Therapeutic Vaccination Against Glioblastoma Multiformae Using CMV gB/pp65 eVLPs Formulated With GM-CSF

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ABSTRACT

Glioblastoma multiformae (GBM) is presently an incurable brain tumor with 75% of patients dead two years after diagnosis. Approximately 15,000 new GBM diagnoses arise in the US each year, and 2-3 people/100,000 each year in most European countries.

A limitation of past immunotherapeutic vaccines against GBM has been the difficulty in inducing a potent tumor-specific response, due at least in part to the inherently poor immunogenicity of tumor-associated antigens, the means of formulation/delivery of the vaccine, or a combination of both.

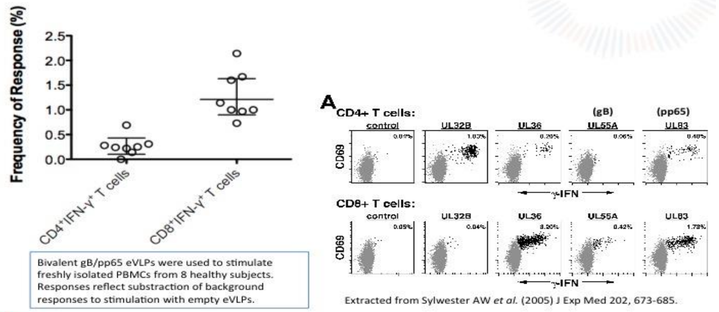
Human cytomegalovirus (CMV) is a ubiquitous, generally asymptomatic virus that is present in over 90% of GBM tumors. Memory CD4+ and CD8+ T cells are most frequently directed against the gB and pp65 antigens, respectively. Thus, CMV gB and pp65 represent attractive new "neoantigen" components of a vaccine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

We are developing a novel enveloped virus-like particle (eVLP) vaccine for treatment of GBMs. Our eVLPs are produced after transfection of HEK 293 cells with plasmid encoding murine leukemia virus (MLV) Gag plasmid fused in-frame with the CMV pp65 antigen, which gives rise to the particles. Plasmid expressing CMV gB antigen is co-transfected such that particles budding from the surface of the cells incorporate the gB protein into the lipid bilayer while the CMV pp65 antigen remains internal to the particles. Pilot (10L) scale production at a GMP-compliant CMO is underway.

Using peripheral blood mononuclear cells (PBMCs) from healthy subjects, we have found that gB/pp65 eVLPs restimulate IFN-γ-secreting CD4+ and CD8+ T cells in all subjects examined (n=8) at mean frequencies of 0.27% and 1.28%, respectively. When formulated with GM-CSF, gB/pp65 eVLP stimulation of PBMCs from healthy subjects (n=4) and GBM patients (n=4) induces both IFN-g and CCL3 secretion at comparable levels between these groups.

A mouse study is underway to determine optimal doses, route of administration, and formulation of GM-CSF and eVLPs. A pre-IND meeting is planned with FDA in Q4 2015.

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

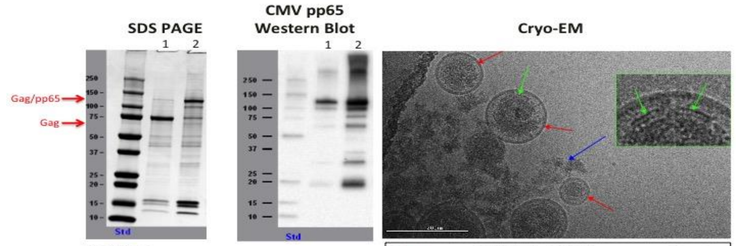


Bivalent gB/pp65 eVLPs were used to stimulate freshly isolated PBMCs from 8 healthy subjects. Responses reflect substraction of background responses to stimulation with empty eVLPs.

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

Characterization of Purified eVLPs

Analytical data confirm good quality of bivalent gB/pp65 eVLPs produced and purified by a CMO



Samples:
Lane 1: VBI bivalent gB/pp65 eVLPs
Lane 2: CMO bivalent gB/pp65 eVLPs

Green arrows highlight the lipid bilayer seen on intact eVLPs, while green arrows highlight the electron dense core structure comprised of MLV Gag and CMV pp65.
Bar scale = 200nm.

