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TREM1 Agonist Antibody PY159 Promotes Myeloid Cell Reprogramming and Unleashes Anti-tumor Immunity

Vladi Juric, Erin Mayes, 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

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

Background: Tumor-associated myeloid cells can impede productive anti-tumor immunity. One strategy for targeting immunosuppression is myeloid reprogramming, which drives immunosuppressive myeloid cells to acquire an immunostimulatory phenotype. Triggering receptor expressed on myeloid cells-1 (TREM1) is an immunoglobulin superfamily cell surface receptor expressed on neutrophils, subsets of monocytes and tissue macrophages. TREM1 associates with the DAP12 adaptor protein and induces proinflammatory signaling, amplifies innate immune responses, and is implicated in the development of acute and chronic inflammatory diseases. TREM1 is also enriched in tumors, specifically on tumor-associated myeloid cells. To investigate the potential of TREM1 modulation as an anti-cancer therapeutic strategy, we developed PY159, an afucosylated humanized anti-TREM1 monoclonal antibody, and characterized it in the pre-clinical assays described below.

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, respectively. 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. A Transwell system was used to evaluate PY159 effects on neutrophil chemotaxis. 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 Receptor

TREM1: Triggering receptor expressed on myeloid cells 1

Localization: Cell surface and soluble

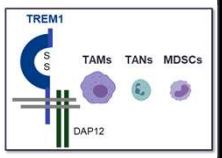
Expression: Neutrophils, monocyte subsets, macrophages -upregulated on TAMs, TANs and MDSCs

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



PY159 is an Afucosylated Anti-human TREM1 Antibody With Enhanced FcγR Binding

PY159 Antibody
Human IgG1κ
Monoclonal
Afucosylated Fc

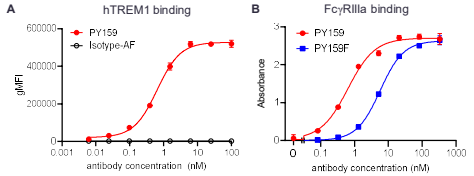


Figure 1. (**A**) PY159 binding to cell surface expressed human TREM1 (hTREM1) was evaluated using HEK 293 cells recombinantly expressing human TREM1 and DAP12. Antibody binding was detected by flow cytometry after staining with an APC-labeled secondary anti-human IgG antibody. (**B**) PY159 and PY159F (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.

PY159 Promotes Signaling Through TREM1/DAP12

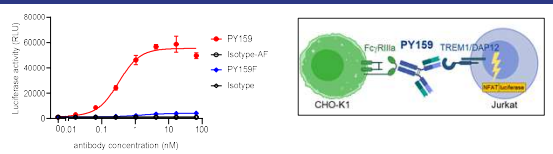


Figure 2. Activity of PY159, PY159F (fully fucosylated version of PY159), or corresponding isotype controls in TREM1/DAP12 reporter assay, depicted on the right. CHO-K1 cells, expressing human FcγRIIIa, and Jurkat cells, expressing human TREM1/DAP12 and the NFAT-luciferase reporter, were co-cultured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

