Tuning the Tumor Myeloid Microenvironment by Targeting MARCO Positive Myeloid Cells to Unleash Anti-tumor Immunity

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Abstract

The tumor microenvironment (TME) contains suppressive myeloid cells that contribute to checkpoint inhibitor (CPI) resistance. Pionyr Immunotherapeutics' Myeloid Tuning™ approach alters the composition and/or the function of myeloid cells in the TME to induce or augment an anti-tumor immune response. Myeloid cell reprogramming converts myeloid cells from immunosuppressive to immunostimulatory, and represents one Myeloid Tuning™ strategy. We identified the Macrophage Receptor with a Collagenous Structure (MARCO) as a potential myeloid reprogramming target, considering its immunomodulatory function and expression on tumor-associated macrophages (TAMs) and monocytic myeloid derived suppressor cells (mMDSCs) in the TME. In addition, correlation of MARCO expression with immunosuppressive myeloid signatures and with poor patient survival in several tumor types supports its role in promoting immunosuppression and facilitating tumor progression.

To investigate the potential of MARCO modulation as an anti-tumor therapeutic strategy, Pionyr developed a humanized anti-MARCO monoclonal antibody (mAb), termed PY265, that binds with high affinity to human and cynomolgus MARCO. PY265 induced reprogramming of M2-like human monocytes derived macrophages (hMDMs) to M1-like hMDMs by inducing transcriptional activation of pro-inflammatory pathways and secretion of pro-inflammatory cytokines and chemokines. PY265m, a surrogate antibody specific to mouse MARCO, demonstrated significant anti-tumor activity both as a single agent in CPI-sensitive syngeneic tumor models and in combination with anti-PD-1 in CPI-resistant syngeneic tumor models. Pharmacodynamic studies in syngeneic mouse models using transcriptomic profiling, flow cytometry immuno-profiling, and immunohistochemistry suggests that PY265m induced immune activation by reprogramming pro-tumorigenic, M2-like TAMs and mMDSCs to pro-inflammatory macrophages and monocytes, leading to an increase in intratumoral infiltration of activated CD8⁺ T cells and NK cells. These preclinical results suggest that PY265 in combination with anti-PD-1 can convert CPI-resistant tumors into treatment responsive tumors.

Targeting the MARCO Receptor

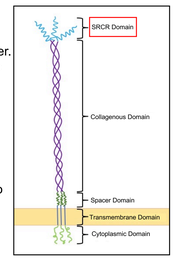
MARCO: **M**acrophage **R**eceptor with **Co**llagenous Structure

Structure: Trimeric Type II receptor with five structural domains. The SRCR (Scavenger Receptor Cysteine-Rich) domain is the ligand-binding functional domain.

Functions as a scavenger receptor and immune modulator in innate immunity
- Expressed on tissue-resident macrophages in the spleen marginal zone, lymph node medullary zone, lung, liver.
- Functions in anti-microbial host defense by binding and up-taking various ligands including foreign polyanionic ligands, bacteria, and endogenous lipoproteins.
- Plays an important role in macrophages and dendritic cells migration, signaling, and TLR-induced activation.

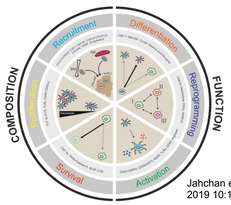
Genetics: MARCO KO mice are viable and fertile, but show increased susceptibility to bacterial infection, impaired macrophage phagocytosis and abnormal spleen marginal zone morphology

Relevance to Immuno-Oncology
- MARCO is enriched on immunosuppressive TAMs and mMDSCs in the TME, and is upregulated in response to IL-10 and TGF-β
- Targeting MARCO activates anti-tumor immunity by switching TAMs from an immunosuppressive phenotype to proinflammatory, and restoring the cytotoxic properties of NK cells and T cells



PY265 is a humanized IgG1 mAb that binds the SRCR domain of human and cyno MARCO with high affinity

PY265m is a mouse surrogate IgG2a mAb that binds the SRCR domain of mouse MARCO with high affinity



PY265 (MARCO)

Jahchan et al.; *Front Immunol.* 2019 10:1611

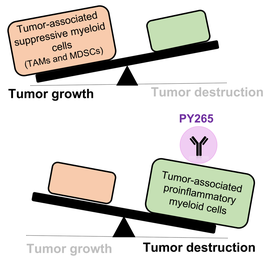


Figure 1. Pionyr Myeloid tuning approach involves modulating inhibitory myeloid populations in the TME with high precision and selectivity. PY265 (anti-MARCO) "re-tunes" the TME by reprogramming the immunosuppressive myeloid cells to acquire proinflammatory phenotype and generate effective anti-tumor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

