Tuning the Tumor Myeloid Microenvironment (TME) by Targeting TREM2⁺ Tumor-Associated Macrophages to Overcome Resistance to Immune Checkpoint Inhibitors

Nadine Jahchan, Mikhail Binnewies, Joshua L. Pollack, Ranna Mehta, Subhadra Dash, Pamela Canaday, Christine Tu, Erick Lu, Xiaoyan Du, Kevin P. Baker, and Venkataraman Sriram

Pionyr Immunotherapeutics, 2 Tower Place, Suite 800, South San Francisco, CA 94080

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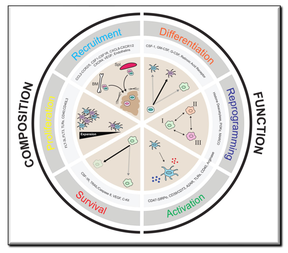
Abstract # LB071

Abstract

The tumor microenvironment (TME) often contains high levels of suppressive myeloid cells that may contribute to innate checkpoint inhibitor (CPI) resistance. Pionyr's Myeloid Tuning™ approach involves altering the composition and/or the function of myeloid cells in the TME. To this end, therapeutic targeting of tumor-associated macrophages (TAMs) is a promising strategy to increase CPI response rates in solid tumor indications, as well as to overcome resistance to CPI therapies. Pionyr and others identified the transmembrane protein triggering receptor expressed on myeloid cells-2 (TREM2) as a highly enriched TAMs target.

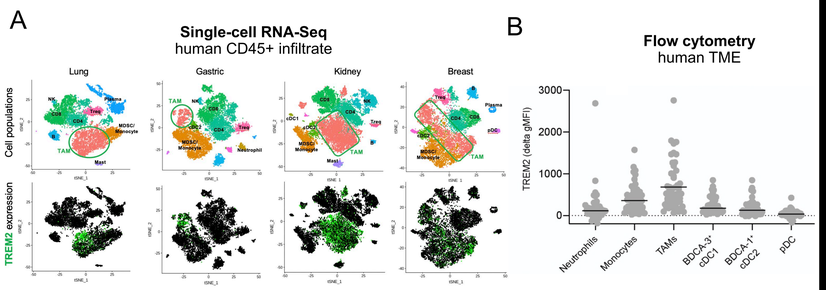
Pionyr developed a lead anti-TREM2 monoclonal antibody, termed PY314, as well as a murinized version of PY314, termed PY314m. PY314m demonstrated significant anti-tumor activity either as single agent in CPI-sensitive syngeneic tumor models or in combination with anti-PD-1 in CPI-resistant syngeneic tumor models. Mechanistically, PY314m reduced the pro-tumorigenic MHC class II-low, M2-like TAMs, induced pro-inflammatory cytokine production, and significantly increased CD8⁺ T cell infiltration into the TME. These findings suggest that PY314 therapy could be used to overcome CPI resistance in humans. To select patients most likely to benefit from PY314 therapy, Pionyr developed a qualitative IHC assay that detects TREM2 expression levels in formalin-fixed, paraffin-embedded human tumor tissues. Screening for TREM2 expression in tumor tissues demonstrated that TREM2⁺ TAMs were present in multiple solid tumor indications and their number increased with disease grade in a selected set of indications. The TREM2 IHC assay will be used to test our hypothesis that patients with tumors with high level of TREM2+ TAMs are most likely to benefit from PY314 treatment.

PIONYR's lead therapy, PY314, targets tumor-associated myeloid cells



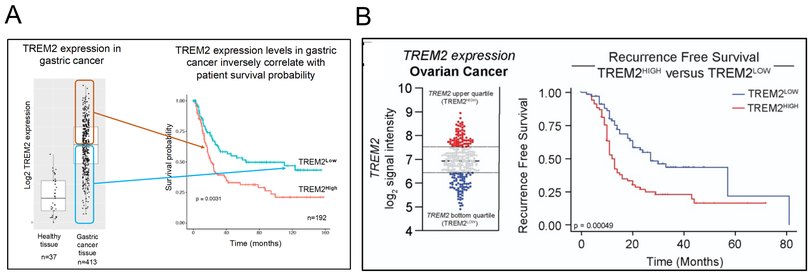
Jahchan et al.; Front Immunol. 2019 10:1611

