Therapeutic targeting of TREM2⁺ tumor-associated macrophages as a means of overcoming checkpoint inhibitor resistance

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Abstract # C104

Introduction

Tumor-associated macrophages (TAMs) are a major source of checkpoint inhibitor (CPI) resistance, as they subvert anti-tumor immunity through immunosuppression and support of tumor growth. In patients, high levels of TAMs predict poor prognosis across multiple solid tumor indications. Therefore, therapeutic targeting of TAMs by impacting their survival and/or modulating their suppressive function is a promising strategy to augment response rates in solid tumor indications, as well as overcome resistance to CPI therapies. We and others have identified the transmembrane protein triggering receptor expressed on myeloid cells-2 (TREM2) as a highly enriched TAM target.

Triggering Receptor Expressed on Myeloid Cells 2 (TREM2)

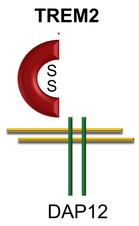
Function: Inhibitory receptor for macrophages. Mutations in humans can result in Nasu-Hakola disease, which is associated with dementia

Structure: Ig-like V-type domain. Small ICD, forms complex with DAP12

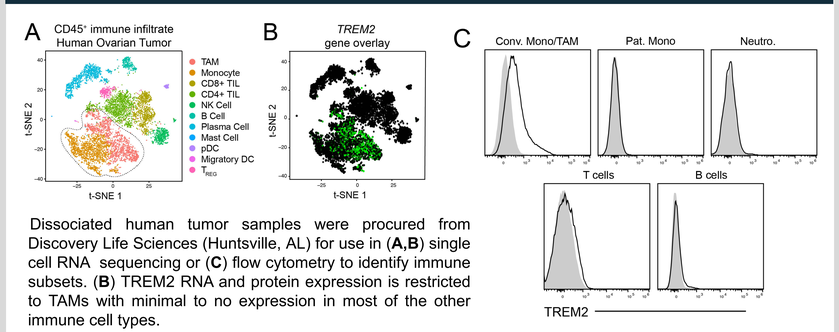
Expression: Macrophages, immature myeloid DCs, and osteoclasts

Ligands: Polyanionic lipids, ApoE

Genetics: KO mice exhibit mild osteopenia, less severe IBD, *in vitro* macrophages display increased responsiveness to TLR ligands. Human LOF mutations linked to Nasu-Hakola disease (dementia).

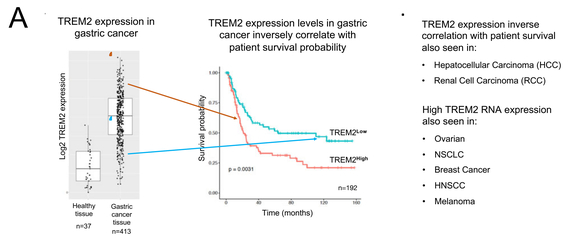


TREM2 is Enriched on TAMs in the Human TME



Dissociated human tumor samples were procured from Discovery Life Sciences (Huntsville, AL) for use in (**A,B**) single cell RNA sequencing or (**C**) flow cytometry to identify immune subsets. (**B**) TREM2 RNA and protein expression is restricted to TAMs with minimal to no expression in most of the other immune cell types.

Increased TREM2 Expression in Multiple Solid Tumors Inversely Correlates with Patient Survival



TREM2 expression inverse correlation with patient survival also seen in:

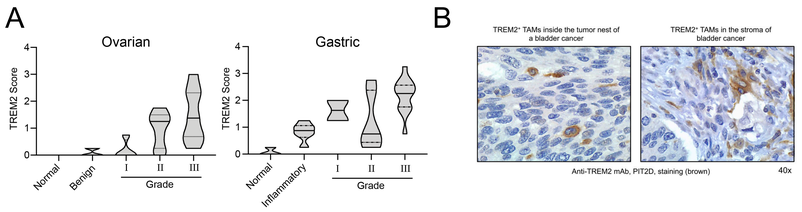
- Hepatocellular Carcinoma (HCC)
- Renal Cell Carcinoma (RCC)

High TREM2 RNA expression also seen in:

- Ovarian
- NSCLC
- Breast Cancer
- HNSCC
- Melanoma

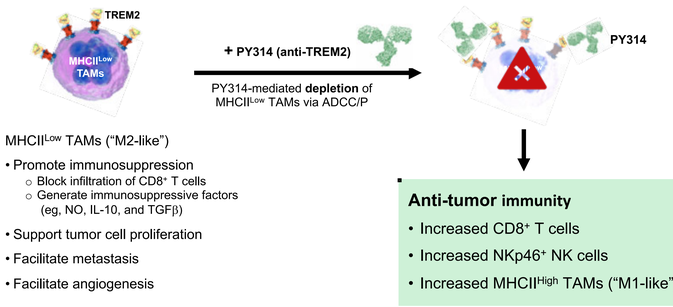
(A) RNAseq data from the TCGA gastric cohort was analyzed. RSEM values for TREM2 from both tumor and adjacent normal samples were converted to log 2 counts per million and plotted in R (panel 1). Normalized TREM2 expression profiles were downloaded from GEO (GSE15459) and divided into two cohorts based on median level of TREM2. Kaplan-Meier survival curves were plotted for each cohort and the associated logrank test was carried using the *survival* and *survminer* packages in R (panel 2).

