Therapeutic targeting of MARCO with PY265 antibody promotes myeloid cell reprogramming and unleashes anti-tumor immunity

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Abstract

Background: The tumor microenvironment (TME) contains immunosuppressive myeloid cells that contribute to checkpoint inhibitor (CPI) resistance. One approach of Pionyr Immunotherapeutics' Myeloid Tuning strategy is to reprogram immunosuppressive myeloid cells to acquire an immunostimulatory anti-tumor phenotype. Macrophage receptor with collagenous structure (MARCO) is an attractive target for myeloid reprogramming, considering its immunomodulatory function, and its expression on tumor-associated macrophages (TAMs) and monocytic myeloid-derived suppressor cells (mMDSCs) in the TME. Pionyr has developed a humanized IgG1k anti-MARCO monoclonal antibody (mAb), PY265, to investigate the potential of MARCO modulation as an anti-cancer immunotherapeutic strategy.

Methods: MARCO expression on TAMs and mMDSCs from multiple solid tumor indications was determined by single-cell RNA sequencing (scRNAseq) and immunohistochemistry (IHC). PY265 levels and activity on human monocyte-derived macrophages (hMDMs) were evaluated by transcriptional profiling, phosphoprotein array, flow cytometry, and measurements of cytokines and chemokines. In vivo efficacy and pharmacodynamic (PD) studies were performed with a surrogate anti-mouse MARCO antibody (PY265m) as single agent or in combination with anti-PD-1 in mice with syngeneic tumors.

Results: MARCO is specifically enriched in a cluster of TAMs and mMDSCs that correlates with immunosuppressive signatures in different types of human solid tumors. IHC studies have shown that MARCO is often expressed in the TME, in metastatic lesions, and in chemo- and CPI-treated tumors. PY265 induces reprogramming in hMDMs in vitro, through induction of rapid phosphorylation events, transcriptional activation of pro-inflammatory pathways, production of cytokines and chemokines, and upregulation of cell surface activation receptors. PY265m has significant anti-tumor activity in syngeneic mouse models, as a single agent in CPI-sensitive models, and in combination with anti-PD-1 in CPI-resistant models. PD studies suggest that PY265m induces immune activation by reprogramming pro-tumorigenic, M2-like TAMs and mMDSCs to proinflammatory anti-tumor M1-like macrophages and monocytes, leading to increased infiltration of the tumor, spleen, and tumor-draining lymph nodes by effector cells. In a non-human primate toxicokinetic study, PY265 was generally well tolerated at all dose levels tested.

Conclusions: Our studies demonstrate that targeting MARCO reprograms myeloid cells and remodels the TME to unleash anti-tumor immunity and convert CPI-resistant tumors into treatment-responsive tumors. Collectively, these preclinical data support PY265 immunotherapy, alone or in combination with a CPI, in patients with cancer resistant or refractory to CPI therapies, to potentially improve rates of overall response and the durability of response. First-in-human testing of PY265 will be initiated in 2023.

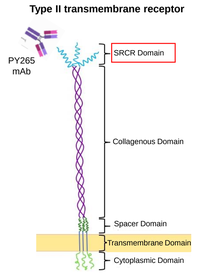
Targeting the MARCO Receptor with PY265

MARCO: Macrophage Receptor with Collagenous Structure

Function: scavenger and immunomodulatory receptor
- Defense against microbial pathogens via binding to ligands including foreign polyanionic ligands, bacteria, and endogenous lipoproteins
- Role in macrophage phagocytosis, adhesion, migration, and TLR-induced activation

Genetics: MARCO-knockout mice are viable and fertile, but have increased susceptibility to bacterial infection and inhaled particles, impaired macrophage phagocytosis, and abnormal spleen marginal zone morphology

Relevance for Immuno-Oncology
- MARCO is enriched on immunosuppressive TAMs and mMDSCs, and is upregulated in response to IL-10 and TGF-β
- Targeting MARCO reprograms TAMs and monocytes from an immunosuppressive to a proinflammatory phenotype and restores the cytotoxic properties of NK and T cells



