Abstract # C105

# Myeloid Cell Reprogramming Unleashes Anti-Tumor Immunity

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## Introduction

The tumor microenvironment contains diverse types of myeloid cells, including tumor-associated macrophages (TAMs), tumor associated myeloid-derived suppressor cells (MDSCs), and tumor-associated neutrophils (TANs). TAMs and 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 checkpoint inhibitor (CPI)-resistant tumors into CPI-sensitive tumors.

## TREM1 Background

| | |
|---|---|
| TREM1: | Triggering receptor expressed on myeloid cells 1 |
| Localization: | Cell surface and soluble |
| Expression: | Macrophages, monocyte subsets, neutrophils<br>-upregulated on TAMs, TANs and MDSC |
| 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 |


## TREM1 is Expressed by Tumor-Infiltrating Myeloid Cells Across Diverse Human Tumor Types

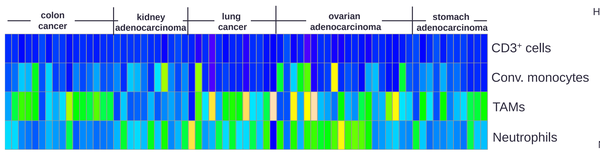
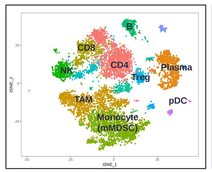
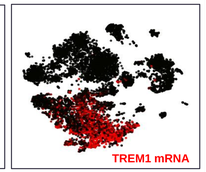
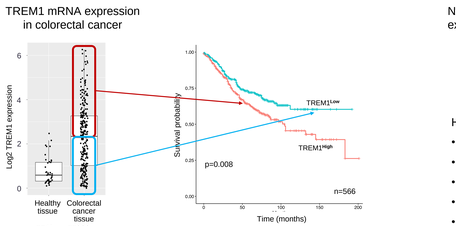



**Figure 1.** Heat map (**top panel**) depicts levels of cell surface TREM1 on CD45+ leukocyte subsets isolated from the indicated human tumor tissues assessed by flow cytometry. Geometric mean fluorescence intensities (gMFI) were adjusted to account for isotype control staining background levels. The adjusted gMFI values were normalized across leukocyte subsets and tumor types to highlight leukocyte subsets with high or low TREM1 expression.
A tSNE plot (**right panel**) summarizes the results of single-cell RNA sequencing performed on CD45+ leukocyte infiltrate sorted from **human ovarian tumor** (neutrophils are not included in the analysis).




## TREM1 Expression in Human Tumors Negatively Correlates With Patient Survival




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

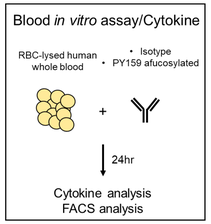
- Breast cancer
- Pancreatic cancer
- SCC

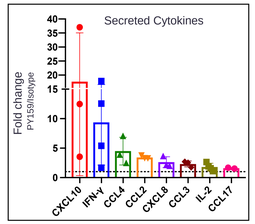
High TREM1 mRNA expression seen in:

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

**Figure 2.** 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. Kaplan-Meier survival curves (**right panel**) were plotted for each cohort and the associated log-rank test was carried using the *survival* and *survminer* packages in R. Besides colorectal cancer, similar analysis was performed for breast cancer, pancreatic cancer and squamous cell carcinoma (SCC) (data not shown), revealing negative correlation between TREM1 mRNA and patient survival.

