Tuning Tuning the tumor microenvironment by reprogramming TREM1⁺ myeloid cells to unleash anti-tumor immunity in solid tumors

Nadine Jahchan, Johanna Ramoth, Vladi Juric, Erin Mayes, Shilpa Mankikar, Ranna Mehta, Mikhail Binnewies, Subhadra Dash, Rachael Palmer, Joshua L. Pollack, Joshua Rudolph, Pamela Canaday, Linnea Haeggblom, Carlos Santamaria, Xiaoyan Du, Leonard Reyno, Kevin P. Baker and Linda Liang

Abstract # 1

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

PionyrTX.com

Abstract

Background: The tumor microenvironment (TME) often contains high levels of suppressive myeloid cells that 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. Myeloid reprogramming alters the function of immunosuppressive myeloid cells to acquire an immunostimulatory phenotype. Triggering receptor expressed on myeloid cells-1 (TREM1) is an immunoglobulin superfamily cell surface receptor enriched on tumor-associated myeloid cells. To investigate the potential of TREM1 modulation as an anti-cancer therapeutic strategy, Pionyr developed an afucosylated humanized anti-TREM1 monoclonal antibody termed PY159 and characterized it in pre-clinical and translational biomarker assays described below.

***Materials and methods*:** PY159 responses in human whole blood and dissociated primary tumor cells *in vitro* were evaluated by flow cytometry and measurement of secreted cytokines and chemokines by MSD. TREM1 expression in human tumors was validated by scRNAseq, flow cytometry, and immunohistochemistry (IHC). *In vivo* efficacy and pharmacodynamic studies of a surrogate anti-mouse TREM1 antibody, termed PY159m, were evaluated using syngeneic mouse tumor models, either as a single agent or in combination with anti-PD-1. To select tumor types and patients most likely to benefit from PY159 therapy, Pionyr developed qualitative and quantitative monoplex and multiplex IHC assays that detect TREM1 expression levels in human tumor tissues.

Results: PY159 treatment *in vitro* induced signaling, upregulated monocyte activation markers, and induced proinflammatory cytokines. In human tumors, TREM1 was detected on tumor-associated neutrophils, tumor-associated macrophages, and monocytic myeloid-derived suppressive cells. The surrogate PY159m anti-mouse TREM1 antibody exhibited anti-tumor efficacy in several syngeneic mouse tumor models, both as single-agent and in combination with anti-PD-1. Screening for TREM1 expression in tumor tissues demonstrated that TREM1⁺ tumor associated myeloid cells were highly enriched in the TME of multiple solid tumor indications. The monoplex and multiplex IHC assays offered insights into the localization of TREM1⁺ myeloid cells and their spatial relationship with other immune cells present in the TME to determine what immune composition will be more favorable for response to PY159.

Conclusions: Collectively, the available nonclinical data support PY159 as a TREM1 agonist that reprograms myeloid cells and unleashes anti-tumor immunity. PY159 safety and efficacy are currently being evaluated in first-in-human clinical trial (NCT04682431) involving select advanced solid tumors patients resistant and refractory to standard of care therapies alone and in combination with a CPI. The monoplex TREM1 IHC assay is successfully being used on FFPE archival tumor tissues from enrolled patients to determine TREM1 expression levels.

Targeting the TREM1 Receptor

TREM1: Triggering receptor expressed on myeloid cells 1

Localization: Cell surface and soluble

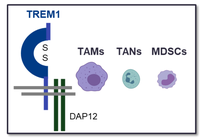
Function: Activating receptor implicated in innate immunity

Signaling: Through association with ITAM-containing DAP12

Genetics: *Trem1*$^{-/-}$ mice have a reduced susceptibility to colitis, reduced neutrophil infiltration following *Leishmania major* infection, increased morbidity from *Influenza* infection, and reduced susceptibility to inflammation-induced cancer

Ligands: Peptidoglycan recognition protein 1 (PGLYRP1), others

Expression: Neutrophils, monocyte subsets, macrophages- upregulated on TAMs, TANs and mMDSCs in multiple tumor indications



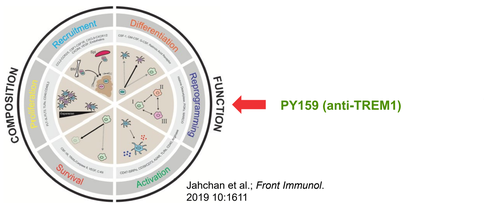


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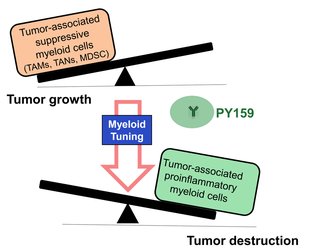
Figure 1. Pionyr Myeloid tuning approach involves modulating inhibitory myeloid populations in the TME with high precision and selectivity. PY159 (anti-TREM1) tunes by changing the function of immunosuppressive myeloid cells within the TME to generate effective anti-tumor immunity.



