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Triple checkpoint blockade targeting PD-1, TIM-3, and LAG-3 reinvigorates ovarian cancer-infiltrating T cells by increasing T cell polyfunctionality and effector function

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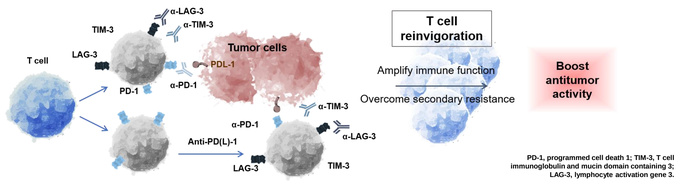
BACKGROUND

Figure 1. Therapeutic concept



PD-1, programmed cell death 1; TIM-3, T cell immunoglobulin and mucin domain containing 3; LAG-3, lymphocyte activation gene 3.

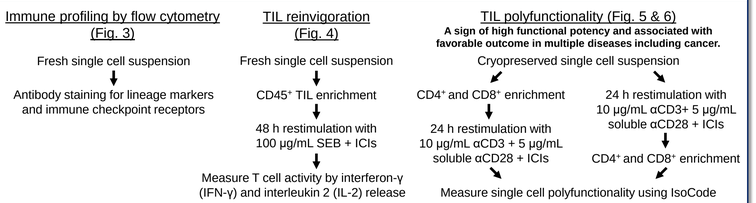
T cells upregulate multiple checkpoint receptors in response to chronic activation, including PD-1, TIM-3, and LAG-3, eventually resulting in a dysfunctional or exhausted phenotype. Therefore, triple blockade targeting all three of these immune checkpoint receptors may be more effective at T cell reinvigoration. In addition, increases in TIM-3 and LAG-3 expression have been observed in the context of acquired resistance to PD-1/PD-L1 axis blockade, which may be overcome by combination checkpoint blockade[1–4]. Furthermore, TIM-3 and LAG-3 have been described to modulate activity of other cell types, including natural killer and dendritic cells, suggesting potential additional mechanisms of action during triple immune checkpoint blockade.

OBJECTIVE

Triple immune checkpoint blockade using inhibitory antibodies targeting PD-1, TIM-3, and LAG-3 has previously been found to improve tumor control and favorably modulate the composition of immune infiltrates in various syngeneic and humanized mouse models[5–6]. Dostarlimab (TSR-042), TSR-022, and TSR-033 are immune checkpoint inhibitors (ICIs) in clinical development that target PD-1, TIM-3, and LAG-3, respectively[7]. Here, we assessed how *ex vivo* triple combination treatment with these antibodies modulates the function of tumor-infiltrating leukocytes isolated from primary ovarian cancer resections.

METHODS

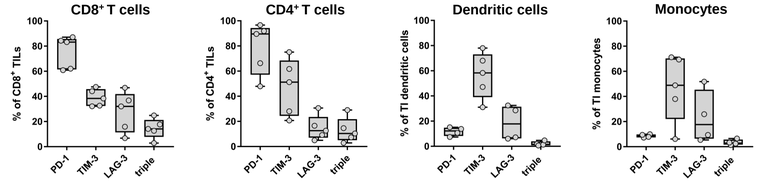
Figure 2. Experimental outline



Primary resections from patients with ovarian cancer were dissociated into single cell suspensions within 18 h of collection. Cells were used immediately for flow cytometry or for CD45+ tumor infiltrating lymphocyte (TIL) enrichment by magnetic bead isolation followed by *ex vivo* restimulation with *Staphylococcus aureus* enterotoxin B (SEB). Cytokine release into the supernatant was quantified by cytometric bead array. T cell polyfunctionality was measured from cryopreserved TILs using a microfluidic IsoCode chip technology[8–9] and a 32-plex cytokine/chemokine panel. *Ex vivo* treatment with ICIs was performed at 10 µg/mL per antibody.

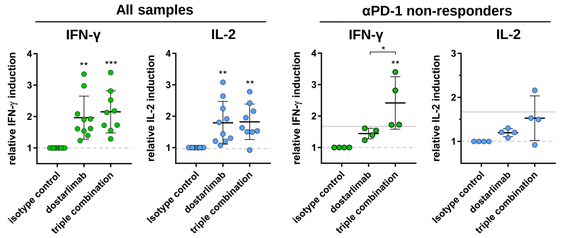
RESULTS

Figure 3. PD-1, TIM-3, and LAG-3 are expressed on ovarian cancer TILs and are frequently co-expressed on T cells.