## PY159 Induces a Highly Selective Set of Anti-Tumor Cytokines and Cell Surface Receptors



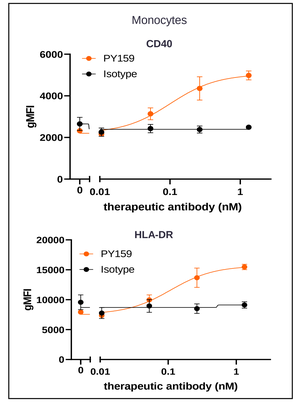
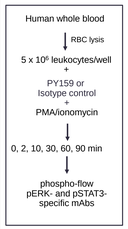
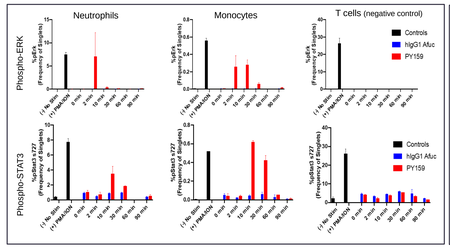




**Figure 3.** Human peripheral blood was subjected to RBC lysis and leukocytes were treated for 24-hours with 1 μg/ml PY159 or the isotype control. Supernatants were harvested for cytokine analysis by MSD, and the cells were stained with a panel of leukocyte lineage markers, anti-CD40 and anti-HLA-DR for downstream analysis by flow cytometry. PY159-induced increase in expression of CD40 and HLA-DR activation markers was notable on monocytes. Cytokines detected at >10 pg/ml and/or increased by PY159 treatment in multiple healthy blood donors are shown above. Cytokines that did not meet the 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 Phospho-Signaling in TREM1-Expressing Cells



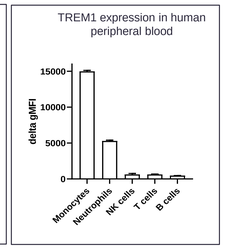




**Figure 4.** RBC-lysed human whole blood was stimulated with 5 μg/ml of PY159 or the isotype control antibody. After the indicated incubation times, cells were fixed, permeabilized and stained with antibodies against phospho-ERK, phospho-STAT3 and leukocyte lineage markers for downstream flow cytometry (**left and middle panel**). PMA/ionomycin was used as a positive control for phosphorylation signaling. TREM1 expression in human peripheral blood (**right panel**) was assessed by cell surface staining of RBC-lysed human blood with mouse anti-TREM1 antibody or the corresponding isotype control, alongside with antibodies against leukocyte lineage markers. TREM1 expression on each leukocyte subset is presented as TREM1 gMFI adjusted to account for isotype control staining background (delta gMFI).

## PY159m Exhibits Anti-Tumor Activity as a Single-Agent and in Combination with Anti-PD-1

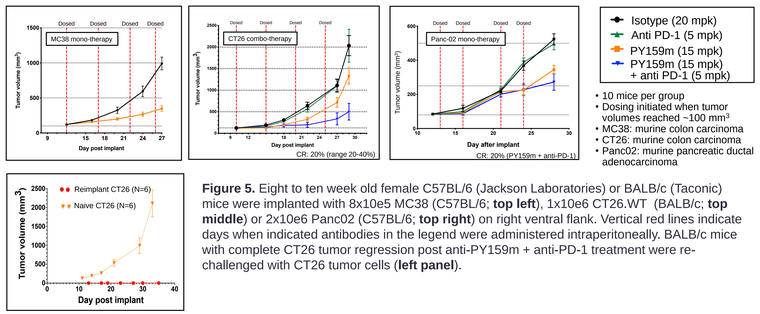
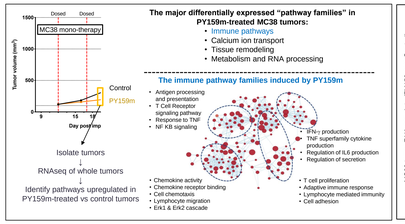






**Figure 5.** Eight to ten week old female C57BL/6 (Jackson Laboratories) or BALB/c (Taconic) mice were implanted with 8x10e5 MC38 (C57BL/6; **top left**), 1x10e6 CT26.WT (BALB/c; **top middle**) or 2x10e6 Panc02 (C57BL/6; **top right**) on right ventral flank. Vertical red lines indicate days when indicated 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 Induces Pro-inflammatory Responses with T Cell-Activation Signature in Mice and Humans



