A preclinical model of acquired anti-PD-1 resistance is responsive to SEA-TGT, an effector-function enhanced anti-TIGIT monoclonal antibody

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Background

- Immune checkpoint inhibitors (CPI) targeting the PD-(L)1 axis induce robust antitumor immunity in a subset of patients with cancer. However, challenges remain for patients who do not respond to or experience disease progression after treatment with CPIs.
- There is an interest in understanding whether immunomodulatory agents targeting other immune checkpoints, such as **SEA-TGT**, an investigational, nonfucosylated mAb directed to TIGIT with enhanced Fc effector function, may elicit activity in patients with anti-PD-1 (αPD-1) resistant malignancies.
- Here, we developed and characterized a murine CT26 tumor model of acquired αPD-1 resistance (CT26.PD1R) through serial implantation and treatment with increasing levels of αPD-1.
- We also evaluated anti-tumor activity of **SEA-TGT,** a mIgG2a version, as monotherapy and in combination with an αPD-1 agent in CT26.PD1R tumors.
- These preclinical data demonstrate the ability of SEA-TGT to work in tumor microenvironments that are refractory or resistant to checkpoint therapies and suggest SEA-TGT may re-sensitize these tumors to αPD-1.

Development of an αPD-1 resistant tumor model (CT26.PD1R)

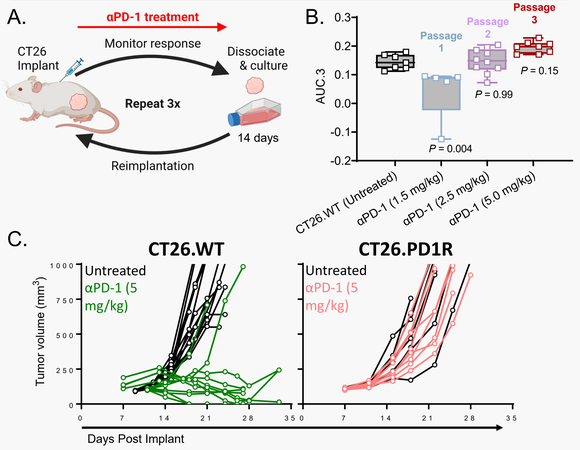

Figure 1. Establishing a syngeneic model of acquired αPD-1 resistance. (A) Schema depicting method to derive αPD-1 resistance. Wild type CT26 tumors (CT26.WT) were passaged *in vivo* and treated (Q3dx4) with an αPD-1 mAb. Non-responsive tumors were excised, dissociated, and cultured prior to reimplantation. **(B)** The average slope of the growth curves in log space (AUC.3)[5] calculated at each passage. Statistics were completed via one-way ANOVA with Dunnett's post-hoc multiple comparisons (*P* values compare CT26.WT with each test group). **(C)** Treatment of CT26.PD1R tumor-bearing mice with an αPD-1 mAb (Q3Dx4) failed to elicit anti-tumor activity.

CT26.PD1R tumors display minor changes in tumor-infiltrating leukocytes

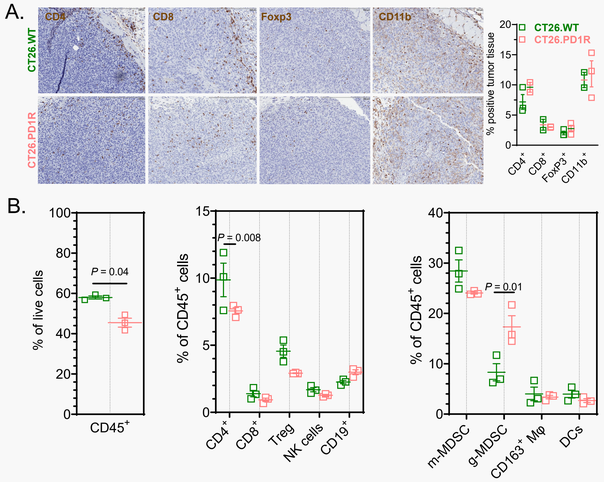

Figure 2. Immunohistochemical (IHC) staining and flow cytometric analysis demonstrate minor immune cell changes in CT26.PD1R tumors. (**A**) Untreated CT26.WT and CT26.PD1R tumors (100mm^3) were stained by IHC. Representative images (left) and quantification of tumor-infiltrating immune cells are depicted (right). (**B**) Flow cytometric immunophenotyping revealed reduced CD45+ leukocytes in CT26.PD1R tumors compared to control tumors. Minor immune composition changes were observed in resistant tumor tissue, including, a reduction in lymphocytes and an increase in granulocytic myeloid cells.
Statistics to compare untreated CT26.PD1R to control CT26.WT tumors were completed via two-way ANOVA with Šidák's post-hoc multiple comparisons test (figures 2 and 3).