MARCO Expression is Elevated in Multiple Cancers and Correlates with Poor Patient Survival

A

- Inverse correlation with survival
  - Colorectal
  - Renal
  - Neuroblastoma
- High tumor MARCO mRNA expression and good correlation with high IL-10
  - Breast (Basal-like)
  - Lung
  - Mesothelioma
  - Pancreatic
  - Ovarian
  - Thyroid
  - DLBCL
- MARCO association with disease
  - High levels of MARCO in CPI refractory melanoma patients
  - MARCO correlation with hypoxia signature

B

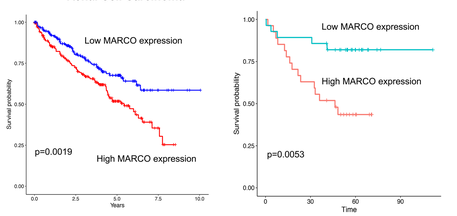


Figure 2. (**A**) Table summarizing the RNA expression and survival data for MARCO on multiple tumor types and publicly available datasets. **(B)** Survival associations in renal cell carcinoma and colorectal cancer based on median-split of MARCO mRNA expression. Pre-normalized expression values for MARCO were downloaded from either the NCBI's GEO website (colorectal cancer) or The Broad Institute's TCGA repository (renal cell).

MARCO-Expressing Monocytes and Macrophages in Human Tumors Have an Immunosuppressive Signature

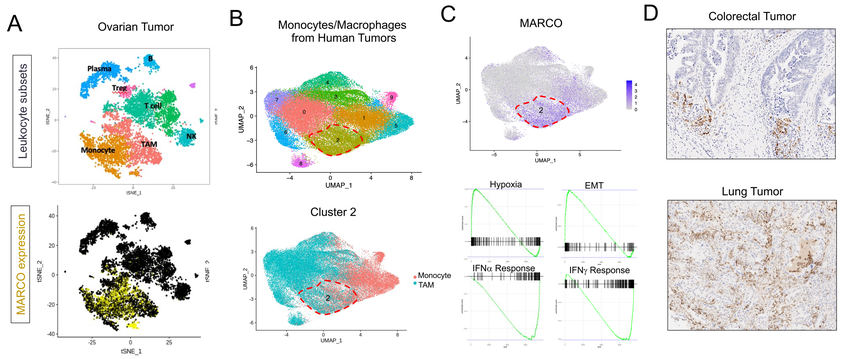
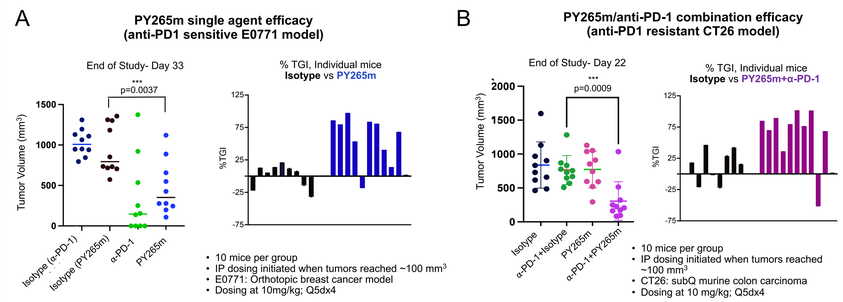


Figure 3. (**A**) Single-cell RNAseq of CD45+ cells from a human ovarian cancer. UMAP plots depict distinct leukocyte subsets (top) and MARCO expression in yellow (bottom). **(B)** Aggregated TAMs and monocytes derived from single cell sequencing (Pionyr Immunotherapeutics) of human immune cells from multiple tumor types (Top) yield a single cluster (cluster 2) with intermediate macrophages and monocytes (bottom). **(C)** Cluster 2 has substantially elevated MARCO expression (top) correlating with immunosuppressive, matrix-associated gene sets (e.g., hypoxia, EMT) and downregulated for inflammatory, interferon-based pathways using Gene Set Enrichment Analysis (GSEA) of Hallmark pathways (MARCO-rich cluster 2 vs. all other macrophages and monocytes. All significant (False Discovery Rate < 0.05) pathways depicted. **(D)** Representative images of colorectal tumor tissue (top) and lung tumor tissue (bottom) stained with an IHC compatible anti-human MARCO antibody. The brown color indicates positive staining of myeloid cells expressing MARCO.