TREM1 is Expressed on Several Tumor-infiltrating Myeloid Populations In Human Tumors

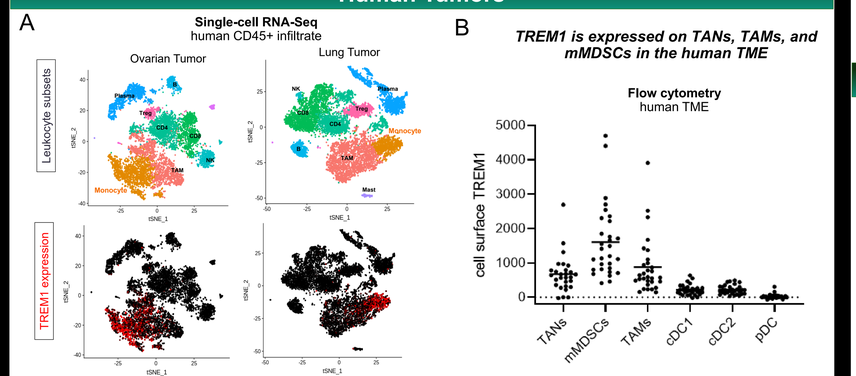


Figure 2. (**A**) Single-cell RNAseq of CD45+ cells from a human ovarian cancer and human lung cancer. UMAP plots depict distinct leukocyte subsets (top) and TREM1 expression in red (bottom). (**B**) TREM1 staining on TANs, mMDSCs, TAMs, cDC1, cDC2, and pDCs in 30 dissociated human tumor samples by flow cytometry. Tumor types include breast, bladder, endometrial, head and neck, ovarian, and renal cancer.

PY159 is Afucosylated Anti-human TREM1 Antibody That Promotes Signaling Through TREM1/DAP12

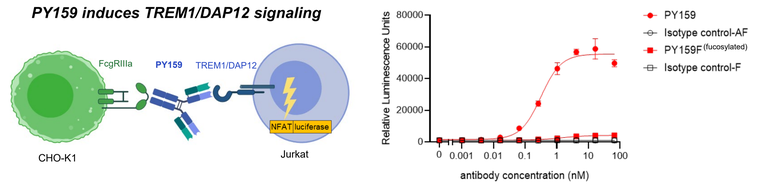


Figure 2. Activity of PY159, PY159F (fully fucosylated version of PY159), or corresponding isotype controls in TREM1/DAP12 reporter assay. CHO-K1 cells expressing human FcgRIIIa, and Jurkat cells expressing human TREM1/DAP12 and the NFAT-luciferase reporter, were co-cultured for 6 hours in the presence of a dose titration of antibodies. Reporter activity was detected by luminescence (RLU, relative light units).

Induction of Proinflammatory Cytokines and Chemokines by PY159

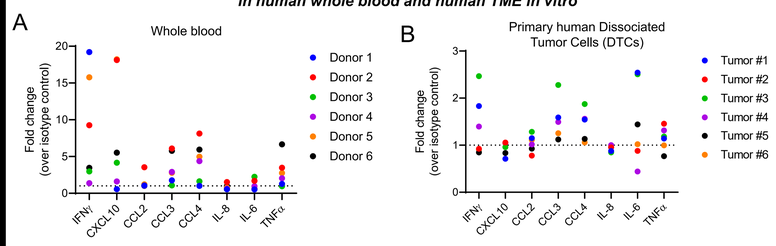


Figure 3. (**A**) Whole blood from six healthy human donors was treated for 24hrs with 25nM PY159 or isotype control. Plasma cytokines and chemokines were measured using the MSD platform. The graph represents PY159-induced cytokines as fold increases relative to the effects of isotype control in individual donors. (**B**) Single cell suspensions from human lung tumor tissues were treated with 30nM PY159 or isotype control (Afucosylated hIgG1) for 24hrs, and secreted cytokines and chemokines were measured by MSD. Cytokine induction by PY159 is presented as fold increase relative to the effects of the corresponding isotype control in individual donors.

