PPARG AMPLIFICATION IS ASSOCIATED WITH LACK OF RESPONSE TO ANTI-PD1 IN MUSCLE-INVASIVE UROTHELIAL CANCER

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Abstract #537

BACKGROUND

Although immune checkpoint inhibitor (ICI) approval has changed the treatment landscape of metastatic urothelial carcinoma, approximately 70% of patients succumb to refractory or acquired resistance[1].

Dysregulation of PPARG signaling is linked to tumor development in urothelial cancer, where PPARG signaling is essential to cell lineage determination in the luminal layers of the normal urothelium[2,3]. Recurrent genetic alterations in *PPARG*, as well as hotspot mutations in its obligate heterodimer-retinoid X receptor alpha (*RXRA*) in Muscle-Invasive Urothelial Cancer (MIUC), are characteristics of the luminal subtype, which responds poorly to ICI[4].

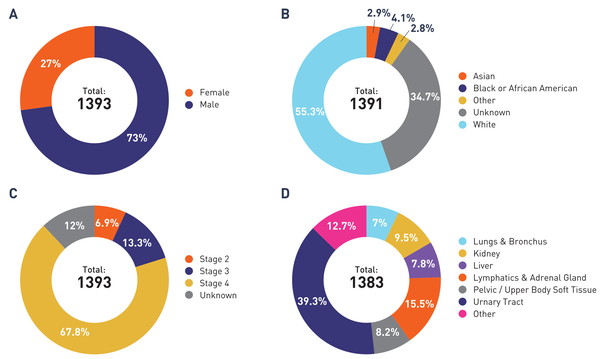
Tumor-cell intrinsic upregulation of PPARG is associated with lack of response to anti-PD1 and an immunosuppressive tumor-microenvironment (TME), characterized by anti-inflammatory cytokine signaling, decreased T-cell infiltration, T-cell dysfunction and increased myeloid-derived suppressive cells[4,5].

METHODS

Analyses were performed on real-world data from 1393 MIUC patients. Tumor samples were sequenced using the Tempus xT assay (DNA-seq of 648 genes at 500x coverage) and RNA-seq (n = 1389 with RNA, n = 1365 with DNA). Within the dataset, 275 patients received anti-PD-1 therapy (248 patients received Pembrolizumab and 27 patients received Nivolumab). Pre-anti-PD1 treatment tissues were analyzed (cut-point of ≤90 days from start-of-treatment to date-of-tissue-collection). PD-L1 expression was assessed using the PD-L1 IHC 22C3 *PharmDx assay* (Combined Proportion Score [CPS] cut-off of 10%). Gene expression values were normalized by transcripts-per-million (TPM). Immune infiltration was quantified with mcpCounter package in R. Patients were binned in "Amplified" (AMP) vs "Non-Amplified" (non-AMP) groups by *PPARG* copy number (CN) cut-off of 3. Kaplan-Meier analyses were performed based on Real-World Progression Free Survival (rw-PFS) Time-to-Next Treatment (TTNT) and *PPARG* amplification.

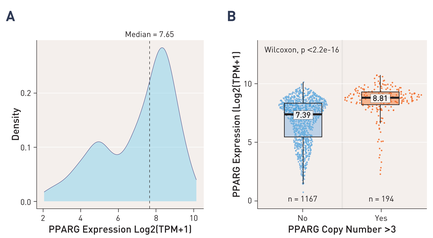
RESULTS

FIGURE 1. Cohort Characterization



The MIUC cohort is composed of 1393 patients. Seventy-three percent of the patients are male, while 27% are females (Figure 1A). The distribution of race shows that approximately half of the patients are white (55.3%; Figure 1B). In addition, the cohort is enriched for stage 4 biopsies (67.8%), followed by stage 3 (13.3%) and stage 2 (6.9%) (Figure 1C). The biopsies assessed for PPARG mRNA expression and PPARG genetic alterations are collected from different sites, such as urinary tract tissues (39.3%), lymphatic and adrenal gland (15.5%), kidney (9.5%), etc (Figure 1D).

FIGURE 2. Amplification of PPARG is Associated with Higher Levels of PPARG Expression



Overall, the median mRNA expression level of *PPARG* in this cohort is 7.65 (Figure 2A). The median mRNA expression level of *PPARG* was significantly higher in the *PPARG* AMP group compared to PPARG non-AMP group (7.39 Log2[TPM+1] vs 8.81 Log2[TPM+1]; p <2.2e-16) [4] (Figure 2B)[6].

