PIONYR
IMMUNOTHERAPEUTICS

Myeloid Tuning™ of the Tumor Microenvironment with PY159 in Cancer Immunotherapy

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TABLE OF CONTENTS

PIONYR'S MYELOID TUNING APPROACHES FOR CANCER IMMUNOTHERAPY

Cancer immunotherapeutic strategies that promote the anti-tumor activities of T cells using checkpoint inhibitors (CPIs), such as antibodies against the T-cell inhibitory receptor PD-1 or its ligand PD-L1, have been transformative in the treatment of some cancers, resulting in complete responses in a subset of patients. However, many patients and tumor types remain resistant to CPI-based therapies.

Further understanding of immune components in the tumor microenvironment (TME) has highlighted the importance of cell types beyond T cells that regulate anti-tumor immunity and may affect the efficacy of CPI therapy. Myeloid cells, in particular, have been identified as important regulators of anti-tumor immune responses. Tumor-associated macrophages (M2-like TAMs), tumor-associated neutrophils (TANs), and monocytic myeloid-derived suppressor cells (mMDSCs) can have suppressive functions that limit anti-tumor responses. Conversely M1-like TAMs can have stimulatory functions that promote antigen presentation and T-cell activation.

While a high level of tumor-associated myeloid infiltrate correlates with shorter survival times of patients with solid tumors (1, 2), profiling studies of human tumors have also associated the functional composition of myeloid cells within the TME with immune responses, tumor progression, and patient survival times. A higher ratio of stimulatory to suppressive cells is associated with better clinical outcome (3, 4).

'Myeloid tuning'™ strategies are designed to shift the balance toward an immune-stimulatory phenotype and are being tested as anti-cancer therapies (5), alone and in combination with CPIs. Pionyr Immunotherapeutics (Pionyr) is developing therapeutics that utilize distinct myeloid-tuning approaches to improve anti-tumor immunity. One strategy is to reprogram myeloid cells in the TME to convert them from immune-suppressive to immune-activating cells (5-8) (see Society for Immunotherapy of Cancer (SITC) 2019 Poster P812). This 'reprogramming' of myeloid cells can be used in combination with CPI-based strategies that remove the suppressive brake on T cells to increase anti-tumor efficacy. We describe an approach for reprogramming suppressive myeloid cells toward a proinflammatory phenotype using a monoclonal antibody, PY159, that targets triggering receptor expressed on myeloid cells 1 (TREM1) (Figure 1).

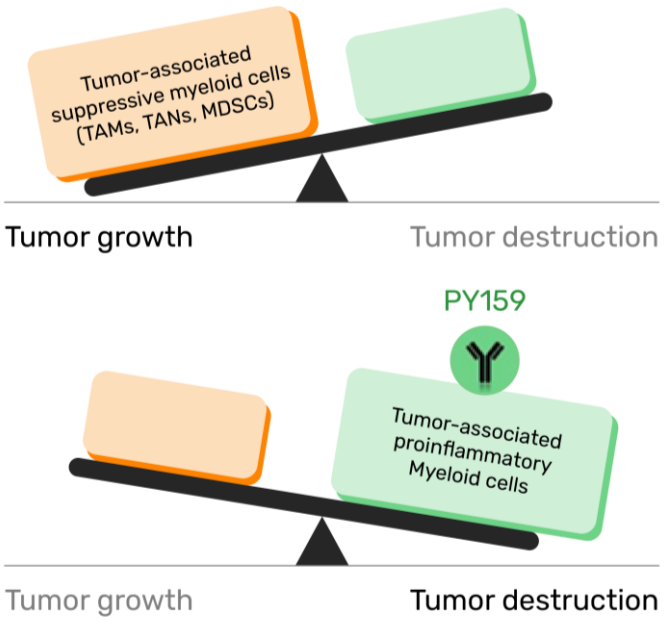


Figure 1. Myeloid Tuning Mechanism

Myeloid Tuning™ involves modulating suppressive myeloid populations in the TME, with precision and selectivity using agents such as PY159, to increase the ratio of stimulatory to inhibitory myeloid cells. This 'rebalancing' occurs via reprogramming of inhibitory myeloid cells and conversion of the TME from an immune-suppressive to an immune-activating microenvironment.