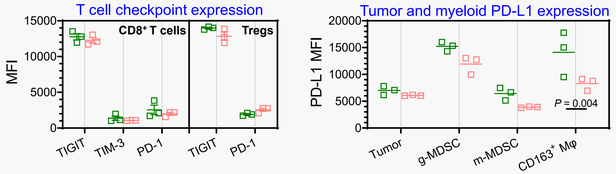

Figure 3. Flow cytometric analysis revealed tumor-infiltrating leukocytes in CT26.PD1R tumors express similar proportions of immune checkpoint ligands. Surface staining of the immune checkpoints TIGIT, TIM-3, and PD-1 was similar on CD8+ T cells and Tregs from CT26.PD1R and control tumors (left panel). Similarly, PD-L1 expression on resistant tumor cells and tumor-infiltrating myeloid cells was largely consistent with control tumors (right panel).

References

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DISCLOSURES: All authors are, or were at the time of study, employees of and/or hold stock in Seagen, Inc. *Work conducted while at Seagen but no longer currently employed at Seagen

Type I interferon (IFN-I) and MHC gene networks are differentially regulated in CT26.PD1R tumors

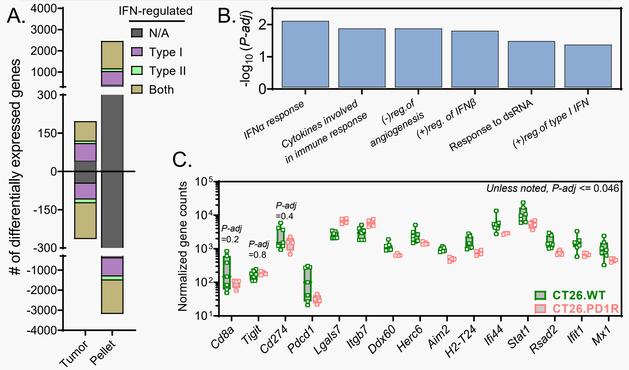

Figure 4. CT26.PD1R tumors have suppressed IFN-I signaling. (A) RNAseq analysis of CT26.WT and CT26.PD1R tumors (100 mm^3) and cell pellets (**Figure 5**) revealed multiple differentially expressed genes (DEGs) associated with IFN signaling determined using the Interferome database[1]. **(B)** Gene Ontology analyses of downregulated DEGs in CT26.PD1R tumors yielded suppressed gene sets involved in IFN-I signaling and innate immune recognition. **(C)** Summary of immune checkpoints and the top IFN-I associated DEGs in CT26.PD1R tumors. No significant changes were found in the expression of the genes encoding PD-L1 (*Cd274*), TIGIT or CD8a.