PY265: a humanized IgG1 mAb that binds the SRCR domain of human- and cyno-MARCO
PY265m: a mouse surrogate IgG2a mAb that binds the SRCR domain of murine MARCO

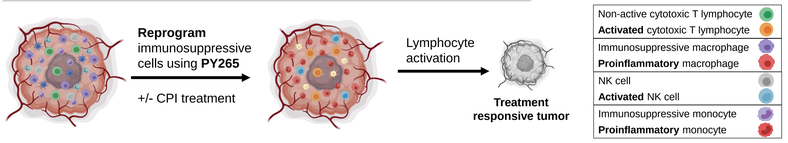


Figure 1. Pionyr's Myeloid Tuning approach involves modulating inhibitory myeloid populations in the TME with high precision and selectivity. PY265 (anti-MARCO) "re-tunes" the TME by reprogramming the immunosuppressive myeloid cells to acquire a proinflammatory phenotype and generate effective anti-tumor immunity.

MARCO-Expressing Monocytes and Macrophages in Human Tumors Have an Immunosuppressive Signature and Correlates with Poor Survival

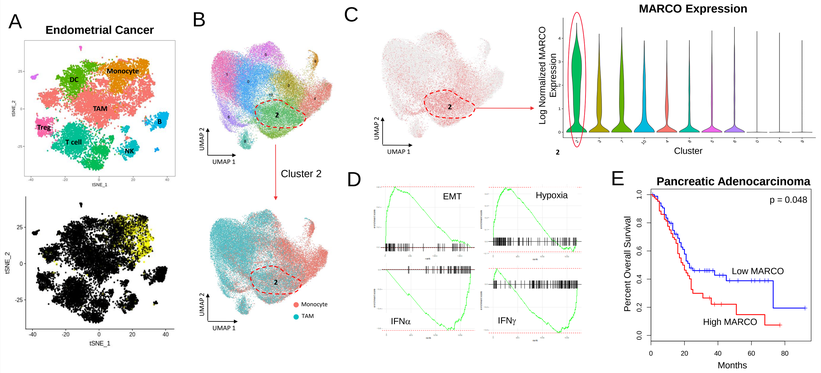


Figure 2. (**A**) scRNAseq of CD45+ cells from a human endometrial tumor. tSNE plots depict distinct leukocyte subsets (top) and *MARCO* expression in yellow (bottom). **(B)** Aggregated TAMs and monocytes were identified by scRNAseq analyses (Pionyr Immunotherapeutics) of immune cells from 13 samples of different human primary tumors (top). Louvain clustering yielded 11 clusters, where cluster 2 contains intermediate macrophages and monocytes (bottom). **(C)** Cluster 2 has significantly enriched expression of *MARCO* compared with the other clusters **(D)** and correlates with immunosuppressive gene expression signatures (e.g., hypoxia, EMT). Cluster 2 correlates inversely with proinflammatory signatures (e.g., interferon-based pathways), as determined by using Gene Set Enrichment Analysis of hallmark pathways (*MARCO*-rich cluster 2 vs all other macrophages and monocytes). **(E)** Differences in overall survival of patients with pancreatic adenocarcinomas that have above-median (high, red) vs below-median (low, blue) expression of *MARCO* mRNA. Pre-normalized expression values for *MARCO* were determined from the GEPIA, which combines data from TCGA.

