Development of PY159, a monoclonal antibody that repolarizes tumor-associated inhibitory myeloid cells, for the treatment of solid tumors

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PIONYR IMMUNOTHERAPEUTICS

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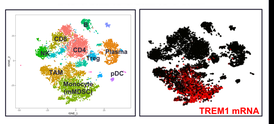
Introduction

To improve the proportion of patients who benefit from checkpoint inhibitor (CPI) therapy additional immune pathways likely need to be targeted. Tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and tumor-associated neutrophils (TANs) exhibit a spectrum of functional phenotypes ranging from immunosuppressive M2-like macrophages or N2-like neutrophils that promote tumor growth to pro-inflammatory M1-like macrophages and N1-like neutrophils that promote anti-tumor immunity. Therapies that shift the balance of inhibitory myeloid cells towards a more pro-inflammatory phenotype are expected to positively impact anti-tumor immune responses and convert CPI-resistant tumors into CPI-sensitive tumors.

The available preclinical and nonclinical data support PY159 immunotherapy, alone or in combination with a CPI, in cancer patients who are resistant or refractory to CPI therapies, to improve both the overall response rates as well as the durability of responses. First in human clinical testing will commence in 2020.

TREM1 Background

TREM1:	Triggering receptor expressed on myeloid cells 1
Expression:	Macrophages, monocyte subsets, neutrophils Upregulated on tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and myeloid-derived suppressor cells (MDSCs)
Function:	Activating receptor implicated 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, PIONYR's Anti-TREM1 mAb, Reprograms Suppressive Myeloid Cells

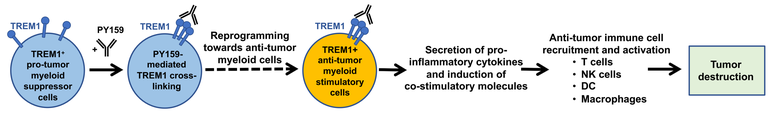


Figure 1. Model of PY159's mechanism-of-action. Cross-linking of cell surface TREM1 on tumor associated myeloid cell populations by PY159 causes downstream signaling that can induce secretion of a specific set of proinflammatory cytokines and chemokines as well as increase surface expression of HLA-DR and CD40. These immune mediators can recruit immune cells including T cells, NK cells, DCs, and macrophages and promote anti-tumor immune cell activation.

TREM1 is Expressed on Suppressive Myeloid Cells From Diverse Tumor Types

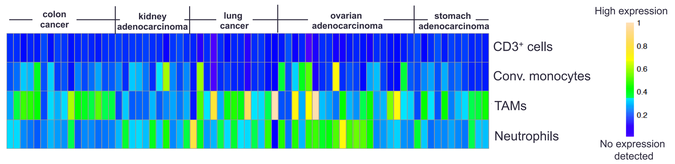
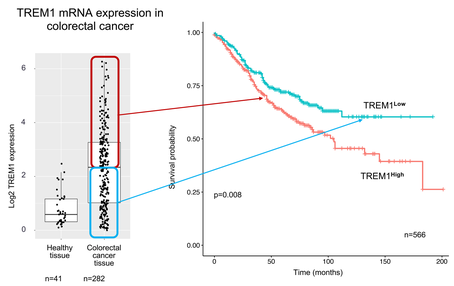


Figure 2. The indicated human tumors were dissociated and TREM1 expression was assessed on CD45+ leukocyte subsets by flow cytometry. The level of TREM1 expression was measured by mean fluorescence intensity (gMFI) adjusted to incorporate isotype background levels. These adjusted gMFI values were then normalized across leukocyte subsets to highlight those subsets which have high or low TREM1 expression across each indication.
1 = high expression, 0 = no expression detected.

TREM1 Expression is Higher in Diverse Cancers, and Inversely Correlates With Patient Survival



Negative correlation between TREM1 expression and patient survival also seen in:

- Breast cancer
- Pancreatic cancer
- SCC

High TREM1 mRNA expression seen in:

- NSCLC (non-SCC)
- HNSCC
- Ovarian
- Stomach
- Bladder

Figure 3. RNAseq data from the TCGA colon cohort were downloaded from the Broad Institute (left panel) and RSEM values for TREM1 mRNA from tumor and adjacent normal samples were converted to log 2 counts per million. Results were plotted in R. Normalized TREM1 expression profiles were downloaded from GEO (GSE35982) and divided into two cohorts based on median level of TREM1 (right panel). Kaplan-Meier survival curves were plotted for each cohort and the associated log-rank test was carried using the *survival* and *survminer* packages in R. Breast cancer, pancreatic cancer and squamous cell carcinoma (SCC) datasets were subjected to similar analysis, also revealing negative correlation between TREM1 mRNA and patient survival (data not shown).

