLPs has been subtracted from all values.

Bivalent gB/pp65 eVLPs Formulated with GM-CSF Induce Desired Immunity in Mice

Induction of CMV pp65 Th1 Response



Induction of CMV gB CTL Response



Mice (n=4/group) were immunized at 0 and 4 weeks, and splenocytes harvested 10 days later. Splenocytes from the above groups were stimulated with recombinant CMV gB or pp65 antigens; responses against empty eVLPs were subtracted from all responses.