Anti-mouse TREM1 Antibody, PY159m, Exhibits Anti-Tumor Activity

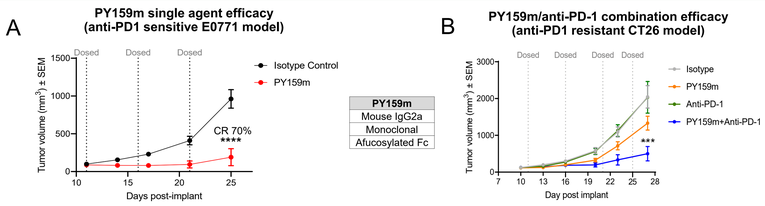


Figure 4. (**A**) E0771 mouse syngeneic breast tumors were grown orthotopically in mammary fat pads of C57BL/6 mice. Dosing with the afucosylated mouse IgG2a isotype control or a surrogate anti-mouse TREM1 antibody (PY159m), was initiated when average tumor volume reached 95 mm^3.. Complete tumor regression (CR) was calculated as % of tumors with TV <50 mm3 at study end. Two Way ANOVA followed by Sidak's multiple comparison test (****P<0001) were used for statistical comparison between the groups. (**B**) CT26 mouse syngeneic colorectal tumors were grown subcutaneously. Dosing with the isotype control, anti-PD-1 (5 mg/kg), PY159m (10 mg/kg), or the combination of anti-PD-1 and PY159m was initiated when average tumor volume reached 110 mm3. Two Way ANOVA followed by Tukey's multiple comparison test (***P=0001) were used for statistical comparison of PY159m and combination groups. For both studies, animals were dosed intraperitoneally (vertical dotted lines) with 10 mg/kg (n=10 mice/group) of the test antibodies

PY159m Induces Proinflammatory Cytokines in Mouse Tumors

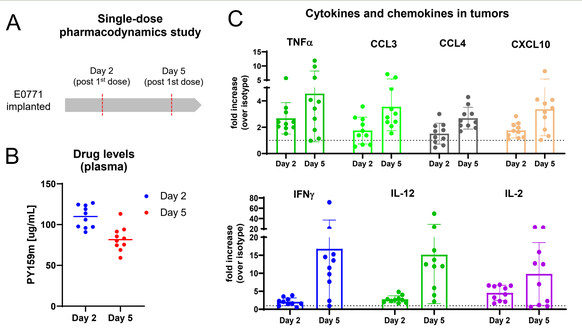


Figure 5. (A) Experiment. al schematic describing a single dose pharmacodynamic study in E0771 tumor-bearing mice, treated with isotype control and PY159m (10mg/kg; ip).
(B) The PY159m drug levels in Plasma at Day 2 and Day 5 after single dose were measured by a PK assay.
(C) Intratumoral cytokines and chemokine levels were measured from E0771 digested tumors (Luminex) and were plotted as a fold increase of PY159m treatment over isotype control.

Optimization and Validation of the TREM1 IHC Assay

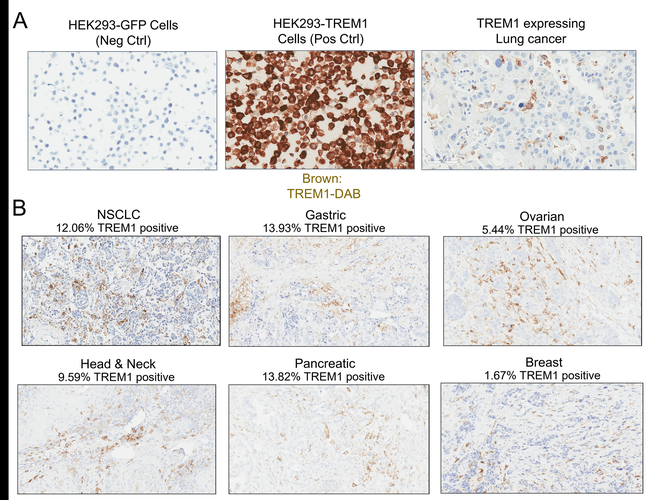


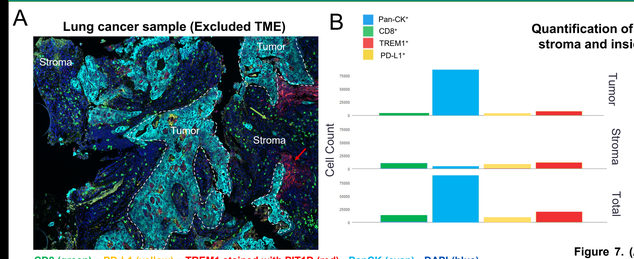
Figure 6. The TREM1 IHC assay was optimized and validated on an automated staining platform using an IHC compatible anti-human TREM1 antibody (custom made).