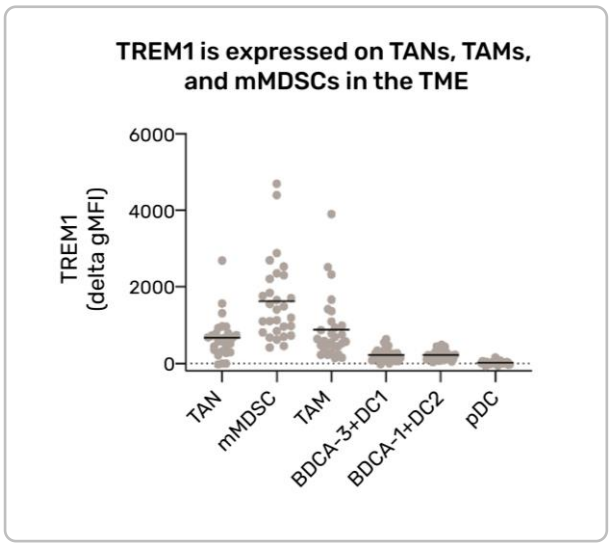
TREM1-MEDIATED REGULATION OF INFLAMMATION

TREM1 is an immunoglobulin (Ig) superfamily transmembrane protein found on the cell surface of monocytes, neutrophils, and a subset of macrophages (9). TREM1 has a short intracellular domain that lacks a signaling motif but forms a complex with the transmembrane adaptor protein DAP12, which contains an activating ITAM signaling domain. Signaling via the TREM1–DAP12 complex amplifies toll like receptor-mediated responses, promotes production of inflammatory cytokines and chemokines by neutrophils and monocytes, induces neutrophil chemotaxis, and upregulates costimulatory molecules on monocytes (10-12). TREM1-knockout mice have reduced inflammation due to decreased infiltration of tissues by inflammatory myeloid cells, and blockade of TREM1 protects mice against microbe-induced shock, indicating that TREM1 is an important mediator of the immune response (10, 13).

EXPRESSION OF TREM1 IN TUMORS AND CORRELATION WITH SURVIVAL

In many tumor tissues analyzed, levels of *TREM1* mRNA are significantly increased compared with adjacent non-tumor tissues (3, 14). Flow cytometric analyses of human tumor-infiltrating myeloid cells isolated from breast cancer, bladder cancer, endometrial cancer, head and neck squamous cell carcinoma, ovarian cancer, prostate cancer, and renal cell carcinoma tissues demonstrated that TREM1 is enriched on myeloid subsets, including mMDSCs, TANs, and TAMs (Pionyr data on file, AACR-NCI-EORTC Virtual International Conference on Molecular Targets and Cancer Therapeutics 2021 abstract no. P104) (15). Immunohistochemical analysis with a proprietary anti-human TREM1 IHC antibody, flow cytometry, and single-cell RNA sequencing (scRNAseq) analyses performed at Pionyr found high expression of TREM1 protein and *TREM1* mRNA, respectively, in TAMs and monocytes in human tumors (EORTC-NCI-AACR 2019 abstract C105, Immunotherapy of Cancer Conference (ITOC) 2021 Abstract P02.11, SITC 2021 Poster 859).

Compared with adjacent tumor-free colon mucosa, expression of TREM1 was increased in mouse and human colorectal tumors (16). Single-cell immune profiling of stage III-C ovarian tumor specimens from patients revealed that TREM1 is expressed in TAM and monocytes in these tumors (AACR-NCI-EORTC INTERNATIONAL CONFERENCE ON MOLECULAR TARGETS AND CANCER THERAPEUTICS 2019 abstract no. C105). Furthermore, TREM1 was expressed on TAMs, TANs, and MDSCs in human lung and ovarian tumors (see Figure 2). In mouse syngeneic tumor models, TREM1 is expressed by tumor-infiltrating MDSC, TAM, and neutrophils (17)—these findings are consistent with internal data generated at Pionyr demonstrating TREM1 expression in the TME of multiple mouse tumor models.