MARCO is Expressed in Aggressive Solid Tumors

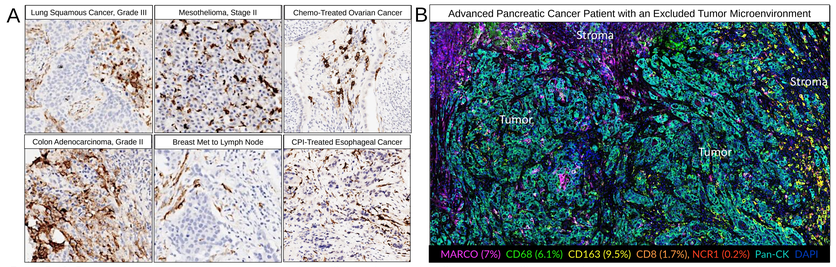


Figure 3. (A) Representative images of tumor tissues of lung (grade III), mesothelium (stage II), colon (grade II), breast metastasized to lymph node, chemo-treated ovarian, and CPI-treated esophagus, stained with an IHC compatible anti-human MARCO antibody. The brown color indicates myeloid cells expressing MARCO in the tumor stroma. **(B)** Representative image of an advanced pancreatic cancer tissue with an excluded TME (defined by high expression of immune cells in the stroma with little infiltration into tumor nests). MARCO+ myeloid cells (pink), CD68+ (green) and CD163+ (yellow) macrophages, CD8+ cytotoxic T cells (orange), NCR1+ NK cells (red), Pan-CK+ tumor cells (cyan) and DAPI+ nuclei (blue) were stained using multiplex immunofluorescent OPAL IHC technology. HALO image analysis was used to quantify the percentage positive cells per marker from the entire tissue.

PY265 Binds With Specificity and High Affinity to Suppressive hMDMs

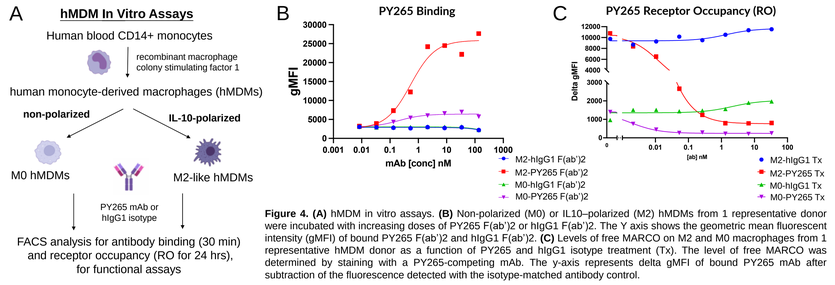


Figure 4. (A) hMDM in vitro assays. **(B)** Non-polarized (M0) or IL10–polarized (M2) hMDMs from 1 representative donor were incubated with increasing doses of PY265 F(ab')2 or hIgG1 F(ab')2. The Y axis shows the geometric mean fluorescent intensity (gMFI) of bound PY265 F(ab')2 and hIgG1 F(ab')2. **(C)** Levels of free MARCO on M2 and M0 macrophages from 1 representative hMDM donor as a function of PY265 and hIgG1 isotype treatment (Tx). The level of free MARCO was determined by staining with a PY265-competing mAb. The y-axis represents delta gMFI of bound PY265 mAb after subtraction of the fluorescence detected with the isotype-matched antibody control.