(A) FFPE HEK-293T cell lines were used as positive (TREM1 expressing) and negative (GFP expressing) controls, and a lung tumor block was selected to use as control tissue, where high levels of TREM1 staining were observed on tumor infiltrating myeloid cells (TAMs and TANs).

(B) The sensitivity of the TREM1 DAB IHC assay was tested on 67 preclinical archival tumor blocks at Mosaic CAP-CLIA IHC Labs and evaluated by pathology guided image analysis using the HALO software. Shown are representative IHC images (20x) of high and low TREM1 expression on FFPE tissues from six solid tumor indications stained with the anti-TREM1 antibody. The percentage of TREM1 expressing myeloid infiltrating cells in the whole tumor area is depicted at the top of each image.

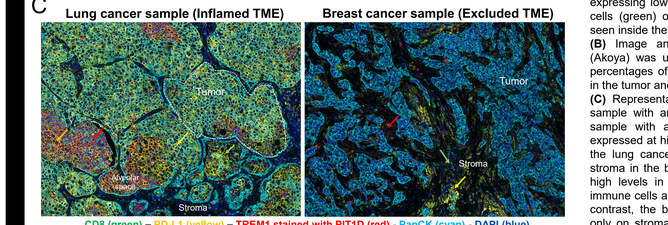
Note: Due to tissue size and heterogeneity of staining, images may not be truly representative of the analysis.

Multiplex IF Analysis to determine TREM1 Levels and the TME Composition



Percentage of positive cells	In Tumor	In Stroma	In Total Tissue
TREM1+ cells	6%	8%	7%
CD8+ T cells	2%	7%	5%
PD-L1+ cells	1%	5%	3%
Pan-Cytokeratin	75%	2%	34%

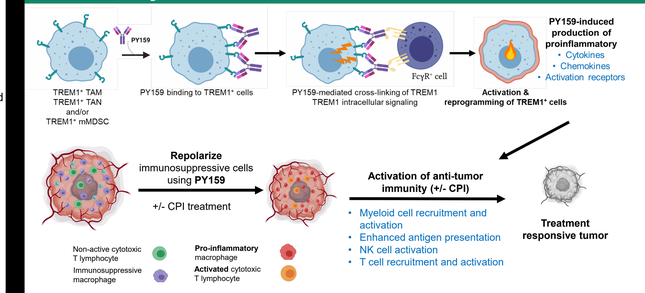
CD8 (green) – PD-L1 (yellow) – TREM1 stained with PIT1D (red) - PanCK (cyan) - DAPI (blue)



CD8 (green) – PD-L1 (yellow) – TREM1 stained with PIT1D (red) - PanCK (cyan) - DAPI (blue)
PD-L1+ TREM1+ cells (orange)

Figure 7. (A) Multiplex Immunofluoresence IF (OPAL from Akoya) staining of an immune excluded lung tumor expressing low levels of PD-L1 and high levels of CD8+ T cells (green) only in the stroma. TREM1+ cells (red) are seen inside the tumor nests and in the surrounding stroma.
(B) Image analysis using the inForm Vectra software (Akoya) was used to determine the total cell counts and percentages of positive expression for each cell phenotype in the tumor and stroma tissue compartments.
(C) Representative multiplex IF images of a lung cancer sample with an inflamed TME (left) and a breast cancer sample with an excluded TME (right). TREM1 (red) is expressed at high levels inside the alveolar luminal space in the lung cancer sample while present in the surrounding stroma in the breast cancer sample. PD-L1 is expressed a high levels in the lung sample on both tumor cells and immune cells and could co-localize with TREM1 (orange). In contrast, the breast cancer sample shows PD-L1 staining only on stromal immune cells. All images are 20x stamps captured on the Vectra 3 microscope (Akoya).

Summary: PY159 is a First-in-Class Anti-TREM1 Therapeutic Antibody



- PY159 is a humanized IgG1 afucosylated mAb that specifically binds human TREM1 and acts as a TREM1 agonist
- PY159 "re-tunes" the tumor microenvironment by reprogramming the immunosuppressive myeloid cells to acquire proinflammatory phenotype
- PY159m, an afucosylated mouse IgG2a mAb against mouse TREM1, demonstrates anti-tumor activity in multiple mouse syngeneic tumor models
- PY159 is currently undergoing testing in Phase 1a clinical trials in advanced solid tumors relapsed or refractory to SOC (NCT04682431)

Acknowledgements: We want to thank the PY159 R&D and clinical teams at Pionyr Immunotherapeutics and our collaborators at Mosaic CAP-CLIA IHC Laboratories (Forest Lake, CA) for their work on the optimization and validation of the anti-TREM1 IHC assay for use in Phase 1 clinical trial.