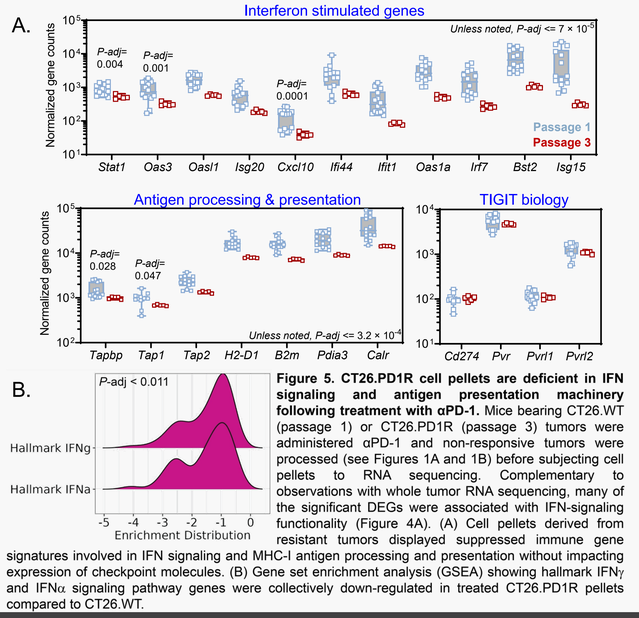

Figure 5. CT26.PD1R cell pellets are deficient in IFN signaling and antigen presentation machinery following treatment with αPD-1. Mice bearing CT26.WT (passage 1) or CT26.PD1R (passage 3) tumors were administered αPD-1 and non-responsive tumors were processed (see Figures 1A and 1B) before subjecting cell pellets to RNA sequencing. Complementary to observations with whole tumor RNA sequencing, many of the significant DEGs were associated with IFN-signaling functionality (Figure 4A). (A) Cell pellets derived from resistant tumors displayed suppressed immune gene signatures involved in IFN signaling and MHC-I antigen processing and presentation without impacting expression of checkpoint molecules. (B) Gene set enrichment analysis (GSEA) showing hallmark IFNγ and IFNα signaling pathway genes were collectively down-regulated in treated CT26.PD1R pellets compared to CT26.WT.

SEA-TGT elicits antitumor activity alone and in combination with αPD-1 in CT26.PD1R tumors

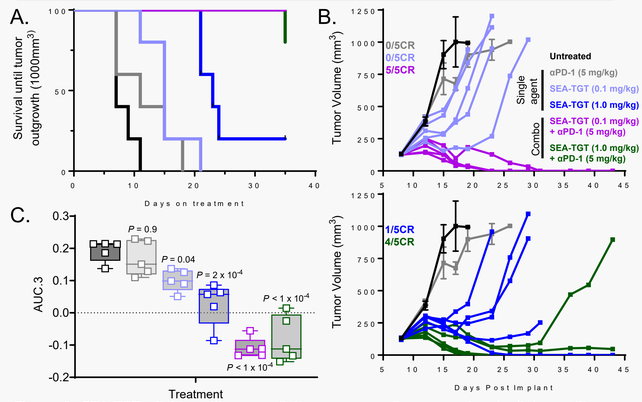

Figure 6. SEA-TGT elicits antitumor activity as a monotherapy and potentiates antitumor activity in combination with an αPD-1 agent. Treatment of CT26.PD1R tumor-bearing mice with SEA-TGT alone or in combination with an αPD-1 mAb (Q3Dx4) elicited improved overall survival **(A)** and antitumor activity **(B)**, suggesting that SEA-TGT has the capacity to re-sensitize resistant tumors to αPD-1 agents. Statistical analysis was performed using Dunnett's test following a one-way ANOVA to compare the AUC values from each treatment group to the untreated control **(C)**.

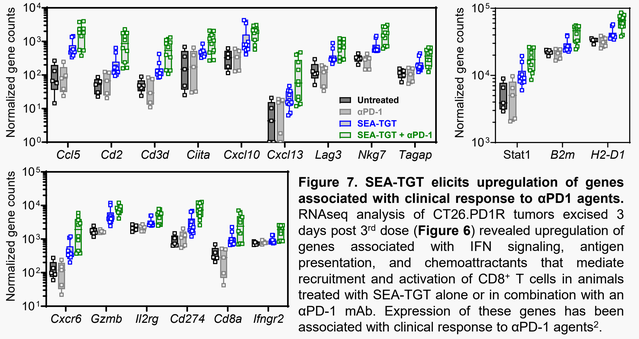

Figure 7. SEA-TGT elicits upregulation of genes associated with clinical response to αPD1 agents. RNAseq analysis of CT26.PD1R tumors excised 3 days post 3rd dose (**Figure 6**) revealed upregulation of genes associated with IFN signaling, antigen presentation, and chemoattractants that mediate recruitment and activation of CD8$^+$ T cells in animals treated with SEA-TGT alone or in combination with an αPD-1 mAb. Expression of these genes has been associated with clinical response to αPD-1 agents[2].

Conclusions

- We characterized and evaluated SEA-TGT alone and in combination with an αPD-1 agent in a newly developed CT26 model of acquired αPD-1 resistance (CT26.PD1R).
- CT26.PD1R tumors displayed significantly downregulated biological pathways involved in IFN signaling and antigen processing and presentation, consistent with clinically relevant mechanisms of αPD-1 resistance[2,3,4].
- The potent activity of SEA-TGT and revival of αPD-1 responsiveness in this model suggests TIGIT targeting may have the potential to be efficacious in tumors that are refractory or resistant to αPD-1 agents.
- Altogether, this preclinical data supports the evaluation of SEA-TGT as a monotherapy and in combination with an αPD-1 agent in the ongoing Phase 1 Study (NCT04254107).

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