PY159 Induces a Highly Selective Set of Anti-tumor Chemokines and Cell Surface Receptors

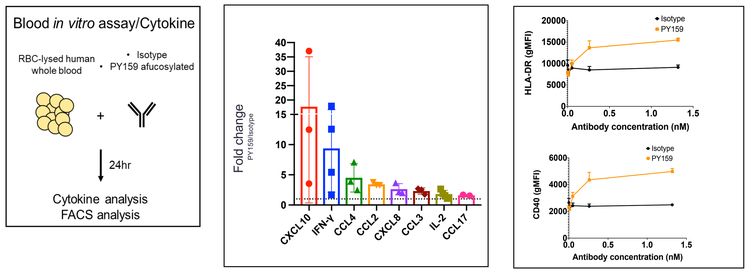


Figure 4. PY159-induced cytokines and activation receptors in human blood. RBC-lysed human whole blood was treated for 24-hours with PY159 (1 μg/ml). Supernatants were harvested for cytokine and chemokine analysis using MSD and cells were stained with a panel of leukocyte lineage markers and with anti-CD40 and anti-HLA-DR. PY159-induced increase in these activation markers was notable on monocytes. Cytokines shown above were detected >10 pg/ml and upregulated across experiments from multiple donors. Cytokines that did not meet criteria: IL-10, IL-12p70, IL-13, IL-4, GM-CSF, IL-1b, IL-6, TNF-a, CCL11, CCL26, CCL13, CCL22, IL-12/23p40, IL-15, IL-16, IL-17A.

PY159 Induces Signaling Downstream of TREM1 and DAP12

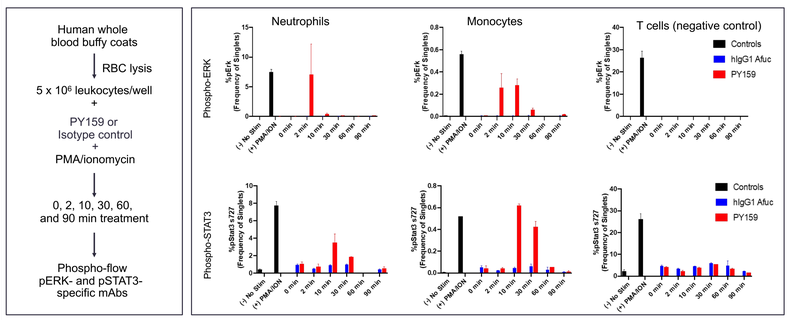


Figure 5. PY159 cross-linking induces signaling pathways downstream of TREM1 and DAP12. RBC-lysed human whole blood was stimulated with 5 μg/ml of PY159 or hIgG1 isotype control, or PMA and ionomycin as a positive control for the indicated times. Cells were fixed, permeabilized and stained with a panel of leukocyte lineage markers and with anti-phospho ERK and anti-phospho STAT3 antibodies. PY159 induces phospho- signaling in TREM1^{+} neutrophil and monocyte populations.

PY159m Induces Anti-tumor Immunity

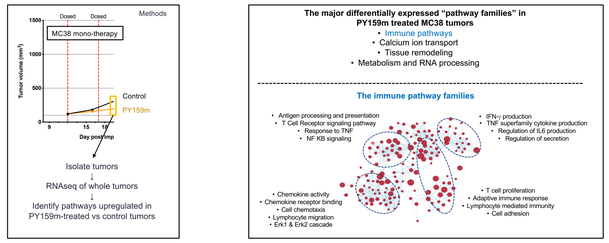
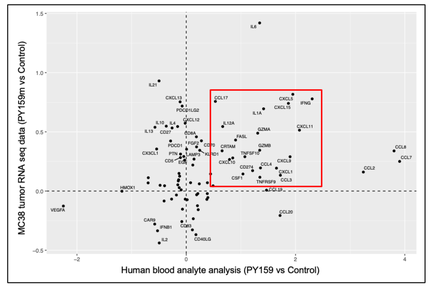


