Therapeutic Targeting of TREM1 With PY159 Promotes Myeloid Cell Reprogramming and Unleashes Anti-tumor Immunity

Erin Mayes, Vladi Juric, Mikhail Binnewies, Pamela Canaday, Tian Lee, Subhadra Dash, Joshua L. Pollack, Joshua Rudolph, Vicky Huang, Xiaoyan Du, Nadine Jahchan, Asa Johanna Ramoth, Shilpa Mankikar, Manith Norng, Carlos Santamaria, Kevin P. Baker and Linda Liang

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

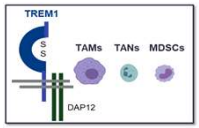
Introduction

Background: Myeloid cells present in the tumor microenvironment can exist in immunosuppressive states that impede productive anti-tumor immunity. One strategy for targeting these immunosuppressive mechanisms is reprogramming of myeloid cells from immunosuppressive to immunostimulatory, resulting in the removal of the immune inhibition and unleashing of anti-tumor immunity. Triggering receptor expressed on myeloid cells-1 (TREM1) is an immunoglobulin superfamily cell surface receptor expressed primarily on neutrophils and subsets of monocytes and tissue macrophages. TREM1 signals through the association with the DAP12 adaptor protein, and mediates proinflammatory signaling, amplifies the host immune response to microbial pathogens, and has been implicated in the development of acute and chronic inflammatory diseases. TREM1 is also enriched in tumors, specifically on tumor-associated myeloid cells.

Materials and Methods: An FcγR binding ELISA and a Jurkat TREM1/DAP12 NFAT-luciferase reporter cell line were used to assess PY159 binding to human FcγRs and TREM1 signaling. PY159 responses in human whole blood *in vitro* were evaluated by flow cytometry, transcriptional analysis of sorted leukocyte subsets, and measurement of secreted cytokines/chemokines by MSD. TREM1 expression in human tumors was validated by scRNAseq and flow cytometry. Anti-tumor efficacy of a surrogate anti-mouse TREM1 antibody, PY159m, was evaluated using syngeneic mouse tumor models, either as a single agent or in combination with anti-PD-1.

TREM1 Background

TREM1:	Triggering Receptor Expressed on Myeloid cells 1
Localization:	Cell surface and soluble
Expression:	Macrophages, monocyte subsets, neutrophils -upregulated on TAMs, TANs and MDSCs
Function:	Activating receptor implicited in innate immunity
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



PY159 Induces Proinflammatory Mediators in Human Tumors

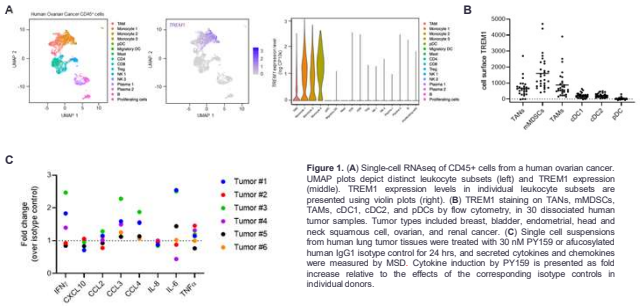


Figure 1. (**A**) Single-cell RNAseq of CD45+ cells from a human ovarian cancer. UMAP plots depict distinct leukocyte subsets (left) and TREM1 expression (middle). TREM1 expression levels in individual leukocyte subsets are presented using violin plots (right). (**B**) TREM1 staining on TANs, mMDSCs, TAMs, cDC1, cDC2, and pDCs by flow cytometry, in 30 dissociated human tumor samples. Tumor types included breast, bladder, endometrial, head and neck squamous cell, ovarian, and renal cancer. (**C**) Single cell suspensions from human lung tumor tissues were treated with 30 nM PY159 or afucosylated human IgG1 isotype control for 24 hrs, 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 controls in individual donors.