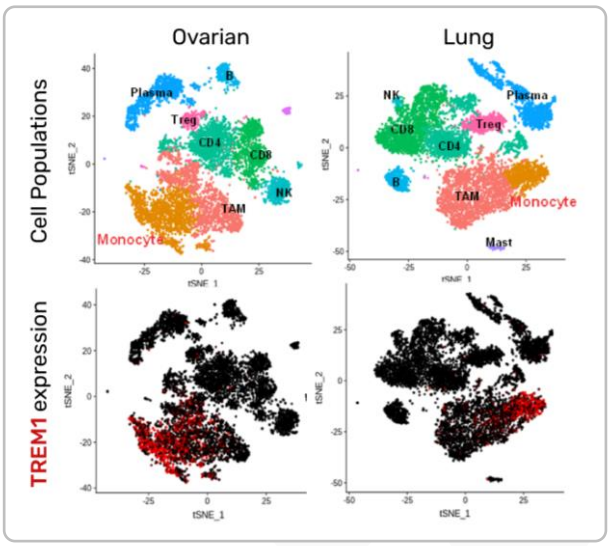


Figure 2: TREM1 expression in the TME

TREM1 is highly expressed by TANs, mMDSCs, and TAMs in the TME (left panel, from flow cytometry). TREM1 is expressed specifically by monocytes in ovarian and lung tumor tissues (right panel, scRNAseq). SITC 2021 Poster 859

Increased levels of TREM1 in tumors correlate with shorter survival times of patients with different types of cancer. Analysis of data from the Cancer Genome Atlas (TCGA) associated *TREM1* mRNA expression with shorter survival times of patients with renal cell carcinoma (14); colorectal, breast, and pancreatic cancers; and squamous cell carcinoma (SITC 2019 Poster P812). Ford et al reported expression of TREM1 on tumor-infiltrating myeloid cells from patients with renal cell carcinoma, along with increased levels of soluble TREM1, and in an additional analysis of TCGA data found that patients with high tumor levels of *TREM1* mRNA had worse outcomes than patients with low tumor levels (17).

RNAseq analyses of breast tumors indicated that *TREM1* mRNA was significantly enriched in MDSCs and M2-like macrophages (18), which promote tumor growth via immune suppression, angiogenesis, neovascularization, as well as stromal activation and remodeling. These observations indicate that strategies to alter TREM1 signaling might convert the TME toward an immunostimulatory environment and be developed for treatment of patients with tumors that are resistant to CPIs.

DEVELOPMENT OF PY159 FOR TREATMENT-RELAPSED OR -REFRACTORY ADVANCED SOLID TUMORS

Because TREM1 mediates inflammatory responses and is expressed by tumor-associated suppressive myeloid cell populations, it is an attractive target for reprogramming myeloid cells within the TME to promote anti-tumor immune responses. Pionyr has developed PY159 to target TREM1 for immunotherapy of solid tumors (SITC 2019 Poster P812, AACR-NCI-EORTC INTERNATIONAL CONFERENCE ON MOLECULAR TARGETS AND CANCER THERAPEUTICS 2019 abstract no. C-105, AACR-NCI-EORTC Virtual International Conference on Molecular Targets and Cancer Therapeutics 2021 Abstract P104, ITOC 2021 Abstract P02.11, SITC 2021 Poster 859).