Expression of immune checkpoints was confirmed on primary ovarian TIL populations. As in other tumor types, PD-1 is the dominant immune checkpoint on T cells , while TIM-3 is the most frequently expressed immune checkpoint receptor on tumor-infiltrating (TI) myeloid cell populations.

Figure 4. Triple immune checkpoint blockade effectively mediates TIL reinvigoration and converts αPD-1 non-responders into responders.

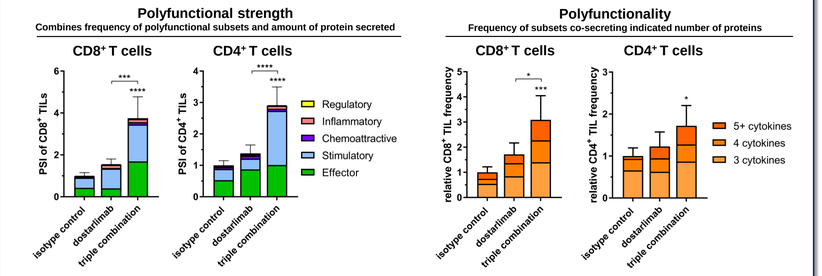


Cytokine secretion after bulk restimulation in presence of the indicated antibodies was quantified and normalized for each donor to isotype control treatment. Data points represent all individual samples tested (left) or only those with < 1.67-fold cytokine induction with dostarlimab treatment alone (non-responders, right). * P < 0.05, ** P < 0.01, *** P < 0.001 by 1-way analysis of variance (ANOVA) with Tukey's multiple comparisons test vs isotype control unless otherwise indicated.

CONCLUSIONS

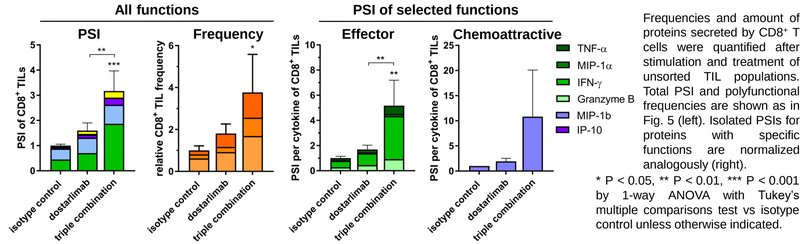
- *Ex vivo* T cell stimulation and dostarlimab treatment either in isolation or in the context of the full tumor microenvironment effectively reinvigorates TILs by increasing cytokine release from polyfunctional cells.
- Triple combination treatment further increases the frequency of highly polyfunctional T cells, contributing to significantly increased overall polyfunctional strength.
- Increased effector polyfunctionality with triple combination treatment supports the concept that simultaneous targeting of multiple T cell checkpoints will provide more effective reversal of T cell exhaustion.
- Increased contribution of chemoattractive factors only with triple combination treatment suggests that additive mechanisms of actions on non-T cells are engaged by TIM-3/LAG-3 blockade.
- Overall, these findings begin to provide a molecular understanding of our previously reported observation that triple combination therapy provides superior *in vivo* efficacy compared to PD-1 monotherapy using preclinical tumor models[5,6] as well as support the concept of treating patients with the triple combination of dostarlimab plus TSR-022 and TSR-033.

Figure 5. Triple checkpoint blockade increases polyfunctional strength by increasing the frequency of highly polyfunctional T cells after *ex vivo* restimulation.



Frequencies and amount of proteins secreted by individual CD8+ or CD4+ T cells were quantified using the IsoCode microfluidics platform after stimulation and treatment of previously sorted TIL populations. A polyfunctional strength index (PSI) is calculated as a summary metric including subsets secreting 2 or more proteins[8–9] and normalized for each donor to the isotype control PSI (left). The frequency of highly polyfunctional populations is normalized analogously (right). * P < 0.05, *** P < 0.001, **** P < 0.0001 by 1-way ANOVA with Tukey's multiple comparisons test vs isotype control unless otherwise indicated.

Figure 6. Triple checkpoint blockade in the context of the full tumor microenvironment increases CD8+ TIL polyfunctionality by increasing effector and chemoattractive functions.



Frequencies and amount of proteins secreted by CD8+ T cells were quantified after stimulation and treatment of unsorted TIL populations. Total PSI and polyfunctional frequencies are shown as in Fig. 5 (left). Isolated PSIs for proteins with specific functions are normalized analogously (right).

* P < 0.05, ** P < 0.01, *** P < 0.001 by 1-way ANOVA with Tukey's multiple comparisons test vs isotype control unless otherwise indicated.

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