PY159 Binds Specifically to Human and Cynomolgus Monkey TREM1

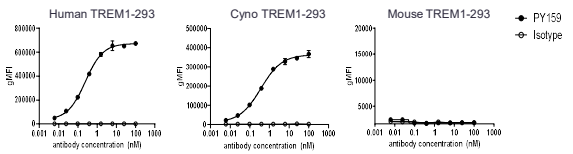


Figure 2. PY159 binding affinity for cell surface expressed TREM1 was measured using HEK 293 cells recombinantly expressing human, cynomolgus monkey (cyno) or mouse TREM1 and DAP12. Cells were incubated with a dose titration of PY159 or afucosylated human IgG1 isotype control. Antibody binding was detected by flow cytometry using an APC-labeled secondary anti-human IgG antibody. PY159 binds to human and cyno TREM1, but not to mouse TREM1.

PY159 is an Afucosylated Anti-human TREM1 Antibody With Enhanced FcγR Binding and Promotes Signaling Through TREM1/DAP12

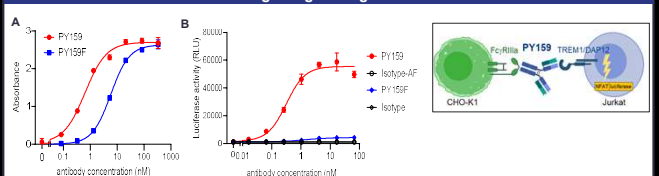


Figure 3. (**A**) PY159 and PY159F (fully fucosylated version of PY159) were tested for binding to immobilized recombinant human FcγRIIIa by ELISA. Antibody binding was detected using a secondary HRP-conjugated goat anti-human F(ab')2 antibody, followed by the measurement of absorbance (optical density) at 450 nm. (**B**) Activity of PY159, PY159F or corresponding isotype controls in the TREM1/DAP12 reporter assay. CHO-K1 cells, expressing human FcγRIIIa, 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).

PY159 Increases Expression of T cell Co-activation Markers on Monocytes

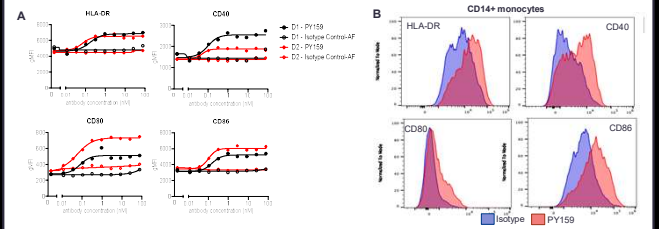


Figure 4. Peripheral blood leukocytes from two healthy human donors, obtained by RBC lysis of whole blood, were cultured for 24 hrs with a dose titration of PY159 or isotype control (AF-afucosylated). The cells were stained with a panel of leukocyte lineage markers, and with anti-HLA-DR, anti-CD40, anti-CD80, and anti-CD86, for downstream analysis by flow cytometry. (**A**) PY159-induced a dose-dependent increase in expression of HLA-DR, CD40, CD80 and CD86 activation markers on CD14+ monocytes (**B**) Histograms depicting expression of CD80, CD86, CD40 and HLA-DR expression on CD14+ monocytes after 24 hrs of treatment with 7 nM isotype control of PY159.

PY159 Promotes Neutrophil Migration

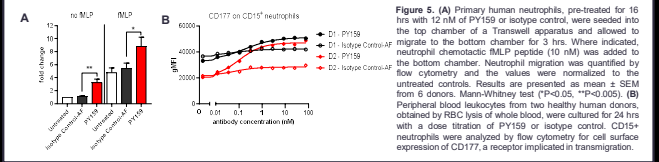


Figure 5. (**A**) Primary human neutrophils, pre-treated for 16 hrs with 12 nM of PY159 or isotype control, were seeded into the top chamber of a Transwell apparatus and allowed to migrate to the bottom chamber for 3 hrs. Where indicated, neutrophil chemotactic fMLP peptide (10 nM) was added to the bottom chamber. Neutrophil migration was quantified by flow cytometry and the values were normalized to the untreated controls. Results are presented as mean ± SEM from 6 donors. Mann-Whitney test (*P<0.05, **P<0.005). (**B**) Peripheral blood leukocytes from two healthy human donors, obtained by RBC lysis of whole blood, were cultured for 24 hrs with a dose titration of PY159 or isotype control. CD15+ neutrophils were analyzed by flow cytometry for cell surface expression of CD177, a receptor implicated in transmigration.