References

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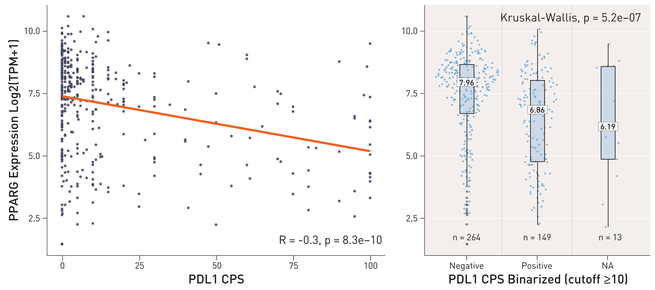
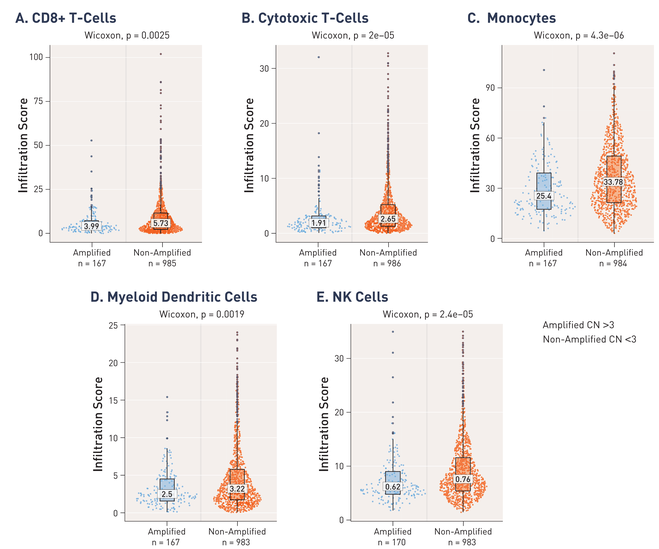
FIGURE 3. Higher PPARG Expression is Negatively Correlated with PDL1 Expression in MIUC



PPARG mRNA expression is negatively correlated with PDL1 protein expression, which was assessed by CPS (R = -0.3, p-value <0.001). PPARG expression was also higher in the PD-L1-negative tumors (CPS <10) compared to PDL1-positive tumors (7.96 Log2 [TPM+1] vs 6.86 Log2 [TPM+1]; p <0.001).

FIGURE 4. PPARG Amplification [CN >3] Correlates with Low Tumor Immune Cell Infiltration



In MIUC tumors, high PPARG mRNA expression is negatively correlated with expression of immune cells (Table 1). Moreover, PPARG AMP tumors exhibited a cold immune-phenotype compared to the PPARG non-AMP tumors, associated with lower CD8+ T-cell infiltration signature score (3.99 Log2[TPM+1] vs 5.73 Log2[TPM+1]; p = 0.0025) and lower expression of other immune cells (Figure 4, Table 2).

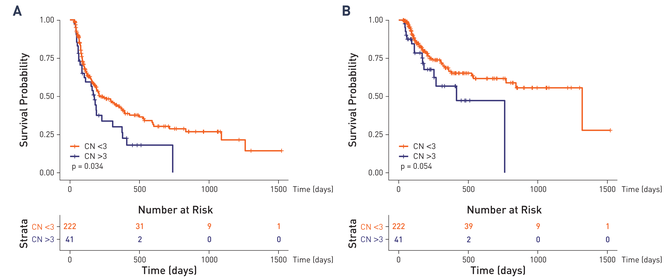
TABLE 1. PPARG Expression Negatively Correlates with Tumor Immune Cell Infiltration

Immune Signatures	Correlation Coefficient (R) with PPARG mRNA Expression	P-Value (Wilcoxon test)
CD8+ T-Cells	-0.17	<0.001
Cytotoxic Lymphocytes	-0.29	<0.001
Natural Killer (NK) Cells	-0.19	<0.001
Monocytes	-0.35	<0.001
Myeloid Dendritic Cells	-0.14	<0.001

TABLE 2. PPARG Amplification Status and Immune Cell Score

Immune Signature	Median RNA Score (Log2[TPM+1])		P-Value (Wilcoxon test)
	PPARG AMP (CN >3)	PPARG Non-AMP (CN <3)	
Cytotoxic Lymphocytes	1.99	2.65	<0.001
Natural Killer (NK) Cells	0.62	0.76	<0.001
Monocytes	25.4	33.78	<0.001
Myeloid Dendritic Cells	2.5	3.22	<0.001

FIGURE 5. PPARG Amplification is Significantly Associated with Shorter rwPFS to anti-PD1



Survival analysis in patients treated with Pembrolizumab (90.5%) and Nivolumab (9.5%) showed significantly shorter rwPFS (p = 0.034) for patients with PPARG AMP (n = 41) compared to the non-AMP group (n = 222) (Figure 5A). Similarly, patients with PPARG AMP and treated with anti-PD1 showed a trend for shorter TTNT (p = 0.054) compared to the non-AMP group (Figure 5B).

CONCLUSION

PPARG overexpression and amplification in a large MIUC cohort correlates with low PD-L1 expression, a cold immune-phenotype and lack of response to anti-PD1. Others have demonstrated the significant role PPARG plays in immune modulation of the TME[5]. FX-909, a first-in-class covalent PPARG inverse agonist that will be evaluated in a Ph1 trial this year, will offer an opportunity to investigate the impact of PPARG inhibition in the TME of MIUC patients[7]. FX-909 combination with ICI agents potentially provides a "one-two punch" strategy to overcome resistance to immunotherapy in MIUC patients with high PPARG expression.

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