Downstream Targets of the Steroidogenic Factor-1 (SF-1) Antagonist OR-449 in PDX Models of ACC

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Introduction 1

- Adrenocortical carcinoma (ACC) is a rare endocrine malignancy frequently diagnosed at an advanced stage when prognosis is dismal (Stage III/IIV 5-year survival <20%).
- Mitotane, the only approved therapy for locally advanced or metastatic disease, is minimally effective. Recent clinical trials with targeted therapies or immune checkpoint inhibitors have been disappointing, prompting a search for novel therapeutics for ACC[1].
- Steroidogenic factor-1 (SF-1, NR5A1), a nuclear receptor and a transcription factor essential for the growth and development of the adrenal gland, has been described as the "master transcription factor" in adult ACC[2].
- Elevated SF-1 expression correlates with poor clinical outcome in adult ACC[3], and SF-1 is consistently amplified at the chromosomal level in pediatric ACC[4].
- OR-449 is a first-in-class small molecule antagonist to SF-1 that inhibits tumor growth in a pediatric ACC patient-derived xenograft (PDX) model (SJ-ACC3, ref 5) and in a rat Leydig tumor cell line (R2C) xenograft model[6,7]. OR-449 is currently undergoing IND-enabling studies in preparation for a Phase I clinical trial in ACC.
- Serum and urine steroid intermediates have been used in the diagnosis of ACC[8]. To identify potential biomarkers for following the action of OR-449 in the clinic, we evaluated the effect of OR-449 on circulating steroids and steroid precursors and on steroidogenic gene expression in pediatric and adult ACC PDX models (**Table 1**).

Table 1. ACC PDX Models

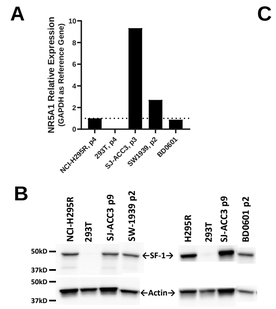
	SJ-ACC3	SW1939	BD0601
Patient sex/age	Male/ 11yr	Male/13yr	Female/54yr
Tumor site/primary/met	Primary Adrenal	Primary Adrenal	Primary Adrenal
SF-1 amplification	Yes	Unknown	Unknown
TP53 status	Yes, G245C	Unknown	Unknown
Where established	St. Jude	UTHSC	BioDuro
Mouse host	CB17 SCID (female)	NSG (female)	NSG (male)
OR-449 30 mg/kg %TGI	105% (ref 6)	65% (Fig 2)	Not tested

% tumor growth inhibition (TGI) = [(C-T)/C] where C and T are mean tumor volume at termination – mean tumor volume on Day 0 for Control and Treated, respectively

SF-1 Expression in SW1939 and BD0601 PDX Models 2

- SW1939 is a new pediatric ACC PDX developed at the University of Texas, San Antonio. BD0601 (BD2000070601) is an adult ACC PDX from BioDuro-Sundia, San Diego, CA.
- SF-1 mRNA measured by qPCR (**Fig 1A**) showing that SF-1 expression in SW1939 is ~3-fold higher than that seen in BD0601 or H295R cells (an adult ACC cell line) and about 25% of that seen in SJ-ACC3[5]. SF-1 expression in 293T (human embryonic kidney cell line) is included as a negative control.
- Immunoblotting using a monoclonal antibody to SF-1 (N1665) showing SF-1 protein expression in H295R, SJ-ACC3, SW1939 and BD0601 lysates (**Fig 1B**). Actin immunoblotting shows that protein loading is similar between samples.
- SF-1 detection by immunohistochemistry in SW1939 and BD0601 using N1665 antibody (**Fig 1C**).

