Poster 2802

DEVELOPMENT OF A SURROGATE TISSUE PHARMACODYNAMIC (PD) ASSAY FOR POTENTIAL CLINICAL USE WITH FX-909, A NOVEL INHIBITOR OF THE UROTHELIAL LUMINAL LINEAGE TRANSCRIPTION FACTOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARG)

Jennifer A. Mertz[1], Stefan A. Kirov[1], Kaylyn E. Williamson[1], Marton Munz[2], Robert J. Sims III[1], Michaela Bowden[1]
[1]Flare Therapeutics, Cambridge, MA; [2]The Bioinformatics CRO, Lake Monroe, FL

BACKGROUND

- Targeting lineage-defined transcription factors with small molecules is a proven and effective therapeutic strategy, as demonstrated by estrogen and androgen receptor therapies for breast and prostate cancers.
- Expression of the transcription factor PPARG is associated with the luminal lineage subtype, which accounts for two-thirds of all advanced urothelial cancer (UC) patients.
- The feasibility of an accessible, less invasive surrogate tissue to evaluate PD biomarkers that support proof of mechanism of drug action, clinical proof of concept and ultimately the selection of optimal dosing, was evaluated.
- FX-909, a covalent PPARG inverse agonist, is projected to initiate Phase 1 clinical studies in 2023 aimed at serving patients with advanced UC.
- *In vivo* mouse xenograft, rat pharmacology and *ex vivo* cultured human explant models were utilized to assess potential biomarkers associated with on-target effects of FX-909.
- Tumor tissue from mouse xenografts, as well as white adipose tissue (WAT) and skin tissue were collected. Changes in known PPARG target gene expression were measured by qPCR and transcriptomic differences by RNA-Seq.

METHODS

***In vivo* studies –** UMUC9 UC xenograft model studies were performed in female NCG mice treated with FX-909 at 0.03 mg/kg to 1 mg/kg BID. All animals were dosed orally twice a day for up to 21 days. UMUC9 xenograft tumor, mouse WAT and skin tissues were collected. Preclinical pharmacology studies were performed in Sprague Dawley male rats. Rats were dosed orally twice a day for 28 days with vehicle or 3 to 30 mg/kg FX-909. Skin and WAT were collected.

Human skin explant studies – Skin tissue collected from abdominoplasty procedures of healthy subjects was used to perform *ex vivo* FX-909 treatment. Using transwell dishes, skin punch tissue was incubated in medium containing FX-909 for 72h at MedPharm.

General RNA-Seq quantification – 50M 150 bp reads were generated from each sample. Raw reads were processed with STAR/RSEM or HISAT2. After genes were filtered to remove low expression (TPM<0.5 and counts <3), the quality of the samples were evaluated according to mapping parameters and based on PCA clustering. Rat data was aligned to Rnor_6.0, mouse to GRCm38 and human to GRCh38 and >11000 genes per sample were quantified.

Differential expression and curve fitting analysis – Dose-dependent expression values (normalized counts) from rat PD study were fit to several models. Genes that fit best consistently both in WAT and skin tissue were filtered. Human data was analyzed with EdgeR comparing DMSO to 50 nM dose after filtering out low expressing genes. Differentially expressed genes were defined as having FDR<0.1 and abs(log2FC)>0.5.

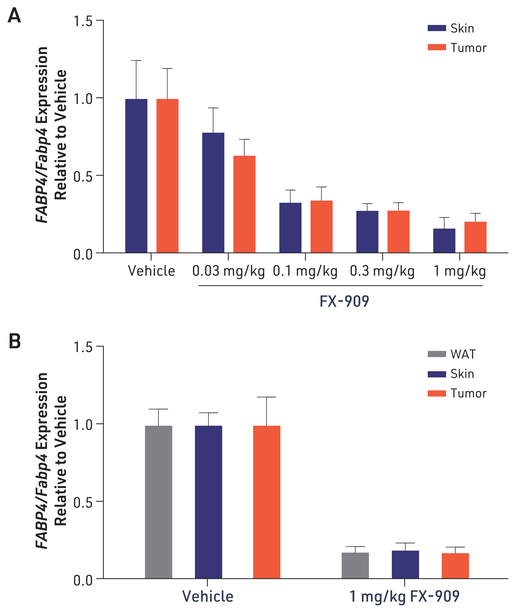
References

1. Konger, R. L. et al. Epidermal PPARγ Is a Key Homeostatic Regulator of Cutaneous Inflammation and Barrier Function in Mouse Skin. *Int J Mol Sci* 22, 8634 (2021).

