Triple checkpoint blockade targeting PD-1, TIM-3, and LAG-3 improves T cell reinvigoration and antitumor efficacy over single and double combinations

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BACKGROUND

Figure 1. Therapeutic concept

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. Triple blockade targeting multiple checkpoint receptors may therefore be more effective at T cell reinvigoration. In addition, increases in TIM-3 and LAG-3 expression have been described in the context of acquired resistance to PD-1/PD-L1 axis blockade, which may be overcome by combination checkpoint blockade. Furthermore, TIM-3 and LAG-3 have been described to modulate activity of other cell types, including NK and dendritic cells, suggesting potential additional mechanisms of action during triple immune checkpoint blockade.

OBJECTIVES

Here, we explore the functional effects of triple blockade of PD-1, TIM-3, and LAG-3 on T cell reinvigoration, tumor immune contexture, and antitumor activity in preclinical models.

METHODS

In vitro exhaustion assay

Splenocytes from MBP-Tracker mice with CD4+ T cell receptor transgenic T cells

72 h peptide stimulation with APL-MBP

Exhausted T cells

72 h restimulation of CD4+ T cells with APL-MBP peptide presented on irradiated APCs with immune checkpoint inhibitors

Measure T cell activity by IFN-γ release

Splenocytes were isolated from mice engineered to express a CD4+ T cell receptor recognizing an epitope of myelin basic protein (MBP). Cells were stimulated with either MBP peptide to generate effector T cells or with a superagonist altered peptide ligand (APL) of MBP to generate exhausted T cells. Immune cells were isolated by density gradient centrifugation and rested for 4 days before restimulation with APL-MBP-loaded and irradiated antigen-presenting cells (APCs). IFN-γ release was quantified in supernatants by ELISA (Figure 3).

Synergic in vivo model for efficacy and pharmacodynamics

Ex vivo TIL reinvigoration

Ovarian cancer tumor resection at clinical site

Dissociation into single cell suspension

TIL enrichment

T cells with APL

Measure T cell activity by IFN-γ and IL-2 release

Primary resections from treatment-naive ovarian cancer patients were dissociated and CD4+ tumor infiltrating lymphocytes (TILs) were enriched by magnetic bead isolation. TILs were then restimulated with S. aureus enterotoxin B (SEB) and cytokine release into the supernatant was quantified by cytometric bead array (CBA, Figure 6).

RESULTS

Figure 2. Co-expression of PD-1, TIM-3, and LAG-3 on human tumor-infiltrating lymphocytes (TILs) isolated from multiple tumor tissues

Fresh tumor tissue samples across 15 different tumor types were collected and dissociated to single cell suspensions before immune profiling by flow cytometry. For each tumor type, the frequency of PD-1, TIM-3, and LAG-3 co-expressing cells was determined for 4 TIL subsets, defined by classical lineage markers.

Figure 3. Triple combination treatment was able to fully reverse the exhausted phenotype in an antigen-specific CD4+ T cell model

(a) In vitro exhausted CD4+ T cells were restimulated for 72 h with a superagonist peptide in the presence of single, double, and triple antibody combinations at 0.32 μM total antibody concentration. For single and double combinations, the remaining thirds of antibody were topped off with isotype control. Means and standard deviations of normalized IFN-γ secretions are shown, n = 4. The dotted line indicates IFN-γ secretion by non-restimulated T cells. *p < 0.05, **p < 0.01, ****p < 0.0001 by one-way ANOVA with Tukey’s multiple comparisons test. (b) A dose-flattening of triple antibody combination and isotype control was performed in the same assay as in (a). Means and standard deviations of normalized IFN-γ secretions are shown, n = 4. The dotted line indicates IFN-γ secretion by non-restimulated T cells. *p < 0.05, **p < 0.01, ****p < 0.0001 over isotype control at the same concentration by two-way ANOVA with Sidak’s multiple comparisons test. One of two representative experiments is shown.

Figure 4. Maximum TGI was observed in the triple combination group in an EMT6 syngeneic breast cancer model

Isotype control
double antibody treatment, EWB

Isotype control + a-PD-1
double antibody treatment, EWB

Isotype control + a-TIM-3
double antibody treatment, EWB

Isotype control + a-LAG-3
double antibody treatment, EWB

Isotype control + a-PD-1 + a-TIM-3
double antibody treatment, EWB

Isotype control + a-PD-1 + a-LAG-3
double antibody treatment, EWB

Isotype control + a-TIM-3 + a-LAG-3
double antibody treatment, EWB

Volumes of subcutaneous EMT6 tumors were monitored every other day during treatment with the indicated antibodies, administered biweekly alone or in combination depending on the treatment group (200 μg per antibody for each mouse). Tumor growth was shown for each animal (one line per animal, n = 10) and tumor growth inhibition (TGI) was calculated.

Figure 5. Triple combination treatment modulated multiple aspects of the tumor microenvironment

Figure 6. Triple combination treatment is able to reinvigorate TILs from human ovarian cancer patients

TILs were isolated from primary ovarian cancer resections from treatment-naive patients. IFN-γ and IL-2 secretion was quantified after restimulation with 100 μg/ml SEB for 48 h in the presence of immune checkpoint inhibitory antibodies targeting PD-1 (TSR-042), TIM-3 (TSR-022), and LAG-3 (TSR-033). All three proprietary antibodies (TESARO, Inc.) are being evaluated in clinical trials. Each antibody was used at 10 μg/ml and cytokine levels were normalized to isotype control conditions. Means, standard deviation, and individual data points are shown, n = 2–3. *p < 0.05, **p < 0.01 over isotype control by one-way ANOVA with Dunn’s multiple comparisons test.

CONCLUSIONS

• Triple blockade of PD-1, TIM-3, and LAG-3 resulted in highly effective reversal of T cell exhaustion in a murine model.
• Triple blockade improved tumor growth control over single or double combinations.
• Targeting immune checkpoints expressed on multiple cell types engaged additional mechanisms of tumor immune control.
• Triple combination treatment with checkpoint inhibitors was able to reinvigorate human TILs most effectively.
• Overall, these findings support the concept of double and triple combinations of antibodies that block PD-1, TIM-3, and LAG-3.

REFERENCES

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