Increased Expression of TREM2 with Higher Grade in Multiple Solid Tumors



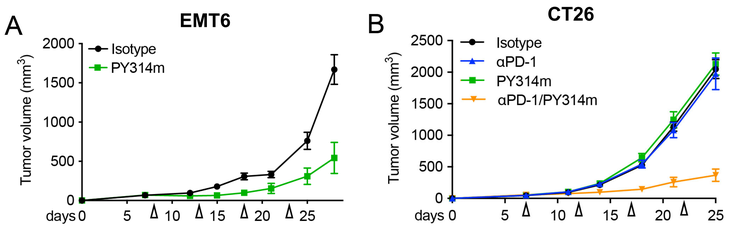
Tumor microarrays (TMAs) of multiple histological types were obtained from Reveal Biosciences. Immunohistochemical analysis of TREM2 expression was evaluated in TMAs using 5 ug per ml PIT2D, an anti-TREM2 mAb developed at Pionyr. (**A**) Semi-quantitative scoring by two investigators took into account both the cells positive for the stain as well as stain intensity. (**B**) Depicts representative staining of TREM2 within tumor nests (left) and in the stroma (right)

PY314, PIONYR's Anti-TREM2 mAb "Surgically" Depletes M2-like TAMs in the TME



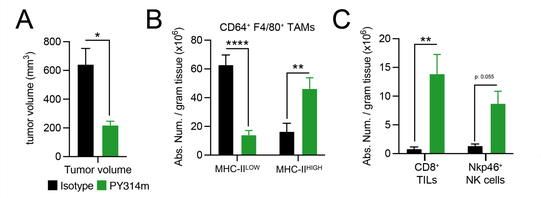
PY314 binds to MHCII-low, TREM2-high M2-like TAMs. Reduction of these immunosuppressive TAMs negates multiple immune suppressive pathways. Concomitant increase in pro-inflammatory M1-like macrophages results in productive anti-tumor immunity accompanied by functional augmentation of CD8⁺ T cells and activated NK cells within the TME.

PY314m Treatment Reduced Tumor Growth as Single Agent or in Combination with anti-PD-1



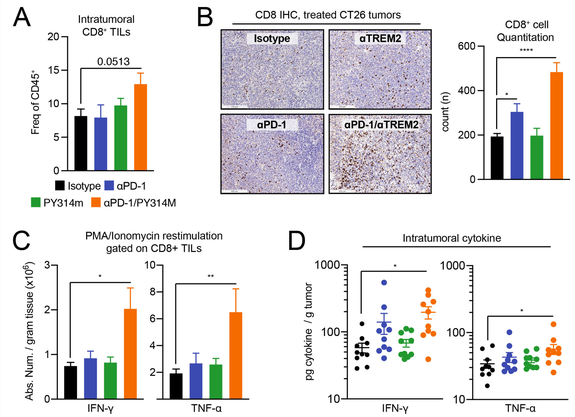
Eight to ten week old female BALB/c (Taconic) were implanted with 1x10e6 EMT6 (BALB/c; **A**), 1x10e6 CT26 (BALB/c; **B**). Vertical points indicate days when indicated antibodies in the legend were administered intraperitoneally.

PY314m Therapy Enhances the TME Immune Landscape



Treatment of EMT6 tumor bearing mice with PY314m (green) alters immune composition in the TME. (**A**) PY314m single-agent therapy reduces tumor growth. (**B**) MHC-II^LOW TAMs are reduced and MHC-II^HIGH TAMs are increased following PY314m treatment. (**C**) CD8⁺ TILs and NKp46⁺ NK cells increase in the TME following PY314m therapy.

PY314m Combined with anti-PD-1 Induces T Cell Expansion and Reactivation in anti-PD-1 Resistant Tumor Models



Treatment of CT26 tumor bearing mice with PY314m in combination with anti-PD-1 augments the abundance and functional capacity of tumor-infiltrating CD8⁺ TILs. (**A**) Frequency of CD8⁺ TILs in CT26 treated with antibodies. (**B**) (left) Representative IHC images of CD8 from treated CT26 tumors. (right) quantification of CD8⁺ cells from IHC. (**C**) Intracellular staining for IFN-γ and TNF-α on restimulated CD8⁺ TILs. (**D**) Intratumoral cytokine levels from CT26 tumors treated with antibodies.

Summary

(1) Pionyr developed anti TREM2 mAb, termed PY314 (humanized IgG1 framework) and PY314m (mouse IgG2a framework) that cross-reacts with human, mouse, and cynomolgus TREM2. TREM is expressed on many different solid tumors, and high TREM2 expression is inversely correlated with poor patient survival probability.

(2) PY314m significantly reduced M2-like, MHCII^Low and Arginase^High TAMs, expanded MHCII^High M1-like TAMs, and was associated with the release of pro-inflammatory cytokines.

(3) PY314m treatment also resulted in an increase in the absolute number of intra-tumor immune cells that are known to drive anti-tumor responses, including cytotoxic CD8⁺ T cells.

(4) PY314m demonstrated compelling anti-tumor activity in combination with an anti-PD-1 mAb in a number of preclinical, anti-PD-1 resistant mouse syngeneic tumor models. Furthermore, PY314m also exhibited strong single-agent activity in a subset of these tumor models. PY314m plus anti-PD-1 mAb combination treatment produced long-term immunological memory as evidenced by the lack of tumor growth upon rechallenge in mice cured of their tumors.

(5) Based on these preclinical findings, we are developing PY314 as a therapeutic agent for monotherapy and/or CPI combination therapy for solid tumors.