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The anti-tumor efficacy of TIM-3 blockade in a murine model of sarcoma

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Background

TSR-022 is an anti-TIM-3 antibody that is currently undergoing clinical development in combination with TSR-042 , an anti-PD-1 antibody (NCT02817633). TIM-3 is an immune checkpoint receptor that negatively regulates T-cell activity and is implicated in resistance to PD-1 blockade. In addition, TIM-3 is also expressed on myeloid cells and has been shown to regulate dendritic cell activity. We previously reported a case study of a patient with leiomyosarcoma who had a partial response to TSR-022 monotherapy [1]. Here we explore the mechanism of the antitumor effect of TIM-3 blockade in a preclinical mouse model of fibrosarcoma.

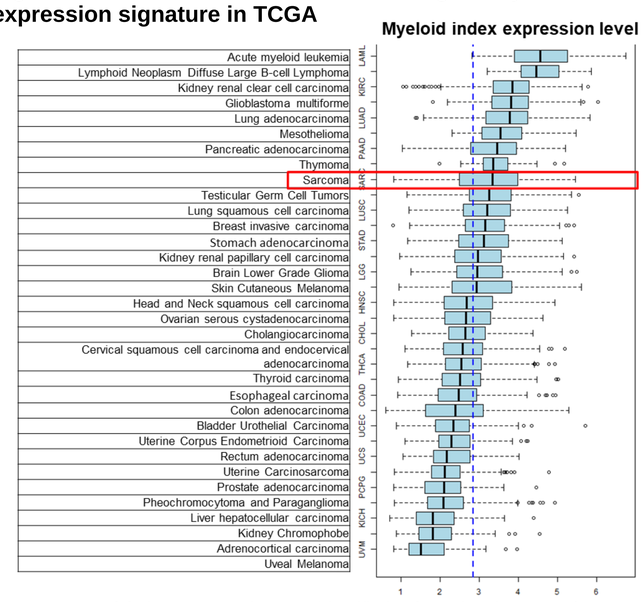
Methods

A/JCr mice were inoculated with murine fibrosarcoma SaL/N cells. Mice were randomized into 4 groups of 8 mice each at tumor volumes of 80-120 mm^3 and treated with isotype control, anti-mouse PD-1, anti-mouse TIM-3, or a combination of both. A total of 5 doses were administered in a twice-weekly schedule. Mice with complete tumor regression were re-challenged with SaL/N cells along with a fresh cohort of 5 mice to serve as a control group. The mice were monitored for tumor regrowth until all the mice in the control group were euthanized due to tumor burden. In a follow-up study to understand the mechanism of efficacy, the mice were randomized at tumor volumes of 200-300 mm^3, treatment initiated, and tumors collected for immunoprofiling using flow cytometry.

Results

Both anti-PD-1 and anti-TIM-3 treatment resulted in antitumor efficacy with 73% and 53% tumor growth inhibition, respectively, which improved to 98% in the combination group. In addition, 2 mice in the anti-PD-1 group and 6 in the combination group showed complete tumor regression. The mice with complete regression were re-challenged and monitored for tumor growth. The tumors in the control cohort of mice grew normally; however, no tumor growth was observed in the re-challenged mice, consistent with the induction of immune memory. Tumor immune contexture correlated with the anti-tumor efficacy seen.

Figure 1. Sarcoma is associated with a higher myeloid index expression signature in TCGA



- Based on TCGA's RNA-seq data, we identified at least four immune clusters, termed lymphoid, myeloid, interferon, and cytokine [2]. The myeloid cluster is enriched for genes related to macrophages, neutrophils, monocytes, etc. The expression index (Myeloid index, e.g.) was calculated for each cluster by averaging the expression of the genes in each cluster (Myeloid, e.g.).
- The box plots of Myeloid index of all tumor samples for each of the 33 TCGA tumor type ordered by Myeloid index. The dashed blue line represents the median of the index of all TCGA tumor samples.