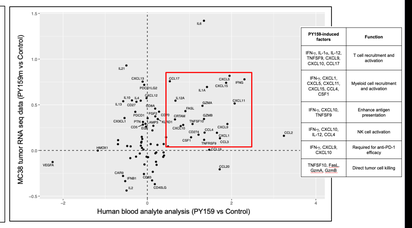




**Figure 6.** Mice bearing MC38 tumors, treated with two doses of PY159m or isotype antibodies (n=10 mice/group), were harvested on day 19 after implant (48 hours post 2nd treatment). Whole tumor mRNA was extracted and sequenced, and pathways differentially induced by PY159m were assessed using the Broad Institute’s Gene Set Enrichment Analysis (GSEA) tool and the c5 collection of gene sets from MSigDB (**left panel**). Significantly upregulated pathways (FDR < 0.1) were visualized using the Enrichment Map module in Cytoscape. Immune families from the resulting network map are shown with cluster-specific pathways highlighted. RNA-based fold changes induced by PY159m in MC38 tumors were compared to protein fold changes from PY159-treated RBC-lysed whole blood (**right panel**). Secreted human cytokine levels were assessed using the O-link multiplex platform.

## PIONYR’s Anti-TREM1 mAb PY159 Reprograms Suppressive Myeloid Cells

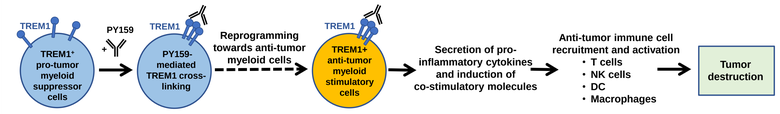



**Figure 7. Proposed mechanism-of-action of PY159.** Cross-linking of cell surface TREM1 on tumor-associated myeloid cell populations by PY159 causes downstream signaling that can induce secretion of a specific set of proinflammatory cytokines and chemokines as well as increase surface expression of HLA-DR and CD40 on MDSCs. These immune mediators can recruit immune cells including T cells, NK cells, DCs, and macrophages and promote anti-tumor immune cell activation.

## PY159 Safety and Pharmacokinetics 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**<br>• 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**<br>• Transient reduction in neutrophils within normal range |
| Repeat dose NHP pilot | • Preliminary findings: **PY159 is well tolerated up to 50 mg/kg for 4 weekly doses**<br>• Transient reduction in neutrophils within normal range |

## Summary

We developed anti-human and anti-mouse TREM1 monoclonal antibodies (mAbs), termed PY159 and PY159m, respectively, to target TREM1+ myeloid cells. These mAbs triggered signaling pathways downstream of TREM1 and induced a highly selective pro-inflammatory cytokine signature in *ex vivo* assays. Additionally, PY159 treatment increased the myeloid cell expression of HLA-DR and the costimulatory molecule CD40, indicating an enhanced potential for costimulation and immune activation. Consistent with *ex vivo* results, *in vivo* treatment with PY159m promoted both innate and adaptive immune pathways in syngeneic mouse tumors revealed by gene expression profiling. These findings suggest that anti-TREM1 therapy re-educates TREM1+ myeloid cells into pro-inflammatory cells. Furthermore, PY159m was sufficient to drive anti-tumor activity as a single agent in a number of syngeneic tumor models. Strikingly, PY159m also converted anti-PD-1 mAb-resistant tumors into treatment-sensitive tumors with combination therapy, demonstrating the utility of targeting TREM1+ myeloid cells as a combination approach to improve CPI therapy responses. Mice cured of their tumors by PY159m/PD-1 mAb combination therapy were resistant to tumor re-challenge, demonstrating that targeting TREM1+ myeloid cells also supports adaptive immunity and induces long-term immunological memory. Based on these preclinical findings, we are developing PY159 as a therapeutic agent for monotherapy and/or CPI combination therapy for solid tumors.