PY265 Induces Proinflammatory Activation In Vitro

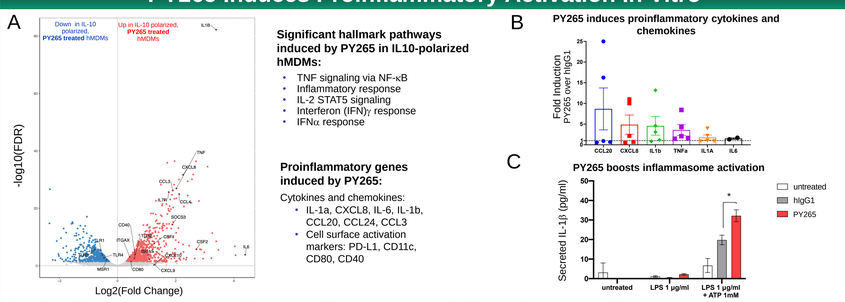


Figure 5. (A) RNA sequencing was performed on IL10-polarized hMDMs treated for 4 hrs with PY265 and hIgG1 isotype mAbs. PY265-induced differentially expressed genes and downstream GSEA pathway analyses yielded a profound increase in proinflammatory chemokines/cytokines, activation markers, and a variety of significantly upregulated pathways associated with macrophage activation/reprogramming and inflammatory responses. **(B)** qPCR mRNA analysis of key proinflammatory genes induced after 4 hrs of treatment (1 μg/ml) is plotted as fold induction over the hIgG1 isotype. Data are presented as mean values from 5 donors ± (SEM). **(C)** IL10- polarized hMDMs were incubated with 5 μg/mL PY265 or hIgG1 isotype for 3 hrs prior to inflammasome priming with 1 μg/mL LPS for 2.5 hrs. hMDMs were then stimulated with 1 mM ATP for 30 mins to activate inflammasome. Secreted IL-1β (pg/mL) was measured in conditioned media by MSD. Data are presented as mean values of technical triplicates ± (SD). *P*<0.05, using Tukey's multiple comparison test.

Anti-mouse MARCO Antibody, PY265m, Exhibits Anti-Tumor Activity as Single Agent and in Combination with Anti-PD1

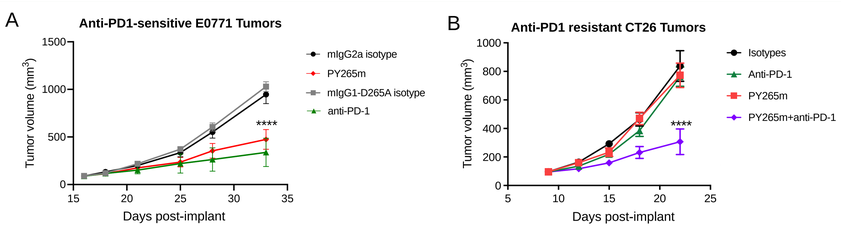


Figure 6. (A) Mice bearing established orthotopic E0771 syngeneic breast tumors were dosed with intraperitoneal (IP) injections of PY265m (10 mg/kg) and/or anti-PD-1 (5 mg/kg), or corresponding isotype control antibodies. Tumor volumes were measured at time points on the X axis, through Day 33. Anti-tumor efficacy of PY265m was significant when compared to its isotype (2-way ANOVA followed by Tukey's multiple comparisons test, ****$P<0.0001$). **(B)** Mice bearing established CT26 syngeneic colorectal tumors were dosed with IP injections of anti-PD-1 (5 mg/kg) and/or PY265m (10 mg/kg), or isotype control antibodies. Tumor volumes were measured at timepoints on the X axis, through Day 22. The combination of PY265m and anti-PD-1 significantly reduced tumor growth vs anti-PD-1 alone, based on 2-way ANOVA followed by Tukey's multiple comparisons test (****$P<0.0001$).

Anti-MARCO Induces Proinflammatory Anti-Tumor Pathways In Vivo and Ex Vivo

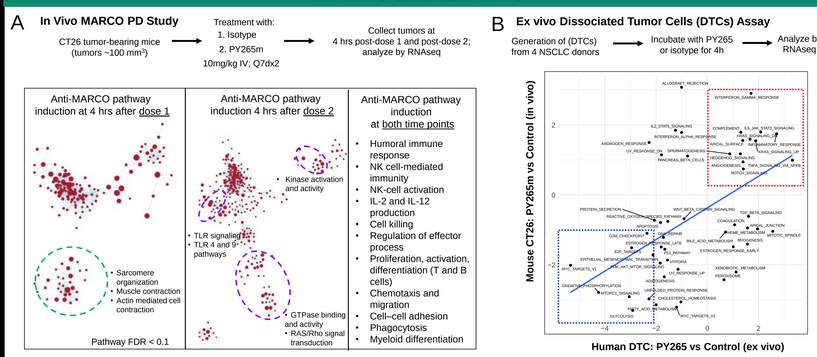


Figure 7. (A) RNA was extracted from CT26 tumors from mice given injections of PY265m or isotype control mAb (single-dose PD study) and sequenced. Differentially regulated pathways are highlighted as spider plots for significant pathways at Dose 1 only (green circle), Dose 2 only (purple circles), and both time points. (**B**) Total RNA was isolated from non-small cell lung cancer (NSCLC) DTCs incubated with PY265 or hIgG1 (isotype control) and sequenced. GSEA-derived enrichment scores from mouse (in vivo) DTC (ex vivo) experiments indicated that the human PY265 mAb and mouse surrogate PY265m induced common proinflammatory and immunostimulatory pathways (red square) and decreased common survival and tumor-growth associated pathways (blue square).