Figure 2. SaL/N study design

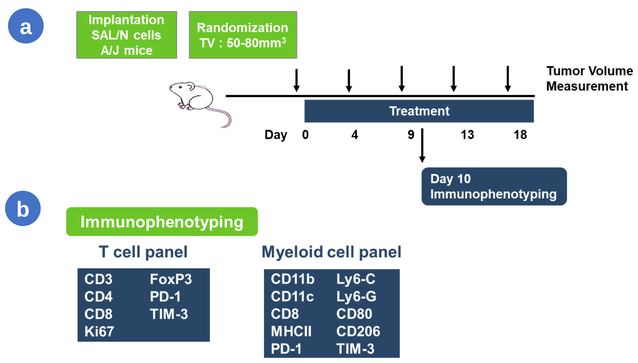
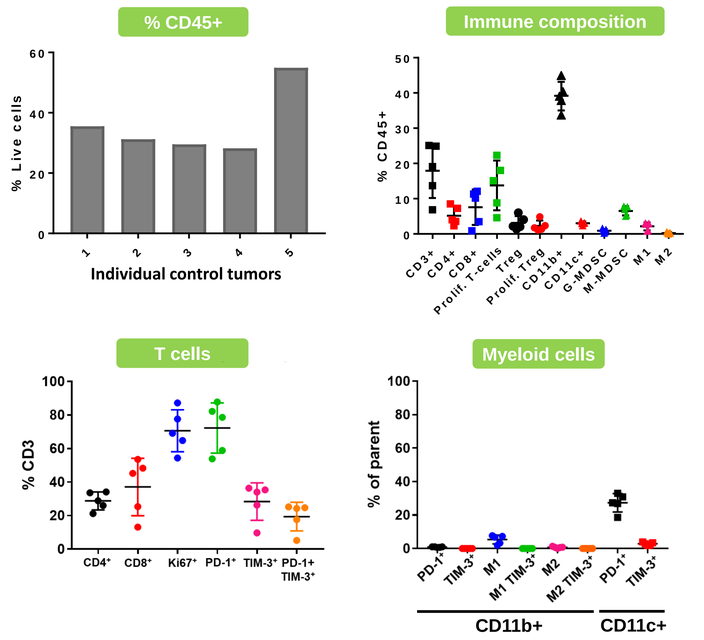
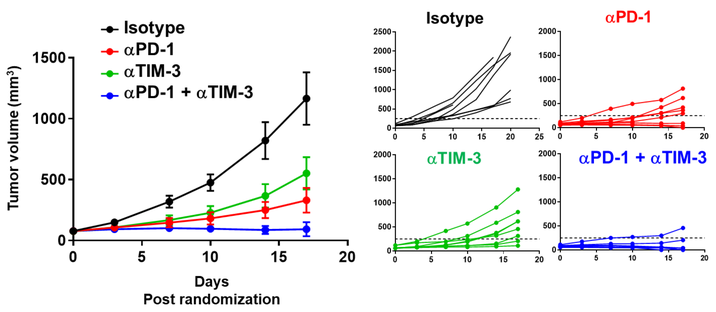


Figure 3. At baseline, Sal/N tumors are myeloid rich with PD-1 and TIM-3 expressed on tumor infiltrating T cells



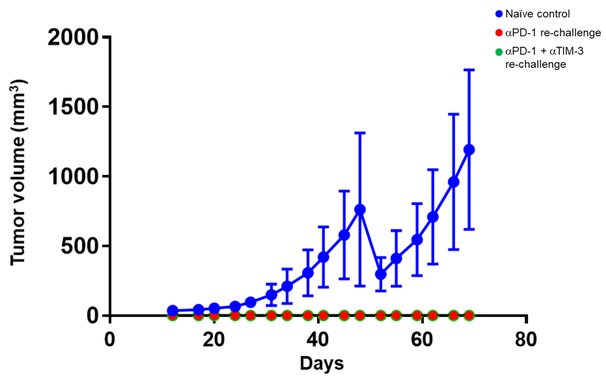
- Sal/N tumors have between 30-50% $CD45^+$ infiltrate at baseline.
- Approximately 40% of immune infiltrate is comprised of cells of the monocyte/macrophage lineage ($CD11b^+$).
- PD-1 and TIM-3 are expressed on tumor infiltrating T cells (TIL) and to a lesser degree on the myeloid infiltrate.

Figure 4. Co-blockade of PD-1 and TIM-3 is more efficacious than either single agent and induces immunological memory