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



- 10 mice per group
- IP dosing initiated when tumors reached ~100 mm³
- E0771: Orthotopic breast cancer model
- Dosing at 10mg/kg; Q5dx4

- 10 mice per group
- IP dosing initiated when tumors reached ~100 mm³
- CT26: subQ murine colon carcinoma
- Dosing at 10 mg/kg; Q5dx4

Figure 4. (**A**) E0771 mouse syngeneic breast tumors were dosed with a mouse IgG2a isotype control or the surrogate anti-mouse MARCO antibody (PY265m) at 10 mg/kg, and with anti-PD-1 or its corresponding isotype at 5mg/kg. Tumor volumes were monitored over time and presented as individual tumor volumes at the end of study on day 33, or as % TGI (Tumor Growth Inhibition). Monotherapy anti-tumor efficacy of PY265m was significant when compared to its isotype. (**B**) CT26 mouse syngeneic colorectal tumors were dosed with the mIgG2a isotype control, anti-PD-1 (5 mg/kg), PY265m (10 mg/kg), or the combination of anti-PD-1 and PY265m. Tumor volumes were monitored over time and presented as individual tumor volumes at the end of study on day 22, or as %TGI. Anti-tumor efficacy of PY265 in combination with anti-PD-1 elicited significant combination therapy when compared to anti-PD-1 alone.

Anti-MARCO Induces Pro-inflammatory Anti-Tumor Pathways *in vivo* and *ex vivo*

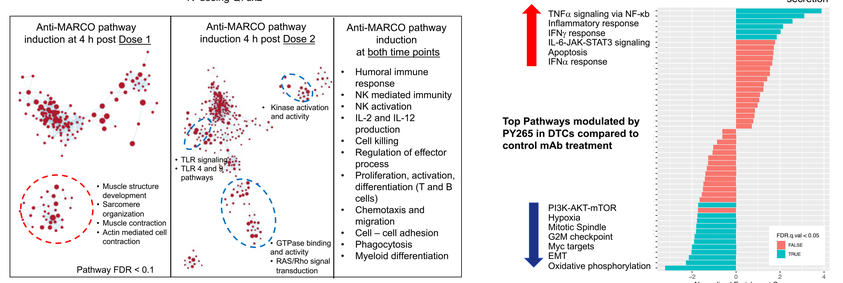


Figure 5. (A) Tumor RNA from PY265m and isotype treated CT26 tumor-bearing mice from a single dose pharmacodynamic (PD) study was extracted and sequenced. Downstream differentially regulated pathways are highlighted as spider plots for significant pathways at Dose 1 only, Dose 2 only, and both time points. **(B)** Total RNA was isolated from PY265-treated dissociated human NSCLC cancers and submitted for RNA sequencing. Gene set analysis (GSEA) of PY265-differentially enriched pathways yielded a variety of gene sets associated with immune activation, inflammation and tumor killing, as well as downregulation of tumor-growth associated pathways. Note the common proinflammatory and immunostimulatory pathways induced by both PY265 and PY265m.

PY265m Elicits Reprogramming and Rapid Activation of Intra-tumoral Immunity in Syngeneic Tumor Models

