TCGA analysis was consistent with IHC analyses, demonstrating a strong correlation of LAG-3 mRNA expression with CD8, PD-1, and CTLA-4 (r=0.81; r=0.87; r=0.69), moderate correlation with PD-L1 and MHCII (r=0.47; r=0.58), and correlation of LAG-3, CD8, and PD-1 mRNA expression with T-cell-inflamed gene signatures across tumor types. Exploratory analyses of clinical trials in RCC and melanoma showed increased mean LAG-3 mRNA expression after nivolumab (anti-PD-1) treatment.

Conclusions

LAG-3 expression correlates with tumor inflammation and is enriched in tumors with $MHCII^{hi}$ tumor cells. Preliminary data suggest that preferential localization of LAG-3-expressing leukocytes to $MHCII^{hi}$ tumor regions potentially serves as a mechanism for LAG-3 checkpoint pathway activation. These findings, and the observation that nivolumab may induce LAG-3 expression, underscore the importance of studies to define predictive biomarker profiles for relatlimab (anti-LAG-3) therapy in PD-1-naive and -progressed patients.

References

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Evaluating immune responses of patients receiving the DPV-001 cancer vaccine

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Background

DPV-001 DRibble® is a dendritic cell-targeted microvesicle (proteasome blocked autophagosome) vaccine derived from adenocarcinoma and mixed histology cancer cell lines. It contains multiple TLR agonists and >130 potential NSCLC antigens, many as prospective altered-peptide ligands or neoantigens. We hypothesize that the efficacy of DRibbles' vaccination can be attributed to tumor-derived short-lived proteins (SLiPs) and defective ribosomal products (DRiPs). SLiPs and DRiPs are typically not processed and presented by professional antigen presenting cells therefor the host may be less tolerant. The large number of potential antigens in the vaccine necessitate new techniques to monitor responses.

Methods

Patients received induction cyclophosphamide, then 7 vaccines at 3-week intervals. First vaccine was given intranodally; subsequent vaccines intradermally. Patients were randomized to receive DRibble alone (A), or with imiquimod (B) or GM-CSF (C). PBMCs and serum were collected at baseline and at each vaccination to assess changes in antibodies (Protoarray, microsphere affinity proteomics (MAP)) and cytokines (Quanterix), peripheral lymphocytes populations (flow cytometry) and TCR repertoires (Adaptive immunoSEQ).

Results

13 pts were enrolled (Arm A: 5; B: 4; C: 4). Serum cytokines (IL1β, IL8, IFNα, IFNγ, IL6, IL17 and TNFα) were measured and normalized and the sum plotted against time. The slope of the resultant trend line was used as an indicator of either increased (positive slope) or decreased (negative slope) systemic inflammation. DPV-001 alone did not change net cytokine load while the addition of the adjuvant immiquimod increased, and the addition of GM-CSF significantly lessened the slope. Vaccination induced or increased IgG Ab responses against targets over-expressed by NSCLC, correlating with activated Th1 cells in whole blood samples. New or augmented Ab responses were observed with continued vaccination. Pts receiving DPV-001 had a significant ($p<0.04$) increase in total (CD4 + CD8) TCRs that increased 10 fold over baseline compared to normal controls (independent from trial, n=3) and the increase in CD4 clones was similar to that seen following Ipilimumab (melanoma pts, independent from trial, n=9). Patients receiving DPV-001 alone had the largest increase in CD8 T cell clones.

Conclusions

Vaccination with DPV-001 increased the number of strong antibody responses to antigens commonly over-expressed in NSCLC and expanded populations of T cells. DPV-001 alone provided the greatest increase in CD8 TCRs. Interval monitoring of PBMCs/serum identified the complexity of the immune response to this vaccine and suggests possibilities to boost or sustain immunity.

Trial Registration

NCT01909752.

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Deep immunoprofiling of rare T-cell populations from clinical samples

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Background

The complexity and heterogeneity of the immune system combined with its central role in tumor biology necessitates sophisticated analytical approaches to reveal molecular mechanisms, novel therapeutic targets and clinically relevant biomarkers. T-cells have significant functional variation in activation states. However, the rare frequency of antigen-specific CD8+ cells, for example, limits transcriptomic and proteomic analysis to identify biomarkers of exhaustion and activation.

Methods

Utilizing a novel integrated workflow, we performed both proteomic and transcriptomic analysis of very rare populations of T-cells. Negatively selected CD3+ cells were derived from whole PBMCs and stimulated *in vitro* with allogeneic, CD40L-activated, viral-antigen presenting B cells for 8 days. The stimulated cell population was stained with HLA-A02:01 MHC Pentamers specific for Influenza A $M1_{58\text{-}66}$ (GILGFVFTL). The cells were then simultaneously labeled with fluorescent markers and 30 different DNA barcoded antibodies. Using the fluorescent markers, antigen-specific and naïve CD8+ T-cells were sorted, lysed, and then the antibody-bound DNA barcodes and the released cellular RNA's were simultaneously measured using the NanoString nCounter® system and analyzed using the nSolver™ software.

Results

By integrating flow cytometry with downstream analysis on the NanoString nCounter system, 30 proteins and 770 RNAs were quantitatively measured on the nCounter from as few as 400 petamer-positive T-cells. Using the nSolver Advanced Analysis software, differences in gene and protein expression between Influenza A $M1_{58\text{-}66}$ specific CD8+ T cells and a pentamer-negative CD8+ T cell population were quantitatively measured. $M1_{58\text{-}66}$ specific cells showed upregulation of extracellular markers of exhaustion and activation consistent with similar proteomic studies including 4-1BB, CD27, CD45, and ICOS. Additionally, normalized