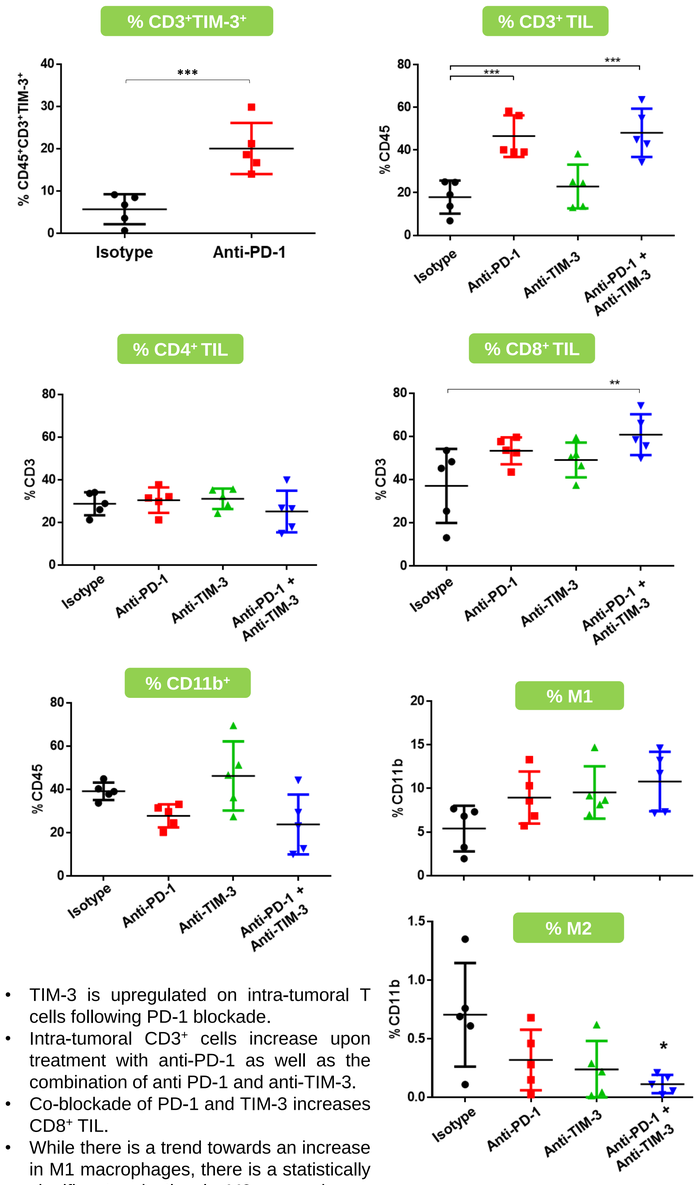
Treatment	TGI	CR
αPD-1	72	2/8
αTIM-3	53	0
αPD-1 + αTIM-3	98	6/8

- Doses (10 mg/kg) were administered twice weekly for a total of 5 doses.
- Both PD-1 and TIM-3 blockade result in modest tumor growth inhibition as monotherapy treatment.
- The combination of anti-PD1 and anti-TIM3 was more efficacious than either as single agents, resulting in complete responses in 6/8 animals.



- Mice with complete tumor regression were re-challenged with SaL/N cells along with a fresh cohort of 5 mice to serve as control group.
- Mice were monitored for 60 days after re-inoculations with SaL/N cells.
- No tumor growth was seen in the re-challenged mice indicating the presence of immunological memory in both the PD-1 treated and combination treated animals.

Figure 5. Co-blockade of PD-1 and TIM-3 increases intra-tumoral $CD8^+$ T cells and decreases M2 macrophages



- TIM-3 is upregulated on intra-tumoral T cells following PD-1 blockade.
- Intra-tumoral $CD3^+$ cells increase upon treatment with anti-PD-1 as well as the combination of anti PD-1 and anti-TIM-3.
- Co-blockade of PD-1 and TIM-3 increases $CD8^+$ TIL.
- While there is a trend towards an increase in M1 macrophages, there is a statistically significant reduction in M2 macrophages following combo treatment.

Conclusions

- Sal/N is a myeloid rich murine model of sarcoma with PD-1 and TIM-3 expression on intra-tumoral T cells and a subset of intra-tumoral myeloid cells.
- While single agent PD-1 or TIM-3 blockade results in a modest inhibition of tumor growth, the combination induces complete regression in most animals treated.
- Tumor regressions are associated with immune memory in both PD-1 treated and combination treated animals.
- Pharmacodynamic analysis shows TIM-3 is upregulated on intra-tumoral T cells following PD-1 blockade.
- Intra-tumoral $CD3^+$ cells increase upon treatment with anti-PD-1 as well as the combination of anti PD-1 and anti-TIM-3 however a significant increase in $CD8^+$ TIL is seen in the combination only.
- While there is a trend towards an increase in M1 macrophages following treatment, there is a statistically significant reduction in M2 macrophages following combo treatment.
- In conclusion, the combination of PD-1 and TIM-3 blockade induces tumor regressions in the syngeneic SaL/N model that is associated with an increase in intra-tumoral CD8+ T cells and a reduction in M2 macrophages.

References

1. Weiss *et al*. A Phase 1 Study of TSR-022, an Anti–TIM-3 Monoclonal Antibody, in Patients with Advanced Solid Tumors. SITC Annual Meeting, 2017.

2. Feng B. & Xiao, Y. TCGA pan-cancer transcriptome-based pathway analysis for cancer therapeutics. EORTC-NCI-AACR Annual Meeting, 2016.