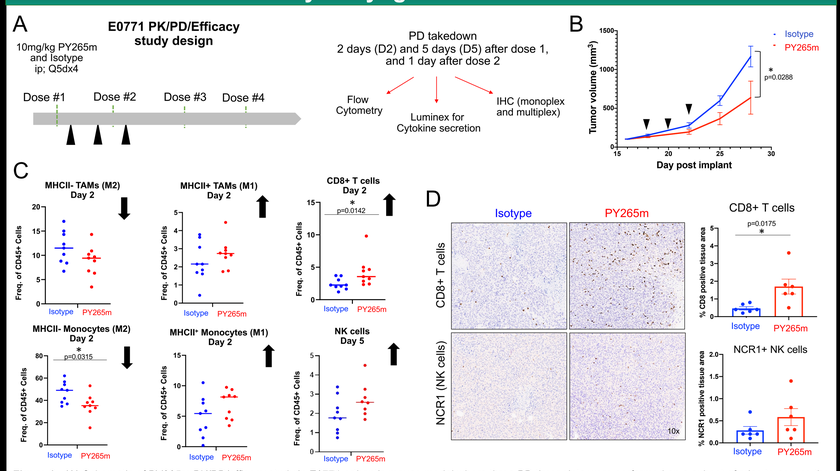


Figure 6 . (**A**) Schematic of PY265m PK/PD/efficacy study in E0771 orthoptic mouse model where three PD timepoints were performed on a subset of mice to assess the myeloid and lymphoid changes. **(B)** The rest of the animals were monitored for anti-tumor efficacy and their tumor volumes presented as averages per group over time. The three PD timepoints are depicted as black triangles. (**C**) Flow cytometry data was analyzed and plotted for relevant myeloid and lymphoid cell surface markers as a frequency of CD45+ cells for the isotype and PY265m treated tumors. **(D)** Representative IHC images of CD8+ T cell and NK cell DAB staining in isotype and PY265m treated tumors harvested 24 hours after dose 2 and HALO image analysis was used to quantify the percentage of CD8+ T cells and NCR1+ cells over the whole tumor area. Data is presented as mean percentage values from 6 mice within each treatment group (Isotype in blue and PY265m in red) ± standard error of the mean (SEM).

PY265 Induces a Pro-inflammatory Signature in Human Suppressive Macrophages

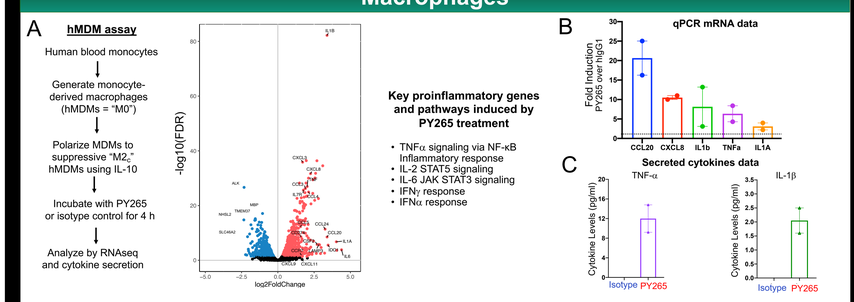
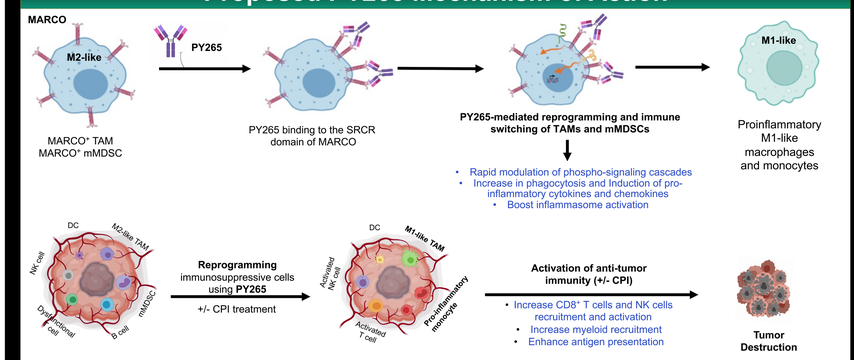


Figure 7. (A) Total RNA was isolated from IL10-treated human monocyte derived macrophages (MDM) and submitted for RNA sequencing. Analysis of PY265-induced differentially expressed genes yielded a profound increase in pro-inflammatory chemokines and cytokines and downstream GSEA revealed a variety of significantly upregulated pathways associated with macrophage activation/reprogramming and tumor reduction. **(B)** qPCR mRNA analysis of key pro-inflammatory genes is plotted as fold induction over the hIgG1 control isotype of one representative donor. **(C)** Cytokine secretion was measured by MSD and data plotted for the levels (pg/ml) of representative pro-inflammatory cytokines. Data are presented as mean values of technical duplicates of the same donor ± standard error of the mean (SEM).

Proposed PY265 Mechanism of Action



In conclusion, our studies demonstrate that targeting MARCO remodels the TME to reinvigorate an anti-tumor immune response (shown by the working MOA model above). Collectively, the available preclinical and nonclinical data support PY265 immunotherapy, alone or in combination with a CPI, in cancer patients resistant or refractory to CPI therapies, to potentially improve both the overall response as well as the durability of response.