Induction of Proinflammatory Cytokines and Chemokines by PY159

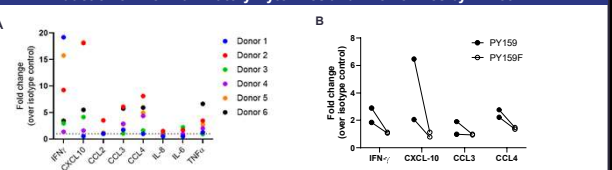


Figure 6. (**A**) Whole blood from six healthy human donors was treated for 24 hrs with 25 nM PY159 or isotype control. Plasma cytokines and chemokines were measured using by MSD. The graph represents PY159-induced cytokines as fold increase relative to the effects of isotype control in individual donors. (**B**) Whole blood from two donors was treated with 10ug/mL of PY159, fully fucosylated PY159F, or the corresponding isotype controls. Cytokine induction by PY159 or PY159F is presented as fold increase relative to the effects of the corresponding isotype controls in individual donors.

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

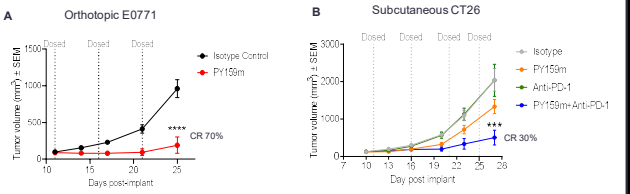
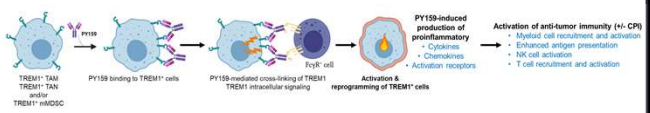


Figure 7. (**A**) E0771 mouse syngeneic breast tumors were grown orthotopically in mammary fat pads of C57BL/6 mice. Dosing with the afucosylated mouse IgG2a isotype control or a surrogate anti-mouse TREM1 antibody, PY159m, was initiated when average tumor volumes reached 95 mm³. Animals were dosed intraperitoneally (vertical dotted lines) with 10 mg/kg (N=10 mice/group) of the test antibodies. 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. Animals were dosed intraperitoneally (vertical dotted lines) (N=10 animals/group). Two Way ANOVA followed by Tukey's multiple comparison test (***P=0001) were used for statistical comparison of PY159m and combination groups.

Proposed Mechanism-of-Action of PIONYR's Anti-TREM1 Antibody PY159



Results & Conclusions

PY159 is an afucosylated humanized IgG1 monoclonal antibody that can activate TREM1/DAP12 signaling due to increased binding affinity for FcγR. In human blood assays, PY159 treatment upregulated monocyte activation markers, promoted neutrophil chemotaxis, and induced proinflammatory cytokines and chemokines, which was dependent on PY159 afucosylation. In human tumors, TREM1 was detected on tumor-associated neutrophils, tumor-associated macrophages, and monocytic myeloid-derived suppressive cells. PY159 induced proinflammatory cytokines and chemokines in dissociated human tumors *in vitro*, demonstrating that PY159 can reprogram tumor-associated myeloid cells. A surrogate anti-mouse TREM1 antibody, PY159m, exhibited anti-tumor efficacy in several syngeneic mouse tumor models, both as single agent and in combination with anti-PD-1. These results show that PY159 reprograms myeloid cells and unleashes anti-tumor immunity. PY159 safety and efficacy are currently being evaluated in a first-in-human clinical trial (NCT04682431) involving patients resistant and refractory to standard of care therapies.