Scan to View this Poster, Abstract 2802

Scan to view press release from oral presentation on FX-909, Abstract ND08

FIGURE 1. FX-909-mediated Gene Expression Changes Correlate Well Between Skin, WAT and Xenograft Tumor Tissues

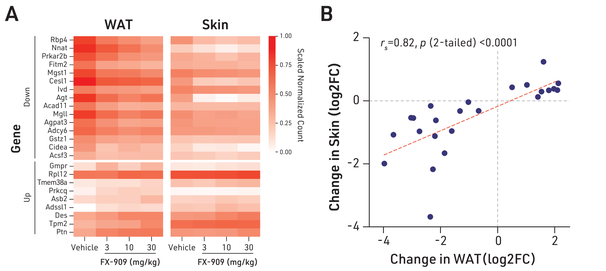


In vivo UMUC9 UC xenograft model studies were performed in NCG mice treated with FX-909 at 0.03 mg/kg to 1 mg/kg BID for up to 21 days. **A.** Quantitative RT-PCR shows dose-dependent suppression of *FABP4/Fabp4* target gene with strong correlation shows strong correlation [r=0.98, p-value=0.003] between tumor and skin tissues. **B.** RNA-Seq analysis of mouse skin, white adipose tissue and UMUC9 xenograft tumor samples treated for 1.5 days with 1 mg/kg BID FX-909 shows robust suppression of *FABP4/Fabp4*.

FIGURE 2. Rat Pharmacology Study Design

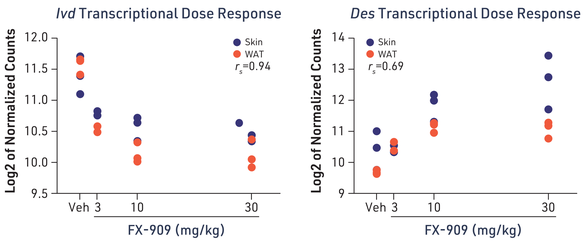
Group	N	Compound	Dosing Regimen
1	6	Vehicle	PO, BID (28 days)
2	6	3 mg/kg FX-909	PO, BID (28 days)
3	6	10 mg/kg FX-909	PO, BID (28 days)
4	6	30 mg/kg FX-909	PO, BID (28 days)

FIGURE 3. Concordance of FX-909-mediated Gene Expression Changes in Rat Skin and White Adipose Tissue



A. 30% of all genes that responded to FX-909 treatment in skin displayed a similar dose-dependent response profile in WAT. **B.** Spearman's Rho correlation between gene expression changes induced by FX-909 (30 mg/kg vs Vehicle) in rat skin and WAT.

FIGURE 4. Correlation of Gene Expression Changes in Rat Skin and WAT



Gene	Tissue	Standard Residual Error	Adjusted p-Value
Ivd	Skin	0.200	<0.0001
	WAT	0.159	<0.0001
Des	Skin	0.662	0.0004
	WAT	0.187	<0.0001

FIGURE 5. Skin Explant Models Bridge the Interspecies Translational Gap for Studying FX-909-mediated Effects

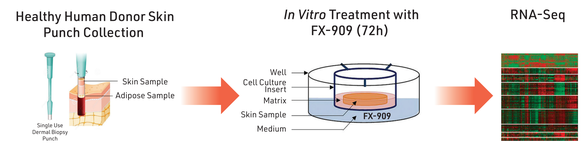
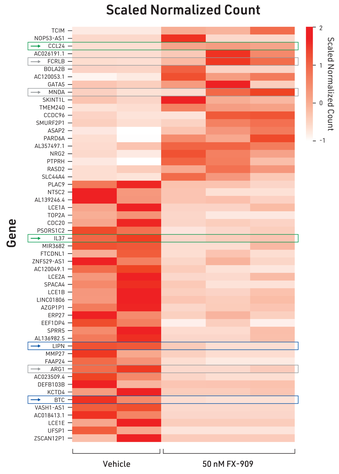


FIGURE 6. FX-909-mediated Transcriptional Changes in Human Skin Explants



- In pilot studies, 36 upregulated and 61 downregulated genes were detected (10% FDR).
- Genes involved in known PPARG-mediated processes such as **adipogenesis and metabolism**, **inflammation** and **immune contexture** are regulated by FX-909 in skin.
- Full scale studies are ongoing.

FIGURE 7. Consistency of PPARG Target Gene Suppression Across Species and Tissues

Species	Tissue	Treatment	*FABP4/ Fabp4*	*IVD/ Ivd*	*GPD1/ Gpd1*	*AGT/ Agt*	*ARG1/ Arg1*
Human	Tumor (UMUC9 xenograft)	FX-909		*		*	*
	Skin (explant)	FX-909			ND		
Mouse	Skin	KO[1]	*			ND	
		FX-909				*	
	WAT	FX-909					
Rat	Skin	FX-909			*		ND
	WAT	FX-909					

-4.1 … -0.57

Log2FC of control vs treated samples; **ND** = Not detected; ***** = Not significant.

SUMMARY

- PPARG target genes, including *FABP4/Fabp4, IVD/Ivd, GPD1/Gpd1, AGT/Agt* and *ARG1/Arg1*, are repressed across different species and/or tissues.
- Based on these preclinical data and confirmatory experiments in the *ex vivo* setting, a novel FX-909 signature is under development to predict target modulation in the tumor.
- The consistent correlation of PPARG target gene suppression in tumor and normal tissues across species provide a strong rationale for a surrogate biospecimen collection, such as a skin punch biopsy, to establish the PK/PD relationship between the dose and response to FX-909 in the clinic.