Poster 5627

PPARG-HIGH CIRCULATING MONOCYTES EXHIBIT AN IMMUNOSUPPRESSIVE PHENOTYPE IN UROTHELIAL CANCER PATIENTS TREATED WITH ANTI-PD1

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BACKGROUND

- Peroxisome proliferator-activated receptor gamma (PPARG) is expressed in a variety of immune cells such as monocytes, macrophages, and lymphocytes, where it plays a role in their maturation and function[1].
- Tumor cell-intrinsic PPARG activation is linked to tumor development in urothelial cancer (UC)[2,3] where upregulation of PPARG is associated with lack of response to anti-PD1 and an immunosuppressive tumor-microenvironment (TME), characterized by anti-inflammatory cytokine signaling, decreased T-cell infiltration, T-cell dysfunction and increased myeloid-derived suppressive cells (MDSCs)[4,5]. Previously, we have shown that PPARG overexpression and amplification in a large muscle-invasive urothelial cancer (MIUC) cohort correlates with low PD-L1 expression, a cold immune-phenotype and lack of response to anti-PD1[6].
- Although PPARG has been implicated in immune-mediated resistance, to date a comprehensive analysis of PPARG expression in peripheral blood mononuclear cells (PBMCs) in UC patients is lacking. Here, we analyzed PPARG expression and associated transcriptomic profiles in PBMCs from UC patients using single-cell RNA-sequencing (scRNA-seq).
- Our results show that PPARG-high circulating classical monocytes (CMs) in UC patients express a subset of genes involved in immunosuppression, macrophage polarization and known target genes of PPARG.

METHODS

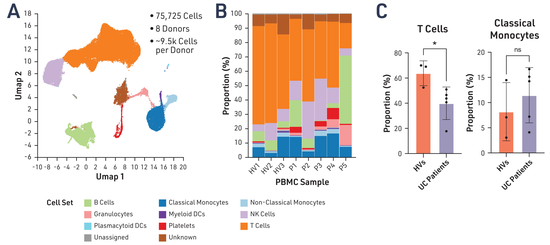
- We performed scRNA-seq of PBMCs from 5 advanced UC patients and 3 normal healthy volunteers (HVs). Real-world samples (Discovery Life Sciences) were collected from patients who received prior Pembrolizumab. Time between treatment initiation and sample collection ranged from 46 to 307 days.
- cDNA libraries were generated using 10X Genomics kit. Count matrices were generated using *Cell Ranger* pipeline (v7.0.0; GRCh38 reference). Downstream data processing, analysis, and visualization were done using *Cellenics* software. GSEA was performed with *fgsea* (v1.24.0) and *msigdbr* (v7.5.1) R packages.
- An average of 12,000 cells (11.8k±4.8k) was recovered per donor. For each donor sample, cells were filtered based on quality metrics such as mitochondrial content, UMIs vs genes detected, and doublets detected. An average of 9,500 cells (9.5k±3.4k) was retained per donor, reflecting an ~80% QC pass rate.
- Expression data were normalized using the *Seurat* 'LogNormalize' method, top 2000 variable genes were selected using *VST*, and data from cells across multiple donors were integrated using *Harmony*.

RESULTS

TABLE 1. Cohort Characteristics

Sample ID	Indication	Stage	Disease Status	Treatment	Time on Treatment (days)	Sex	Race	Age
HV1	Healthy	–	–	–	–	F	White	26
HV2	Healthy	–	–	–	–	M	White	39
HV3	Healthy	–	–	–	–	F	White	37
P1	Urothelial Ca	III-B	Stable	Pembrolizumab	144	F	White	64
P2	Urothelial Ca	IV-B	Stable	Pembrolizumab	307	F	White	85
P3	Urothelial Ca	IV-A	Stable	Pembrolizumab	46	M	White	89
P4	Urothelial Ca	IV	Stable	Pembrolizumab	84	F	White	67
P5	Urothelial Ca	IV-A	Stable	Pembrolizumab	63	M	White	79

FIGURE 1. Cell Phenotype Proportion Comparison Between Urothelial Cancer Patients and Healthy Volunteers

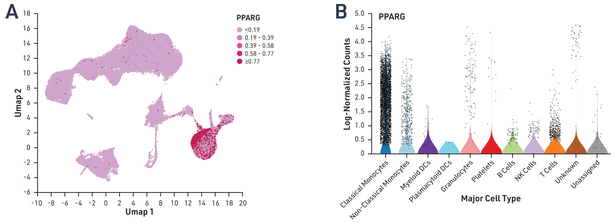


- Expression data from PBMCs were integrated across all 8 donors and 9 major immune cell populations were identified based on Louvain clustering and automated cell-type identification (*ScType*; "Immune System" cell marker database) (Fig 1A-B).
- The proportion of each cell population observed in each donor ranges from 18-70% for T-cells, 5-28% for NK-cells, 0.5-48% for B-cells, 3-17% for classical monocytes, 1-4% for non-classical monocytes, 0.2-14% for granulocytes, 0.1-8% for platelets, and <2% for myeloid and plasmacytoid dendritic cells (DCs) (Fig 1B).

