Conclusions

The impact of TMB may vary across PD-L1 expression subgroups. Rational integration of TMB and PD-L1 expression may identify NSCLCs with the greatest likelihood of response or resistance to ICIs.

Ethics Approval

Clinicopathologic data were collected from patients with advanced NSCLC who had consented to a correlative research study (DF/HCC protocol #02-180).

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248 IMMUNOTHERAPY PERSISTER CELLS UNCOVERED BY DYNAMIC SINGLE-CELL RNA-SEQUENCING

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Background

To understand fundamental mechanisms of immune escape, we leveraged our functional ex vivo platform of murine derived organotypic tumor spheroids (DOTS)1 to determine if drug-tolerant persister cells analogous to oncogene targeted therapies limit efficacy of programmed death (PD)-1 blockade, and to identify therapeutic vulnerabilities to overcome anti-PD-1 (αPD-1) resistance.

Methods

Murine syngeneic cancer models with well-characterized response to αPD-1 therapy were chosen: MC38 (sensitive) and CT26 (partially resistant). Bulk and single-cell (sc) RNA-sequencing (RNA-seq) were performed on αPD-1 treated DOTS. In vitro culture studies were conducted with or without cytokines (100 ng/ml) or drugs (500 nM). In vivo studies in mice bearing MC38 or CT26 tumors evaluated the combinational strategy with PD-1 blockade. We further evaluated our findings in scRNA-seq of an αPD-1 refractory colorectal cancer (CRC) patient tumor.2

Results

Bulk RNA-seq of αPD-1 treated DOTS revealed a mesenchymal resistant phenotype with upregulated TNF-α/NFκB signaling (figure 1). scRNA-seq further identified a discrete sub-population of immunotherapy persister cells (IPCs). These cells expressed a stem-like phenotype including downregulation of E2F targets indicative of quiescence, suppression of interferon-γ response genes, induction of hybrid epithelial-to-mesenchymal state, and active IL-6 signaling (figure 1). Ly6a/stem cell antigen-1 (Sca-1) and Snai1 were found to be differentially upregulated in IPCs resistant to αPD-1 blockade (not shown). Sca-1 positivity was confirmed in pre-existing tumor populations in vitro (figure 2). When enriched via sorting, these cells remained more persistently Sca-1+ at 96 hours in culture of CT26 compared to MC38 cells, related to increased autocrine IL-6 production by CT26 Sca-1+ cells. Indeed, IL-6 supplementation was capable of expanding Sca-1+ cells in culture (figure 2). Sca-1+ cells expressing ovalbumin peptide were refractory to OT-1 T cell mediated killing and failed to upregulate MHC class-1 antigen presentation (H-2Kb) in contrast to interferon-γ (not shown). Analysis of RNA-seq data further identified Birc2/3 as potential targets limiting TNF-mediated apoptosis of these cells (not shown). Notably, Birc2/3 antagonism depleted Sca-1+ IPCs in vitro and significantly potentiated the impact of PD-1 blockade in vivo in MC38, and less robustly in CT26 (figure 3). Evaluation in a microsatellite-instability high CRC patient identified a pre-existent IPC subpopulation within the αPD-1 refractory pre-treatment tumor, with high SNAI1 expression compared to CRC samples in TCGA (figure 4).

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Bulk and single-cell (sc) RNA-sequencing (RNA-seq) of DOTS identifies an anti-PD-1 (αPD-1) resistant subpopulation of persister cells. IgG= isotype control
Abstract 248 Figure 2 Pre-existent population of stem cell antigen-1 (Sca-1)+ cells expands in response to interleukin-6 (IL-6), as characterized by flow cytometry evaluation in murine syngeneic cancer models at baseline and after purification by fluorescence-activated cell sorting (FACS). H = hours

Abstract 248 Figure 3 Combination of anti-PD-1 therapy with Birc2/3 antagonism increases tumor responses and improves survival. CR = complete response

Abstract 248 Figure 4 Single-cell RNA-sequencing (scRNA-seq) of a pre-treatment microsatellite-instability (MSI-H) colorectal cancer (CRC) patient tumor, refractory to anti-PD-1 (αPD-1) therapy, reveals presence of SNAI1-high immunotherapy persister cells

Conclusions High-resolution functional ex vivo profiling identified Sca-1+/SnaI1<sup>high</sup> stem-like ‘immunotherapy persister cells’ and uncovered their anti-apoptotic dependencies targetable with Birc2/3 antagonism to augment αPD-1 efficacy.