IMST-30. EVALUATING THE IN-VITRO EFFECTS OF TUMOR- TREATING FIELDS ON T-CELL RESPONSES

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BACKGROUND: Tumor Treating Fields (TTFields) are low-intensity electrical fields that target proliferating cells by hindering the formation of mitotic spindle and the translocation of charged organelles. Combining TTFields with immune-based therapies is a rational approach as they possess different mechanisms of action (MOA). Conversely, TTFields may potentially abrogate various cellular functions of T-cell responses which we studied the effect of TTFields on select human T cell functions which are pivotal for an effective anti-tumor response. METHODS: Peripheral blood mononuclear cells (PBMC) were isolated from healthy adults. Cells were cultured in culture medium with conditioned medium (CM) and conditioned medium secreted by tumor cells (TUM). RESULTS: TTFields did not alter the functionality of non-activated T cells. Viability, CM-activated T cells treated with TTFields exhibited no change in their PD1 up-regulation (activation), in their IFNγ secretion and in their CD107a surface-expression (cytotoxicity). Viable, CM-activated T cells exhibited reduced proliferation, in line with TTFields known MOA. Polymorphismality, e.g., the secretion of more than one cytokine by a single T cell, is associated with effective anti-pathogen and anti-tumor responses. A polymorphismality analysis of the activated T cells demonstrated that under TTFields conditions, T cells that had lost the capacity to proliferate retained all other polymorphic/combinatorial immune functions. All findings were true both for T helpers and for cytotoxic T cells. CONCLUSIONS: TTFields, as a pivotal T cell response parameter, with the exception of proliferation, were found to be unaffected by TTFields. The data suggests that the integration of TTFields with various immunotherapeutic approaches may be a rational approach to explore for the treatment of brain tumors.

IMST-31. CMV gB/pp65 eVLPs FORMULATED WITH GM-CSF AS A THERAPEUTIC VACCINE AGAINST GBM AND MEDULLOBLASTOMA

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A limitation of past immunotherapeutic vaccines against glioblastoma multiforme (GBM) and medulloblastoma (MED) 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, and the combination of both. Human cytomegalovirus (CMV) is a ubiquitous, generally asymptomatic virus that is present in over 90% of GBM and MED tumors. Memory CD4+ and CD8+ T cells are most frequently directed against the gB and pp65 antigens, respectively. Thus, CMV gB/pp65 envelope glycoprotein (eVLPs) are produced by transfection of HEK 293 cells with a plasmid encoding murine leukemia virus Gag fused in-frame with CMV pp65 antigen (eVLPs) are produced after transfection of HEK 293 cells with a plasmid encoding murine leukemia virus Gag 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 labeling. Production and purification at the scale required for clinical trials (50L) at a GMP-compliant manufacturing facility in order, eVLPs are formulated in IFNγ 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 IFNγ and CCL3 secretion in stimulated PBMCs from healthy subjects (n=4), GBM patients (n=4), and MED patients (n=4) at comparable levels among these groups. This vaccine candidate also induces CD4+ and CD8+ responses in mice. A pre-IND meeting with FDA is planned for H1 2016, with anticipated phase I/IIa trial start in H1 2017.

IMST-32. T CELL EXHAUSTION IN PATIENTS WITH MEDULLOBLASTOMA

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Glioblastoma (GBM) persists as the most common primary brain tumor in adults and is one of few cancers that remain uniformly lethal despite multiple advances in therapy. Despite offering the promise of improved survival, a ceiling in the efficacy of immunotherapy is imposed by GBM's propensity for sabotaging immune responses and propagating T cell dysfunction. Exhaustion is a well-recognized mode of T cell dysfunction whereby cells become hyporesponsive following repeated antigen exposure. While exhaustion is an increasingly recognized impediment to T cell function in cancer, its contribution to immune escape in GBM has not been well-evaluated. We therefore recently characterized a broad array of T cell exhaustion markers in human and murine GBM and murine models of CNS metastasis. TILs and matched peripheral blood from 8 patients with GBM, as well as blood from 8 age-matched controls, were evaluated for expression of T-cell exhaustion markers by flow cytometry. The same assessments were repeated in mice with CT2A and SMA-560 gliomas, Lewis lung carcinomas (LLC), or B16F10 melanomas implanted either intracranially (IC) or subcutaneously (SC). Expression of the exhaustion markers was correlated with overall survival of patients with GBM, and identified new T cell exhaustion markers in murine models of CNS metastasis that did not vary across IC or SC location for a given tumor, while glioma models demonstrated most severe exhaustion phenotypes. Our findings suggest that tumors, and particularly GBM, exhibit characteristic and varying mechanisms for eliciting an exhaustion "fingerprint" among infiltrating lymphocytes that is tumor- and not microenvironment-intrinsic.

IMST-33. ENHANCED T CELL ACTIVATION USING DENDRITIC CELLS PULSED WITH CHIMERIC RNA ENCODING FULL-LENGTH LAMP-1 FUSION CONSTRUCTS

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BACKGROUND: Human CMV antigens have been proposed as novel targets for immunotherapy in glioblastoma (GBM). We previously demonstrated in a pilot randomized and blinded clinical trial, that vaccination of patients with newly-diagnosed GBM with autologous dendritic cells (DC) pulsed with RNA encoding the CMV antigen pp65 led to the expansion of pp65-specific T cells compared to vaccination with LAMP-1 protein. This finding is consistent with previous studies showing that T cell responses are improved in patients treated with RNA viral vaccines compared to protein vaccines. RESULTS: We have extended these studies in the context of a phase 1 clinical trial based on vaccination against CMV pp65 demonstrated significant improvement in overall survival of patients with GBM, and identified chemokine CCL3 as a correlate of efficacy. Enveloped virus-like particles (eVLPs) are produced after transfection of HEK 293 cells with a plasmid encoding murine leukemia virus Gag fused in-frame with CMV pp65 antigen (eVLPs) are produced after transfection of HEK 293 cells with a plasmid encoding murine leukemia virus Gag 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 labeling. Production and purification at the scale required for clinical trials (50L) at a GMP-compliant manufacturing facility in order, eVLPs are formulated in IFNγ 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 IFNγ and CCL3 secretion in stimulated PBMCs from healthy subjects (n=4), GBM patients (n=4), and MED patients (n=4) at comparable levels among these groups. This vaccine candidate also induces CD4+ and CD8+ responses in mice. A pre-IND meeting with FDA is planned for H1 2016, with anticipated phase I/IIa trial start in H1 2017.

IMST-34. IMMUNOLOGICAL CHANGES DURING TUMOR PROGRESSION FROM PRIMARY TO RECURRENT GLOBLASTOMA

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