TREM2 is Enriched on TAMs in the Human TME



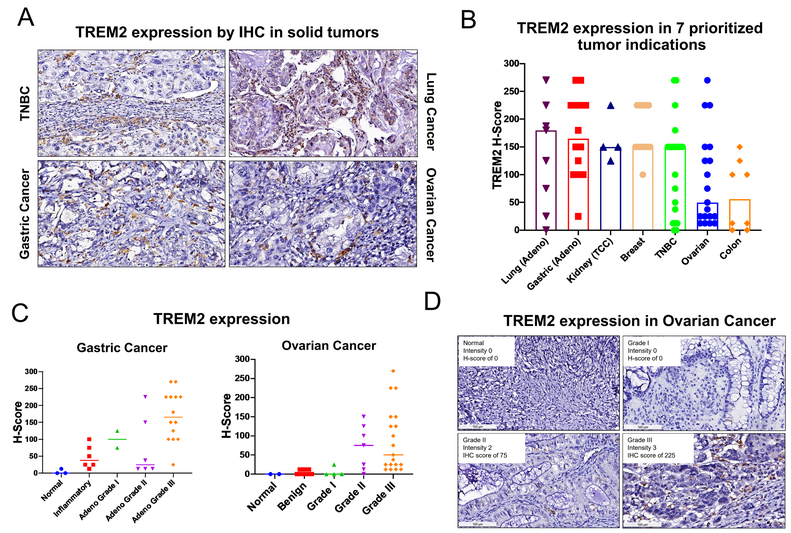
Dissociated human tumor samples were procured from Discovery Life Sciences (Huntsville, AL) for use in single cell RNA sequencing (**A**) or flow cytometry to identify immune subsets on multiple solid tumor indications (**B**). TREM2 RNA and protein expression is restricted to TAMs with minimal to no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

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



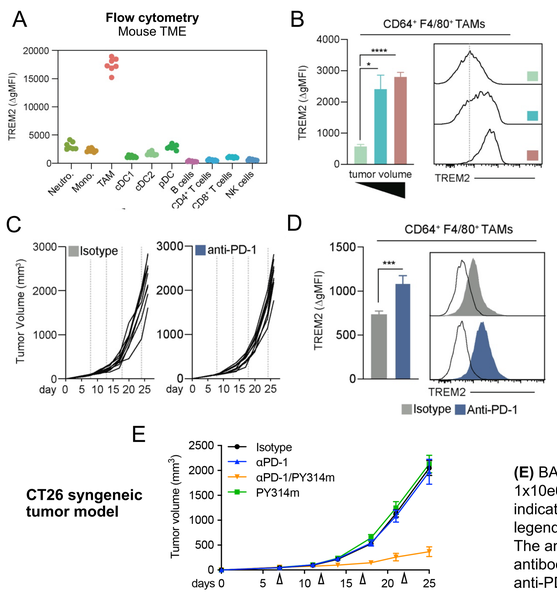
(A) RNAseq data from the TCGA gastric cohort was analyzed and normalized TREM2 expression profiles were downloaded from GEO (GSE15459) and divided into two cohorts based on median level of TREM2 (panel 1). Kaplan-Meier survival curves were then plotted for each cohort (panel 2). **(B)** Analysis of an ovarian cancer dataset that contains both expression data and recurrence free survival data. Patients in the upper and lower quartile of TREM2 mRNA expression were identified (left) and assessed for recurrence-free survival (right).

Profiling TREM2 in Multiple Solid Tumors by IHC Shows Increased Expression in Higher Tumor Grade



IHC analysis of TREM2 expression was evaluated in TMAs from multiple tumor indications using PIT2D, an anti-TREM2 mAb developed at Pionyr. **(A)** Shown are representative images of TREM2 in grade III tumor cores from multiple indications stained with PIT2D (DAB Brown stain). TREM2 could be seen in the tumor-associated stroma and within the tumor nests. **(B)** The median H-scores (semi-quantitative scoring by a board-certified pathologist evaluating the percentage of positive cells as well as stain intensity) for PIT2D staining of TREM2 per indication from the advanced tumor grades of the 7 prioritized tumor indications. Higher TREM2 expression correlated with disease grade in various tumor types, including gastric adenocarcinoma and ovarian cancer, as shown by the TREM2 H-score graphs **(C)** and representative images of normal, grade I, grade II, and grade III ovarian cases stained with PIT2D **(D)**.

