PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARG) STATUS DEFINES THE LUMINAL LINEAGE IN MOLECULAR PROFILES OF ADVANCED UROTHELIAL CANCERS (UC)

Poster 113

William Motley[1], Sumaiya Islam[2], Ken Eagle[1], Joshua Bell[2], Robert Sims[1], Michaela Bowden[1]

[1]Flare Therapeutics, Cambridge, MA, USA; [2]Tempus, Chicago, IL, USA

BACKGROUND

- About 20,000 patients are diagnosed with muscle-invasive UC annually, where the five-year survival rate is approximately 5% in metastatic cases.
- UC molecular classification has generally identified five subtypes; three of which have markers of luminal differentiation.
- PPARG is a nuclear receptor-family transcription factor with an important role in cell lineage determination in adipose tissue and the luminal layers of the normal urothelium.
- The transcription factor PPARG is associated with the luminal lineage subtype reflecting ~65% of all advanced UC patients.
- Recurrent genetic alterations in PPARG, including focal amplification, missense mutations, and fusions, as well as hotspot mutations in its obligate heterodimer, retinoid X receptor alpha (RXRA) are characteristic of this molecular subtype.

MATERIALS AND METHODS

- Tumor tissue collected from over 3,000 patients with muscle-invasive UC sequenced using the Tempus xT assay.
- Molecular classification as luminal (luminal papillary, luminal, and luminal infiltrated subtypes) or non-luminal (basal-squamous and neuronal subtypes) was performed using non-negative matrix factorization (NMF) rank 5 following the Robertson method[1], excluding PPARG from the gene set to eliminate the inference of bias.

RESULTS

TABLE 1: PPARG Expression Level is a Defining Feature of Urothelial Carcinomas that are Classified as Luminal

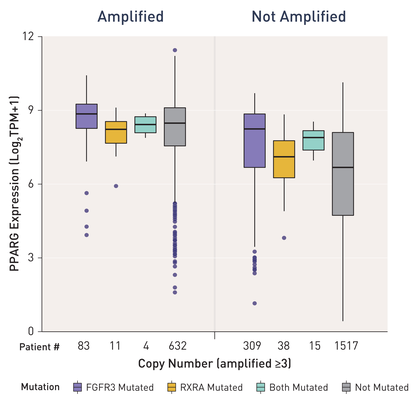
Molecular Subtypes[1]	Luminal Lineage			Non-Luminal Lineage	
	Luminal Papillary	Luminal	Luminal Infiltrated	Basal-Squamous	Neuronal
Patients (n)	644	436	607	611	311
Average PPARG mRNA Expression Level Log_2(TPM+1) (95% CI)	8.72 (8.66-8.77)	7.51 (7.38-7.64)	6.98 (6.87-7.09)	5.2 (5.05-5.35)	6.02 (5.75-6.28)
Average PPARG mRNA Expression Level Log_2(TPM+1) (95% CI)	7.78 (7.71-7.85)			5.47 (5.34-5.61)	

Abbreviations: **CI** – Confidence interval; **TPM** – Transcripts per million

TABLE 2: Alterations in PPARG, RXRA and FGFR3 are More Frequently Observed in Tumors that are Classified as Luminal

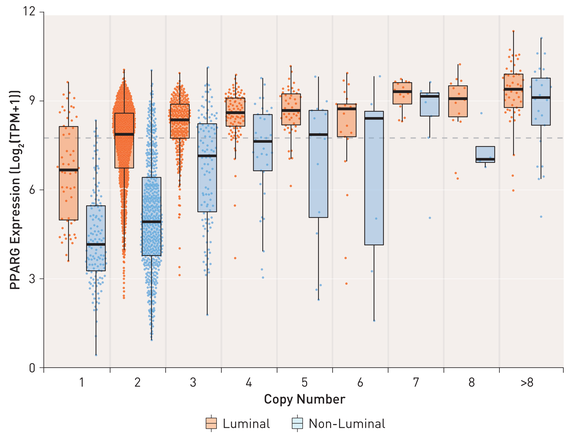
Molecular Subtypes[1]	Luminal Lineage			Non-Luminal Lineage		P-Value	Odds
	Luminal Papillary	Luminal	Luminal Infiltrated	Basal-Squamous	Neuronal		
Patients Profiled	1687			922			
PPARG Amplification (Copy Number ≥3)	30.90%			22.60%		<0.00001	1.54
RXRA Hotspot Mutations (p.Ser427Tyr, p.Ser427Phe)	3.60%			0.80%		<0.00001	4.9
FGFR3 (Single Nucleotide Variants and Small Insertions/ Deletions, and Fusions)	18%			11.60%		<0.00005	1.67