PY159 is an afucosylated, humanized IgG1κ monoclonal antibody (mAb) that specifically binds human TREM1 and cross-reacts with cynomolgus monkey TREM1. Consistent with the afucosylated IgG1κ framework, PY159 has enhanced binding to Fc gamma receptors (FcγR). PY159 can therefore effectively crosslink TREM1 molecules to induce signaling through the TREM1–DAP12 complex. PY159 functions as a TREM1 agonist, inducing phosphorylation of molecules downstream of TREM1-DAP12. This results in upregulation of costimulatory molecules (CD80 and CD86) and cell-surface CD40 and MHC class II on monocytes, and production of cytokines (including CXCL10 and IFNγ) and chemokines (including CCL2, CCL3, and CCL4). These data indicate that PY159 converts suppressive tumor-associated myeloid populations into inflammatory cells that promote anti-tumor immune responses (see Figure 3).

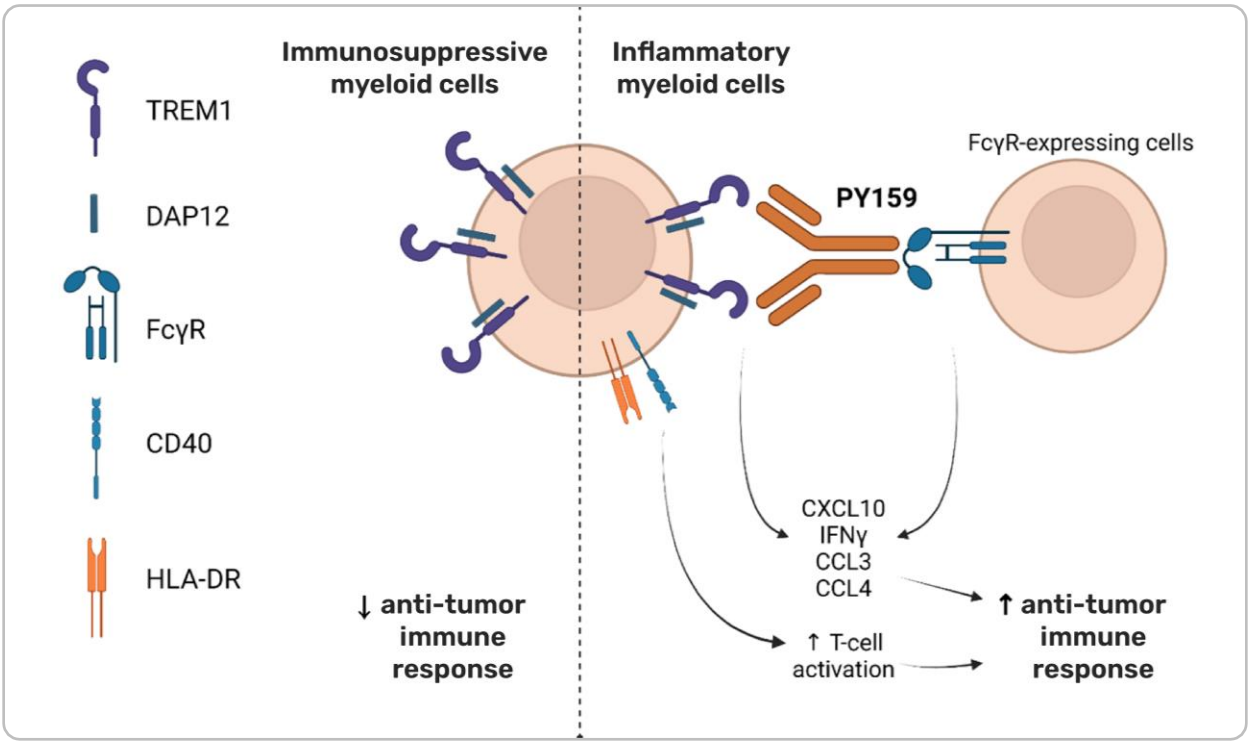


Figure 3. PY159 Mechanism

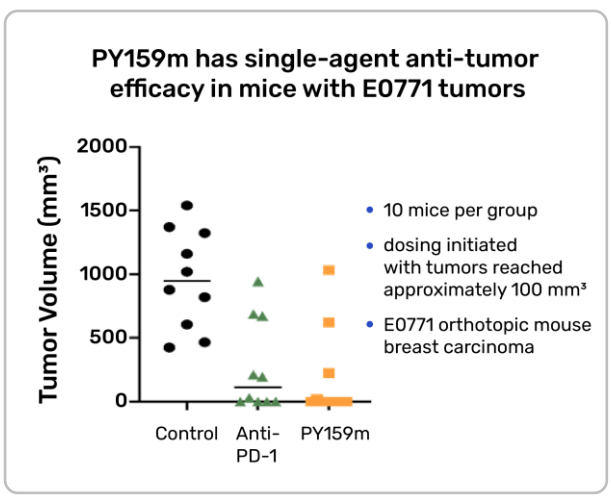
Immune-suppressive myeloid cells express TREM1–DAP12. PY159 is an anti-TREM1 antibody with enhanced binding to FcγR. PY159 reprograms TREM1-expressing myeloid cells to a proinflammatory state: they produce chemokines such as CXCL10, CCL3, and CCL4 and upregulate costimulatory molecules. This results in activation of T cells and promotes an anti-tumor inflammatory response.

The safety of PY159 was evaluated in cynomolgus monkeys, in single-dose and repeat-dose studies that included a drug-free recovery period, to assess its pharmacokinetics, tolerability, and toxicity. PY159 has a long serum half-life and was well tolerated (Liang presentation, 2021 PEGS symposium).

INDUCTION OF INFLAMMATORY RESPONSES BY PY159