PY265m Elicits Anti-Tumor Activity and Reprogramming of TAMs and mMDSCs with Rapid Activation of Intra-tumoral Immunity

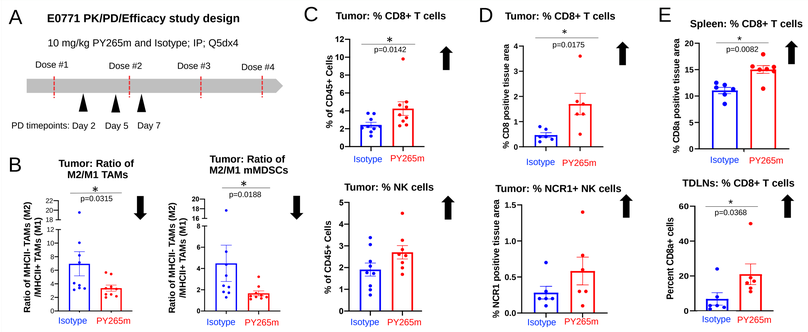
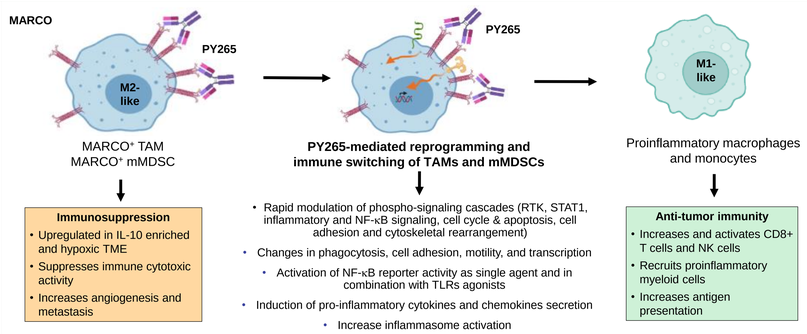


Figure 7. (**A**) PY265m PK, PD, and efficacy study in mice with E0771 orthoptic tumors given IP injections of PY265m or isotype control mAb; samples were collected at 3 timepoints for PD analysis from a subset of mice to assess myeloid and lymphoid changes. (**B**) Flow cytometry data showing differences in myeloid populations (as M1/M2 ratios on Day 2), and **(C)** CD8+ cells (Day 2), and NK cells (Day 5), as a frequency of CD45+ cells. **(D)** IHC data from tumor tissues collected at Day 7 after dose 2. Image analysis was used to quantify the percentages of CD8+ T cells (top) and NCR1+ cells (bottom) over the whole tissue area. **(E)** IHC image analysis data showing the percentage CD8+ T cells (Day 5) in the marginal zone of the spleen (top) and in the paracortical regions of tumor-draining lymph nodes (TDLNs) (bottom). Data are presented as mean percentage values from 6 mice within each group ± standard error of the mean (SEM). Mann Whitney unpaired *t* test was used for statistical analysis.

Proposed Mechanism for PY265 Activity



- PY265 is safe and generally well tolerated in pilot NHP toxicology studies.
- Terminal half-life of PY265 PK is typical of mAbs.
- No significant cytokine release observed in whole blood or PBMCs in response to PY265.
- PY265 first in human clinical trials are planned for 2023 in patients with advanced solid tumors refractory or relapsed to standard care, as single agent and in combination with CPI.