Background

In a murine model of 5-week established (~200mm^3) melanoma, we previously reported that combined treatment with radiation and intratumor (IT) injection of anti-GD2 hu14.18-IL2 immunocytokine (IC) results in an *in situ* vaccine effect, rendering most mice disease-free and eliciting tumor-specific T cell-dependent immunologic memory. In the treatment of larger 7-week (500 mm^3) melanomas, combining this *in situ* vaccine with anti-CTLA-4 resulted in greater complete tumor regression (73%) and enhanced survival compared to dual combinations of radiation, IT-IC, and anti-CTLA-4. All mice (17/17) rendered disease-free by this triple combination therapy exhibited immunologic memory. Here, we evaluate whether this combination may trigger an endogenous anti-tumor antibody response.

Methods

GD2$^+$ B78 melanoma cells were injected subcutaneously on the flank of C57BL/6 mice. After 5 weeks, B78 tumors were treated with 12Gy, IT injection of hu14.18-IL2 on days 6-10 after radiation, and IP injection of anti-CTLA-4 on days 3, 6, and 9 after radiation. Blood was drawn from mice via facial vein bleeds prior to treatment, at 10 day intervals thereafter to day 50, and from disease-free animals >90 days after radiation. Serum from these samples was tested by flow cytometry against the closely related GD2$^-$ B16 melanoma (parental to B78) and the unrelated GD2$^+$ Panc02-GD2 pancreatic tumor lines for the presence of tumor-specific IgM and IgG antibodies. Functional capacity of tumor-specific serum was tested using Complement-Dependent Cytotoxicity (CDC) assays.

Results

A tumor-specific endogenous IgG response was observed against GD2$^-$ B16 melanoma in untreated tumor-bearing, untreated mice, and levels of this tumor-specific IgG declined over the first ~20 days following combined treatment with 12Gy + IT-IC + anti-CTLA-4. Beginning 20-30 days after this treatment, a tumor-specific IgM response against GD2$^-$ B16 melanoma was identified in most mice. T and this briefly peaked and then declined by day 50. A renewed tumor-specific IgG response was observed in serum from mice rendered disease-free with combined *in situ* vaccine and anti-CTLA-4, and the level of this tumor-specific IgG increased modestly following subcutaneous re-challenge of these mice with B78 cells.

Conclusions

In this preclinical melanoma model, combined treatment with 12Gy + IT-IC + anti-CTLA-4 augments an endogenous antibody response to the B78 tumor, resulting in a memory humoral response in animals rendered disease-free. This may suggest an opportunity to improve this treatment regimen by using the endogenous anti-tumor B cell response as a biomarker of adaptive anti-tumor immunity.

P38

A strategy to assess contributions of individual agents in combination immunotherapy trials

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Background

A recent FDA workshop sought discussion on how to demonstrate the contribution of each individual agent in combination immunotherapy trials. Here we outline a strategy of coordinated assessment of B and T cell response kinetics to a complex cancer vaccine as a possible model for such an evaluation. This strategy was applied to assess patients receiving DPV-001 DRibbles®, a dendritic cell-targeted microvesicle (proteasome blocked autophagosome) vaccine derived from adenocarcinoma and mixed histology cancer cell lines. It contains multiple TLR agonists and > 200 potential NSCLC antigens, many as prospective altered-peptide ligands or neoantigens. Based on preclinical studies where anti-OX40 significantly (P<0.05) improved survival and apparent cures, a clinical trial of DPV-001 plus anti-OX40 is planned and will use the proposed strategy to monitor the impact of this combination for cancer immunotherapy.

Methods

Patients received induction cyclophosphamide followed by 7 vaccines at 3-week intervals. The first vaccine was given intranodally; subsequent vaccines intradermally. Patients were randomized to receive DRibble alone (A), or with imiquimod (B) or GM-CSF (C). Thirteen pts were enrolled (Arm A: 5; B: 4; C: 4). PBMCs and serum were collected at baseline and at each vaccination to assess changes in antibodies (Ab) (Protoarray, microsphere affinity proteomics (MAP)), cytokines (Quanterix), PBMCs (flow cytometry) and TCR repertoires (Adaptive immunoSEQ).

Results

IgG levels to 5000 proteins was assessed thrice prior to vaccination and at 3-week intervals. For some antigens, IgG responses peaked and then returned to baseline with new Ab responses developing or being augmented at each time point. In others, Ab responses were maintained at multiple time points. Ab responses were detected against proteins whose genes were commonly upregulated in NSCLC, in some cases this upregulation was associated with significantly reduced survival (TCGA). Evaluation of CD4 and CD8 T cell clones by TCRSeq identified significantly (p=0.002) increased clonal expansion compared to normal controls (n=3). Similar to Ab responses, T cell clonal expansion exhibited expansion followed by apparent contraction.

Conclusions

This monitoring strategy identified that continued vaccination was associated with induction of new IgG Ab responses, inferring that new CD4 T cell responses were developing with repeated vaccination. Expansion and apparent contraction of CD4 and CD8 T cell clones, consistent with basic immunological principles, provides insights into combination immunotherapy strategies that might be used to augment response as well as a method to monitor for that effect.

Trial Registration

ClinicalTrials.gov Identifier NCT01909752

P39

Use of *ex vivo* histoculture to identify potential predictive biomarkers for the ICOS agonist antibody, JTX-2011

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Background

ICOS (Inducible T cell CO-Stimulator) is a co-stimulatory molecule expressed primarily on T lymphocytes. Clinical and preclinical data suggest that ICOS plays an important role in the immune response to cancer. Therefore, we generated JTX-2011, an ICOS agonist antibody currently in clinical development in advanced solid tumors in the ICONIC trial. In preclinical studies, single agent efficacy correlates with the percentage of ICOS-expressing T cells within the tumor. Thus, ICOS expression is being used as a biomarker to enrich for patients in Phase 2 of the ICONIC trial. Building on our biomarker-driven strategy, we have explored additional potential predictive biomarkers using *ex vivo* tumor histoculture which allows for *in vivo*-like analysis of therapies using patient intact tumor tissue. Herein, we report on the results of such analysis, including assessment of the induction of an IFN-gamma gene signature.

Methods

Tumor processing: Fresh human tumor samples were obtained post-surgery through the Cooperative Human Tissue Network. A section of each tumor was cut and fixed for IHC. 300 mM slices of remaining