PY159 engagement of TREM1 induces signaling, measured using a cell line reporter assay, and phosphorylation of ERK1/2 and STAT3, determined by phospho-flow analysis, in neutrophils and monocytes (AACR-NCI-EORTC INTERNATIONAL CONFERENCE ON MOLECULAR TARGETS AND CANCER THERAPEUTICS 2019 abstract no. C-105). Consistent with TREM1 signaling, PY159 can induce upregulation of costimulatory molecules including CD80 and CD86, MHC class II, and CD40 on monocytes and human monocyte-derived macrophages, as well as production of inflammatory cytokines. PY159-mediated production of inflammatory cytokines and chemokines, including IFNγ, CXCL10, CCL3, and CCL4, was observed in ex vivo whole-blood systems and in dissociated human tumor cells.

Studies of the pharmacology of TREM1 signaling in mice used a surrogate anti-mouse TREM1 antibody (PY159m). Similar to PY159, PY159m is an afucosylated IgG2a antibody, with enhanced binding to mouse FcγR. PY159m demonstrated anti-tumor activity as a single agent and in combination with anti–PD-1 in multiple syngeneic tumor models, including anti-PD-1 resistant models (8) (SITC 2019 Poster P812, AACR-NCI-EORTC Virtual International Conference on Molecular Targets and Cancer Therapeutics 2021, ITOC 2021 Abstract P02.11). Pharmacodynamic studies with PY159m and anti-PD-1 demonstrated augmented anti-tumor activity (SITC 2021 Poster 859). Mice that had completely rejected tumors, furthermore, developed durable immunologic memory, in that they were able to reject the same tumors when re-challenged (SITC 2021 Poster 859 and Figure 4).



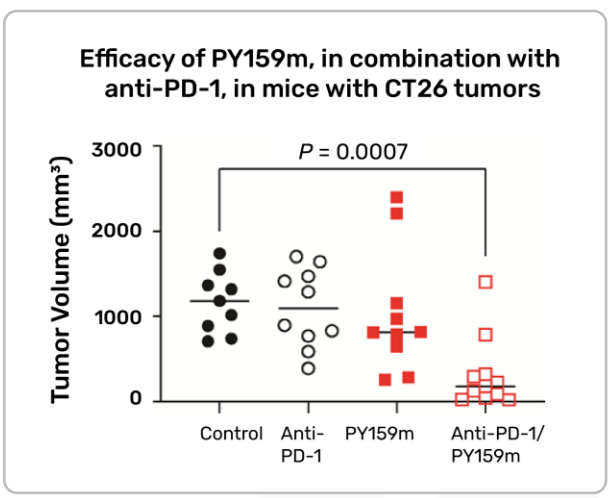


Figure 4. Anti-Tumor efficacy of PY159m

PY159m shows monotherapy efficacy in mice with orthotopic E0771 syngeneic breast tumors and PY159m has anti-tumor efficacy in combination with anti-PD-1 in mice with the subcutaneous CPI-resistant, CT26 colon tumors.