CMV as a NeoAntigen

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

- Glioblastoma (GBM)[1]
- Breast cancer[2,3]

CLINICAL EVIDENCE SUGGESTS CMV VACCINATION CAN BE SUCCESSFUL (DUKE DATA)[4]

- Dendritic cell priming combined with CMV vaccination significantly extended overall survival of GBM patients relative to SoC

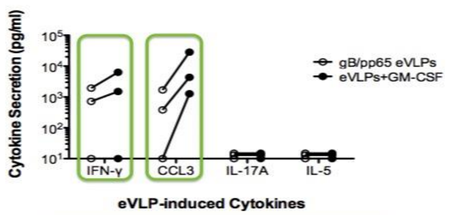
GBM UNMET MEDICAL NEED

- Over 20,000 new patients diagnosed each year
- Only 40% survive longer than 6 months[5]
- GBI Research predicts a market size of $600+ million by 2020

Sources: [1]Cobbs CS(2013) Curr Opin Oncol 25, 682; [2]Taher C(2013) J Clin Virol 54, 240; [3]Harkins LE (2010) Herpesviridae 1, 8; [4]Mitchell DA(2015) Nature 519, 366-369; [5]Ohgaki (2004) Cancer Research, 64:6892

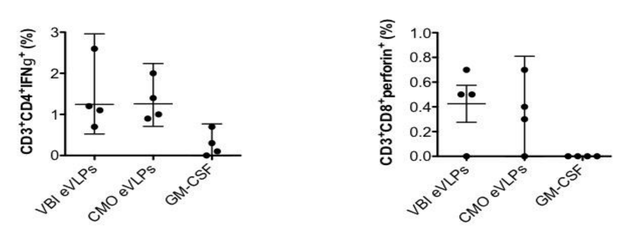
VBI GBM Vaccine Elicits Strong Th1 Immunity & Mobilizes Dendritic Cells when Adjuvanted with GM-CSF

eVLP stimulation elicits desired immuno-phenotype – Correlates with clinical biomarker identified in Duke study *(Mitchell DA(2015) Nature 519, 366-369)*



Bivalent gB/pp65 eVLPs were used to stimulate **freshly isolated PBMCs** from 3 healthy subjects. Cytokine secretion after stimulation with empty eVLPs has been subtracted from all values.

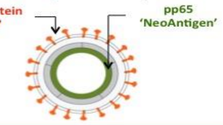
Bivalent gB/pp65 eVLPs Formulated with GM-CSF Induce Desired Immunity in Mice



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.

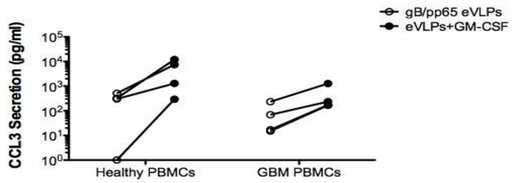
Design of GBM CMV eVLP Vaccine Candidate

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)*

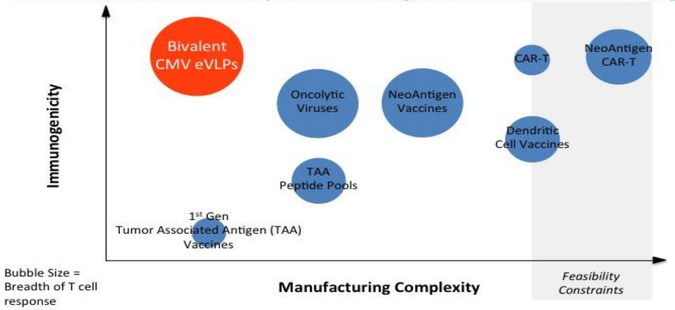
GBM Patient PBMCs Respond to Bivalent CMV eVLP & GM-CSF Stimulation



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.

CMV eVLP "NeoAntigen" Strategy vs Alternate Cancer Vaccine Approaches

eVLPs Balance Potent Delivery of Diverse Antigens with Ease of Manufacturing



Bubble Size = Breadth of T cell response