PY159 Activates Monocytes and Promotes Neutrophil Migration

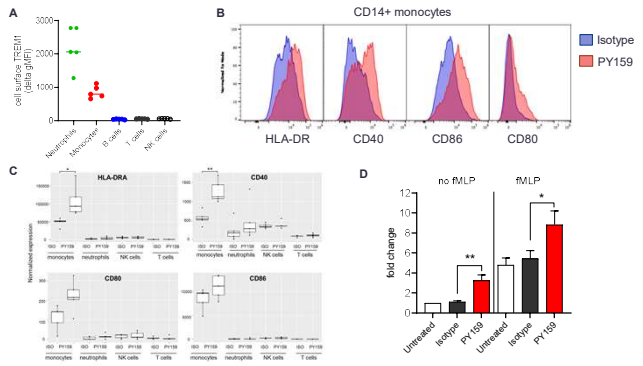


Figure 3. (**A**) Cell surface TREM1 staining in peripheral blood of five healthy human donors by flow cytometry. TREM1 levels are presented as delta gMFI values (isotype gMFI subtracted from TREM1 gMFI) on CD15$^+$ neutrophils, CD14$^+$ conventional monocytes, CD19$^+$ B cells, CD3$^+$ T cells, and CD56$^+$ NK cells. (**B**) Flow cytometry staining of HLA-DR, CD40, CD80, and CD86 on CD14+ monocytes after treatment of human peripheral blood with 7 nM PY159 or isotype control for 24 hrs. (**C**) Whole blood from 5 healthy donors was treated with 7 nM PY159 or isotype control for 16 hours. CD14+ monocytes, CD15+ neutrophils, CD56+ NK cells, and CD3+ T cells were sorted by FACS and subjected to RNA sequencing. Read count normalization and differential gene expression analysis were performed in R using DESeq2. P values were computed using a Wald test in a generalized linear model framework constructed using treatment status and donor of origin as variables of interest. (**D**) 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).

Induction of Proinflammatory Cytokines and Chemokines by PY159

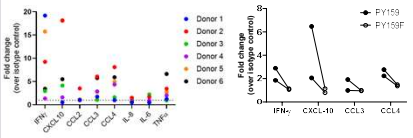


Figure 4. (**A**) Whole blood from six healthy human donors was treated for 24 hrs with 25 nM PY159 or isotype control. Plasma cytokines and chemokines were measured using the MSD platform. The graph represents PY159-induced cytokines as fold increases relative to the effects of isotype controls in individual donors. (**B**) Whole blood from two donors was treated with 10μg/mL of PY159, fully fucosylated PY159F, or corresponding isotype controls. Cytokine induction by PY159 or PY159F is presented as fold increase relative to the effects of corresponding isotype controls in individual donors.

PY159 Induces Proinflammatory Mediators in Human Tumors

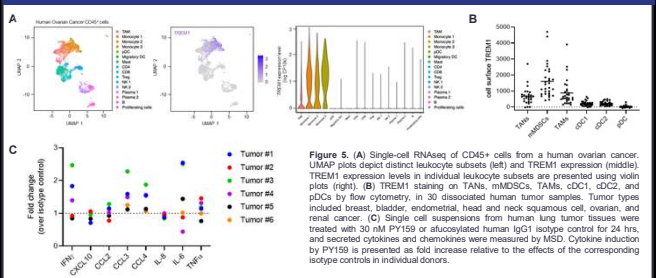


Figure 5. (**A**) Single-cell RNAseq of CD45+ cells from a human ovarian cancer. UMAP plots depict distinct leukocyte subsets (left) and TREM1 expression (middle). TREM1 expression levels in individual leukocyte subsets are presented using violin plots (right). (**B**) TREM1 staining on TANs, mMDSCs, 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.

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

PY159m
Mouse IgG2a
Monoclonal
Afucosylated Fc

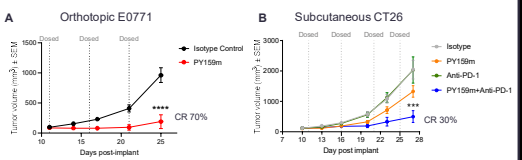
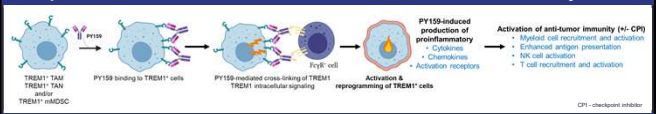


Figure 6. (**A**) E0771 mouse syngeneic breast tumors were grown orthotopically in mammary fat pads of C57BL/6 mice. Dosing with the afucosylated mouse IgG2a isotype control or a surrogate anti-mouse TREM1 antibody,PY159m,was initiated when average tumor volume reached 95 mm^3. Animals were dosed intraperitoneally (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 isotype control, anti-PD-1 (5 mg/kg), PY159m (10 mg/kg), or 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

Afucosylation of PY159 increased its binding affinity for FcγR and its ability to activate TREM1/DAP12 signaling. 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 first-in-human clinical trial (NCT04682431) involving patients resistant and refractory to standard of care therapies.