PY159 IN A PHASE 1 TRIAL OF SUBJECTS WITH CANCER

PY159 is being evaluated, alone and in combination with pembrolizumab, a humanized monoclonal IgG4 antibody against human PD-1, in a phase 1a/1b study (NCT04682431) of subjects with locally advanced (unresectable) and/or metastatic solid tumors that are refractory or relapsed to standard of care (including CPIs, if approved for that indication).

The primary outcomes are incidence of adverse events and dose-limiting toxicities.

There are other companies pursuing programs that target TREM1. However, these are primarily antagonist approaches for inflammatory diseases rather than agonist approaches for oncology (see Table 1).

TABLE 1. COMPOUNDS IN DEVELOPMENT FOR MODULATION OF TREM1

Company	Compound	Molecule Type	Mechanism/ effect	Indication	Phase
Pionyr https://www.pionyrtx.com/pipeline/py159/	PY159	Antibody (afucosylated)	Agonist	Cancer	Phase 1a and 1b
Celsius https://celsiustx.com/programs/	CEL383	Antibody	Blocking?	Inflammatory bowel diseases	IND-enabling
Inotrem https://www.inotrem.com/pipeline/	Nangibotide	Peptide	Inhibitor	COVID-19, septic shock, ischemia–reperfusion injury after acute myocardial infarction	Phase 2
	INO-002	Antibody	Antagonist	Inflammatory bowel diseases	Preclinical
Signablock https://signablok.com/pipeline/		Peptide	Inhibitor	Cancer, autoimmune diseases, COVID-19 and other infectious diseases, retinopathy, atherosclerosis, sepsis, acute respiratory distress syndrome	Discovery

DIFFERENTIATION FROM PIONYR'S ANTIBODY PY314

Pionyr is using multiple myeloid-tuning strategies to improve anti-tumor immunity by shifting the balance of myeloid cells in the TME from immunosuppressive to immune-stimulatory. In addition to PY159, Pionyr's anti-TREM2 antibody, PY314, is in clinical trials for advanced solid tumors. PY314 is an afucosylated antibody, with enhanced ADCC and ADCP activity, that specifically depletes TREM2-positive immunosuppressive TAMs from the TME. In non-clinical studies, a monoclonal antibody against mouse TREM2 (PY314m) depleted TREM2 immunosuppressive TAMs and increased the ratio of immunostimulatory macrophages (characterized by high MHC class II expression) to immune-suppressive cells in syngeneic tumors (19). PY314 is being tested in phase 1a and 1b trials of patients with advanced solid tumors, alone and in combination with anti-PD-1 therapy.

Both PY159 and PY314 promote anti-tumor responses by targeting immunosuppressive myeloid cells. However, they achieve this using distinct mechanisms and by targeting different myeloid populations. PY159 is a TREM1 agonist and promotes anti-tumor immune responses by reprogramming immunosuppressive myeloid cells, including TAMs, TANs, and mMDSCs. PY314, however, depletes TREM2-positive immunosuppressive TAMs to promote anti-tumor immunity. Both antibodies are afucosylated and have enhanced binding to FcγR. (see Table 2)