- *The proportion of T-cells was significantly lower in PBMCs from UC patients compared to normal HVs (mean of 40% vs 64%; p=0.036, Wilcoxon test) (Fig 1C).*
- *No statistically significant differences were observed in other cell types (Wilcoxon test).*

References: **1.** Hernandez-Ouiles et al. (2012) *Front Endocrinol*; **2.** Liu et al. (2019) *Nat Commun*; **3.** Tate et al. (2021) *Nat Commun*; **4.** Galsky et al. (2018) *J Clin Oncol*; **5.** Xiong et al. (2023) *Gut*; **6.** Gjini, Kirov, Bowden (2023) Abstract 537: PPARG amplification is associated with lack of response to anti-PD1 in muscle-invasive urothelial cancer. *JITC*; **7.** Court et al. (2017) *Mol Cell Proteom*; **8.** Liao et al. (2012) *Cancer Sci*; **9.** Pullikuth et al. (2021) *Frontiers in Oncology*; **10.** Sims et al. (2023) Abstract ND08: Discovery of FX-909, a first-in-class inverse agonist of the peroxisome proliferator-activated receptor gamma (PPARG) lineage transcription factor, to potentially treat patients with the luminal subtype of advanced urothelial cancer (UC). *Cancer Res.*

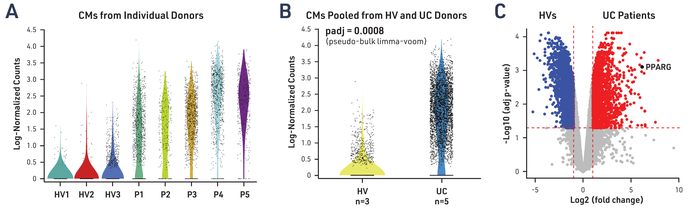
FIGURE 2. PPARG is Expressed in Classical Monocytes



- Fig 2A: PPARG expression (log-normalized counts) overlaid on the UMAP projection shown in Fig 1A. Fig 2B: PPARG expression for each cell type pooled across all 8 donors (each dot is an individual cell).

- *PPARG is highly enriched in classical monocytes (CM) among the 9 major immune cell types.*
  - *52.2% CMs express PPARG vs 1.6% of all other cell types (n=7,389 CMs; n=68,336 other).*
  - *Mean log-normalized PPARG expression is 1.00 in CMs vs 0.02 in all other cell types (padj <0.0001, Wilcoxon test).*

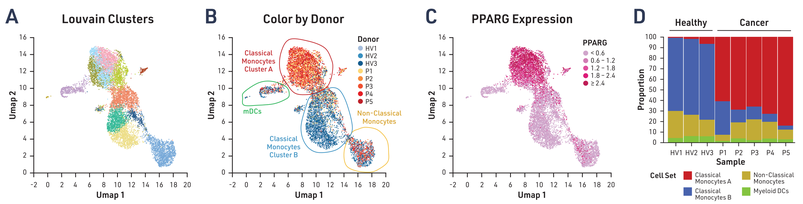
FIGURE 3. Comparison of PPARG Expression in Classical Monocytes of UC Patients and Healthy Volunteers



- Genome-wide differential gene expression analysis comparing CMs from UC patients to CMs from HVs was performed using the pseudo-bulk limma-voom method (Fig 3C).

- *PPARG expression in CMs is significantly higher in UC patients compared to HVs (1.74 vs 0.11 mean log-normalized counts; padj=0.0008).*

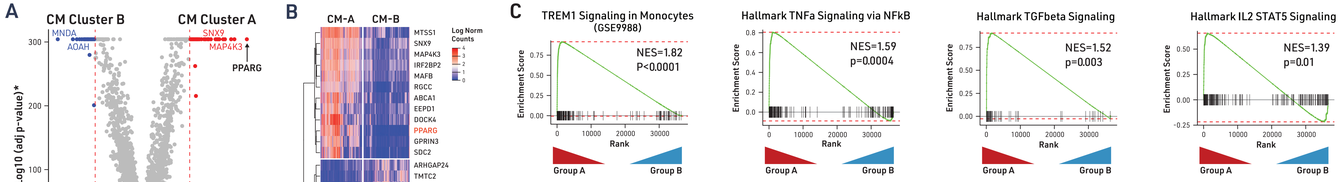
FIGURE 4. Sub-Clustering of Myeloid Cells Further Reveals PPARG-High and PPARG-Low Classical Monocyte Cell Populations



- Sub-clustering of the myeloid cell population comprising of CMs, non-classical monocytes, and mDCs (Fig 4A-C) was performed using Louvain and UMAP clustering, which revealed two major CM sub-clusters named 'A' and 'B' (Fig 4B).

- *In cluster A, 95% of the cells originated from UC samples, while most cells in cluster B (81%) originated from HV samples (Fig 4D).*
- *PPARG expression was higher on the 'A' CMs compared to the 'B' CMs (Fig 4C).*

FIGURE 5. Differential Expression Analysis Reveals PPARG as the Top Upregulated Gene in the Classical Monocyte Sub-Group Enriched in UC Patients



* -Log10(adj P)>308 is presented as 308

- Unbiased differential expression analysis showed that PPARG was the top upregulated gene in cluster A (PPARG-high) vs cluster B (PPARG-low) CMs (1.89 vs 0.23 log-normalized counts; adj P-val<0.0001; Wilcoxon test) (Fig 5A).
- PPARG-high CMs are characterized by a gene signature inclusive of GPRIN3, RGCC, MAFB and IRF2BP (Fig 5B), which have been shown to be upregulated in M2 polarized macrophages.[7]
- Pathway analysis using GSEA revealed that PPARG-high CMs are enriched for TREM1 signaling genes (padj<0.0001; Fig 5C), whose high expression has been associated with immunosuppressive phenotypes in myeloid cells[8] and shorter survival of patients across other cancers.[9]

CONCLUSION

PPARG-high circulating classical monocytes (CMs) in urothelial cancer (UC) patients exhibit a transcriptomic profile associated with immunosuppression and M2 macrophage polarization. FX-909, a first-in-class PPARG inverse agonist, is being evaluated in the clinic and the study includes an exploratory biomarker approach to assess the potential effect of PPARG inhibition on the CM immune-profile of advanced UC patients enrolled in the Ph1 study[10].

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