Figure 6. Mice bearing MC38 tumors (n=10 per group) treated with PY159m and isotype antibodies, respectively, were harvested on day 19 after implant (48 hours post 2nd treatment). RNA was extracted and sequenced. Differentially induced pathways associated with PY159m treatment were assessed using the Broad Institute's Gene Set Enrichment Analysis (GSEA) tool using the c5 collection of gene sets from MSigDB. Significantly upregulated pathways (FDR < 0.1) were visualized using the Enrichment Map module in Cytoscape using default parameters. Immune families from the resulting network map are shown with cluster-specific pathways highlighted.

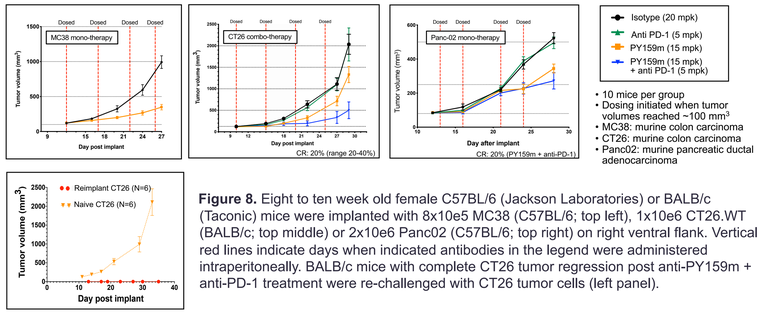
PY159 Produces a Pro-inflammatory Response



PY159-induced factors	Function
IFN-γ, IL-1α, IL-12, TNFSF9, CXCL9, CXCL10, CCL17	T cell recruitment and activation
IFN-γ, CXCL1, CXCL5, CXCL11, CXCL15, CCL4, CSF1	Myeloid cell recruitment and activation
IFN-γ, CXCL10, TNFSF9	Enhance antigen presentation
IFN-γ, CXCL10, IL-12, CCL4	NK cell activation
IFN-γ, CXCL9, CXCL10	Required for anti-PD-1 efficacy
TNFSF10, FasL, GzmA, GzmB	Direct tumor cell killing

Figure 7. RNA-based fold changes induced by PY159m treatment of MC38 tumors were compared to protein fold changes from PY159-treated RBC-lysed whole blood. Cytokine levels were assessed using the O-link multiplex platform.

PY159m has Significant Anti-tumor Activity



- 10 mice per group
- Dosing initiated when tumor volumes reached ~100 mm^3
- MC38: murine colon carcinoma
- CT26: murine colon carcinoma
- Panc02: murine pancreatic ductal adenocarcinoma

Figure 8. Eight to ten week old female C57BL/6 (Jackson Laboratories) or BALB/c (Taconic) mice were implanted with 8x10e5 MC38 (C57BL/6; top left), 1x10e6 CT26.WT (BALB/c; top middle) or 2x10e6 Panc02 (C57BL/6; top right) on right ventral flank. Vertical red lines indicate days when indicated antibodies in the legend were administered intraperitoneally. BALB/c mice with complete CT26 tumor regression post anti-PY159m + anti-PD-1 treatment were re-challenged with CT26 tumor cells (left panel).

PY159 Safety and PK Assessment Summary

Analysis	Summary findings
Rodent PK	• PY159m shows dose dependent PK
Rodent tox	• **PY159m is well tolerated in mice up to 4 weekly doses of 100 mg/kg**
NHP PK	• **Terminal half-life ($T_{1/2}$) range of 9-11 days between 1 and 10 mg/kg** • Volume of distribution (Vd) of 70-80 mL/kg, suggesting distribution beyond the vasculature and into tissues
Single dose NHP pilot	• **Well tolerated up to the top dose tested of 50 mg/kg** • Transient reduction in neutrophils within normal range • No discernable changes in cytokine and chemokine levels were observed for all groups
Repeat dose NHP pilot	• Preliminary findings: **PY159 is well tolerated up to 50 mg/kg for 4 weekly doses** • Transient reduction in neutrophils within normal range • No discernable changes in cytokine and chemokine levels were observed for all groups