TREM2 Expression Correlates With Tumor Size and Anti-PD-1 Resistance in Mouse Models

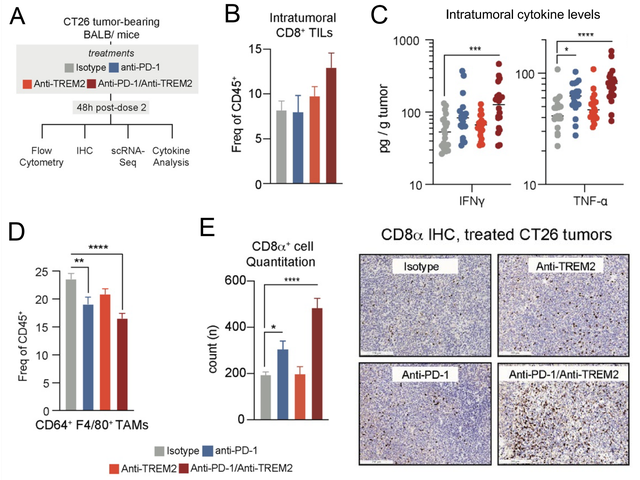


Profiling TREM2 surface levels in the mouse TME by Flow cytometry shows specific TREM2 expression on TAMs **(A)**, which correlates with tumor size as shown by the representative tumor volume histograms **(B)**.

(C) Tumor growth from CT26 tumor bearing mice treated with either isotype or anti-PD-1. **(D)** TREM2 surface levels on TAMs 2 days after the second dose of either isotype or anti-PD-1 (left) and representative histograms (right) shows that TREM2 expression correlates with ant-PD-1 resistance in this model.

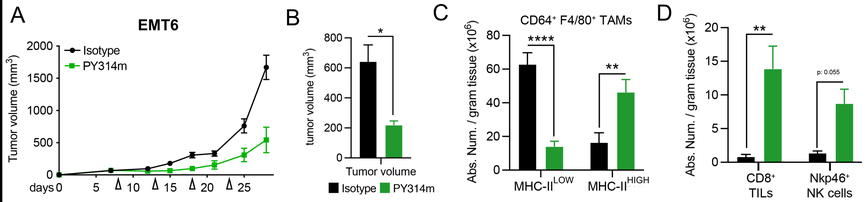
(E) BALB/c females were implanted with 1x10e6 CT26 tumor cells. Vertical points indicate days when the antibodies in the legend were administered intraperitoneally. The anti-TREM2 murinized monoclonal antibody (PY314m) reverses resistance to anti-PD1 therapy.

PY314m Combined with anti-PD-1 Promotes Effector T cell Function in anti-PD-1 Resistant Tumor Models



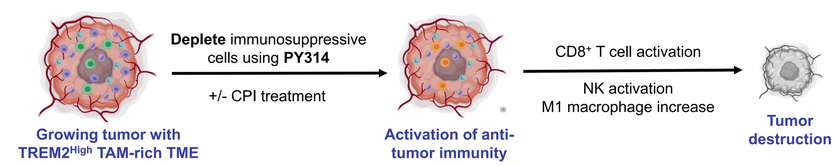
Anti-PD-1 and anti-TREM2 synergize to augment the abundance of intratumoral CD8+ T cells and to promote effector T cell function and remodel the TAM compartment in the TME.
(A) Experimental schematic detailing the approaches to assess pharmacodynamic changes in the TME following mAb therapy. **(B)** Quantified frequency of intratumoral CD8⁺ T cells over total CD45⁺ cells and **(C)** intratumoral cytokine levels from CT26 tumors treated with antibodies. **D)** Flow cytometric proportion of CD64⁺ F4/80⁺ TAMs as a frequency of CD45⁺ tumor immune cells. **(E)** Quantification of CD8+ T cells (left), with representative IHC images of CD8 DAB staining from tumors (right).

PY314m Therapy Reduces Tumor Growth as single agent and Enhances the TME Landscape



Treatment of EMT6 tumor bearing mice with PY314m (green) reduces tumor growth and alters the immune composition in the TME. BALB/c females were implanted with 1x10e6 EMT6 tumor cells and PY314m as a single agent reduces tumor growth in an efficacy study after 4 doses of PY314m **(A)** and in a pharmacodynamic study after 2 doses of PY314m **(B)**. MHC-II-LOW TAMs are reduced and MHC-II-HIGH TAMs are increased following PY314m treatment (**C**), leading to an increase in CD8+ TILs and NKp46+ NK cells in the TME following PY314m monotherapy **(D)**.

Summary: PY314 is a First-in-Class Anti-TREM2 Therapeutic Antibody



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.

Therapy	MOA	Pre-clinical anti-tumor activity	Safety	Manufacturing	Clinical Trial
PY314	**Selective Depletion** of immunosuppressive "M2-like" TAMs	**Broad** activity (As single agent and/or in combination with CPI in 7/9 syngeneic tumor models)	**Excellent** NOAEL at highest dose tested in GLP NHP studies (50 mg/kg)	**Excellent** (~4 grams purified PY314 per liter)	Phase 1a/1b study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of PY314 as a single Agent and in combination with Pembrolizumab in subjects with advanced solid tumors. **Clinical Trial: NCT04691375**