TABLE 2. PY314 VS PY159 PROGRAMS

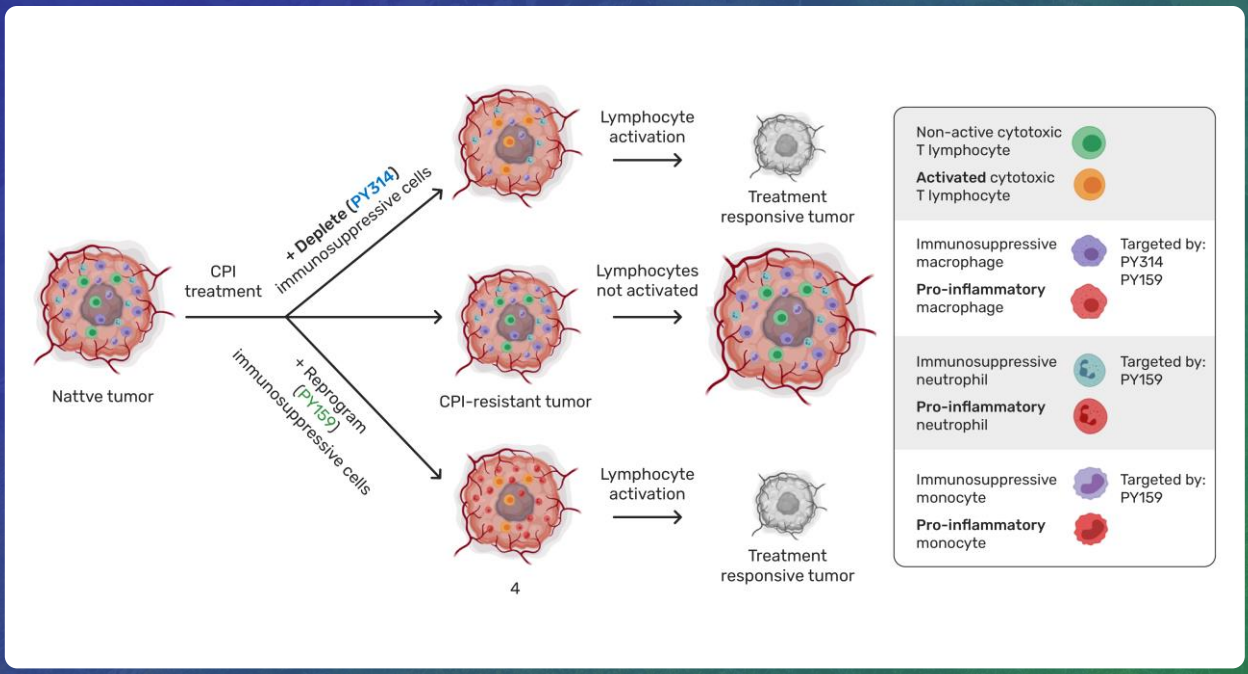
Antibody	Target	Function of Receptor	Cell Targets	Effect of Antibody	Afucosylation required?
PY159	TREM1	Pro-inflammatory	TAMs*, TANs, mMDSCs	Agonist, Reprogramming	Yes
PY314	TREM2	Immune-modulatory	TAMs*	Depletion	Yes

*Expressed on different subsets of TAMs

CONCLUSIONS

TREM1 mediates inflammatory responses and is expressed on tumor-associated suppressive myeloid cell populations. Pionyr has developed PY159 to target TREM1 and thereby reprogram suppressive myeloid cells within the TME to promote anti-tumor immune responses. The mechanism of PY159 differs from that of PY314, which depletes TREM2-expressing, immune-suppressive M2-like TAMs (see Figure 5). Instead, PY159 is a TREM1 agonist that reprograms suppressive tumor-associated myeloid populations toward inflammatory phenotypes, demonstrated by increased production of cytokines and chemokines and upregulation of costimulatory and activation markers. Pionyr is the only company with a clinical-stage antibody against TREM1 for treatment of cancer. The safety and efficacy of PY159 are being tested in a phase 1a and 1b trial of patients with solid tumors as monotherapy and in combination with CPI.

FIGURE 5. TARGETING IMMUNOSUPPRESSIVE MYELOID CELLS TO OVERCOME RESISTANCE TO CPI THERAPY



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