FIGURE 1. PPARG Levels are Increased in Advanced Muscle-Invasive UC Harboring Alterations in PPARG, and FGFR3



- RXRA mutation confers >8-fold biochemical activation of PPARG (Abstract 94, Poster 084, Figure 2), which we hypothesize confers significant pathway activation in the presence of normal levels of PPARG. Consistent with this, patients with RXRA mutation do not have higher levels of PPARG expression.

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



- A PPARG expression threshold (dashed line, 7.75 Log_2[TPM+1]) was derived from logistic regression comparing amplified and diploid patients, where gain of even one copy of PPARG is associated with overexpression.
- Many diploid patients exhibit PPARG expression (7.49 Log_2[TPM+1]) comparable to amplified patients.

FIGURE 3. Distribution of PPARG Expression in Metastatic Tumors is Similar to Distribution in Primary Tumors

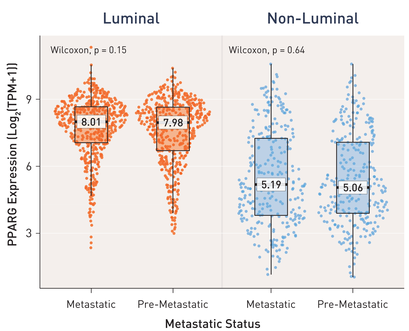
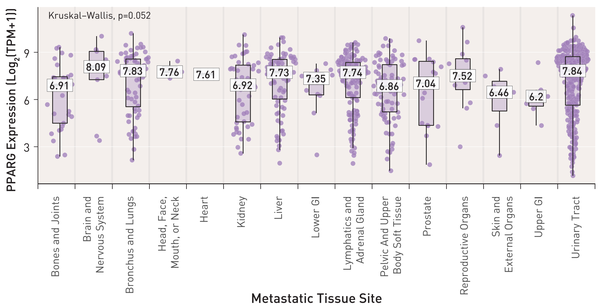


FIGURE 4. PPARG Expression Does Not Significantly Vary by Metastatic Tissue Site



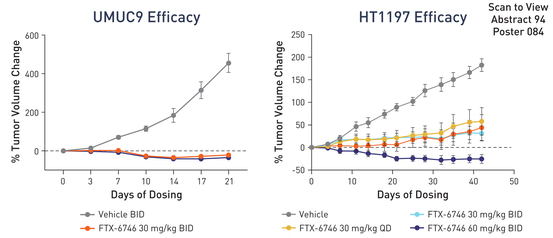
- High PPARG expression was sustained in metastatic samples, and expression did not significantly vary by metastatic tissue site (p>0.05).

CONCLUSION

- PPARG is a defining feature of the luminal phenotype in advanced UC.
- Molecular characterization of UC serves as the foundation for a precision approach to identifying patients most likely to respond to PPARG inhibition.
- Novel inverse agonists under development by Flare Therapeutics represent a promising therapeutic approach to the treatment of UC and have demonstrated robust preclinical activity in xenograft models (see below, Abstract 94, Poster 084).

FROM POSTER 084. FTX-6746 Robustly Suppresses Tumor Growth in UC Xenograft Models with PPARG Amplification and RXRA Mutation

Scan to View Abstract 94 Poster 084



Scan to View this Poster Abstract 333, Poster 113

For more information: wmotley@flaretx.com or mbowden@flaretx.com.

The authors would like to thank Stefan Kirov for help with data analysis and figure preparation.

References: 1. Robertson AG, et al. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. *Cell.* 2017 Oct 19;171(3):540-556.e25. doi: 10.1016/j.cell.2017.09.007.