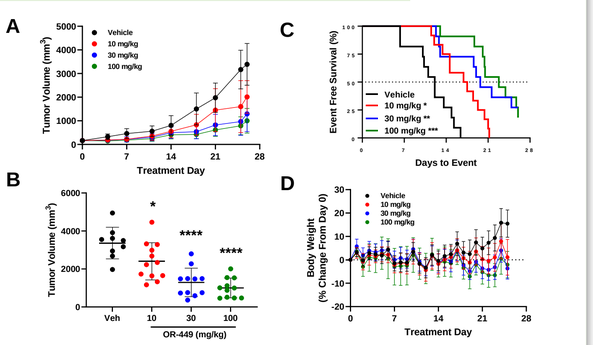
Figure 1. SF-1 mRNA and Protein Expression in SW1939 and BD0601



OR-449 Inhibits SW1939 Tumor Growth *In Vivo* 3

- Immunocompromised female NOD-scid IL2Rγ null mice were inoculated subcutaneously in the right flank with 2x10[6] SW1939 cells in growth factor reduced matrigel.
- When tumors reached 105-250mm[3], mice bearing similarly sized tumors were randomly assigned to treatment groups (n=12) and dosed orally with OR-449 (10, 30 or 100 mg/kg/day) or vehicle for 26 days.
- OR-449 significantly inhibited SW1939 tumor growth (**Fig 2A & 2B**) and improved event-free survival (**Fig 2C**) without affecting body weight (**Fig 2D**).

Figure 2. SW1939 tumor growth, body weight and survival



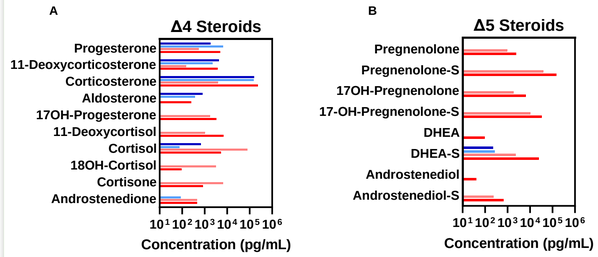
*A, B: Tumor volume was calculated as: (length x width^2) x 0.5. *, p<0.05, ****, p<0.0001*
C: An event is defined as a 4-fold increase in tumor volume., p<0.05, **, p<0.01, ***, p<0.001*
Data shown are mean ± SD. (n=10-12/group)

ACC PDX Tumors Produce Steroid Precursors *In Vivo* 4

Serum steroid profiling by LC-MS/MS demonstrates high levels of Δ4 steroids and steroid precursors (**Fig 3A**) and Δ5 steroids, steroid precursors and sulfates (**Fig 3B**) in mice harboring established SW1939 or BD0601 tumors compared to non-tumor-bearing CD-1 male (M) or CD-1 female (F) mice.

- Notably, steroid precursors and sulfated steroids that are frequently elevated in ACC patients[7], including11-deoxycortisol, pregnenolone sulfate and 17OH-pregnenolone sulfate, are also abundant in ACC tumor-bearing mice.

Figure 3: Serum steroids and precursors in CD-1 and PDX mice

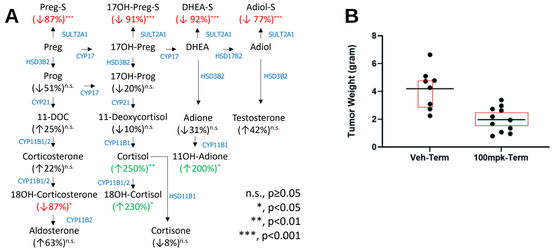


BLOQ in all samples: 11-ketoandrostenedione, 11-Ketoprogesterone, 11-Ketotesterone, 18-Oxocortisol, 21-Deoxycortisol, Estriol, Estradiol, Estrone

OR-449 Inhibits Secretion of Steroid Sulfates *In Vivo* 5

- SW1939 tumor-bearing mice were dosed orally with OR-449 (100 mg/kg) or vehicle for 24 days.
- Serum steroids from 3 mice in each group were profiled by LC-MS/MS and the percent change in concentration relative to vehicle-treated mice is shown for each measured steroid (**Fig 4A**).
- The mean tumor weight in the OR-449-treated group was ~50% of the vehicle group. The 3 mice in each group had tumor volumes close to the group mean (**Fig 4B**).
- The serum levels of individual steroids in vehicle-treated mice are shown in **Fig 4C**.

Figure 4. Steroid profiling in OR-449-treated tumor-bearing mice



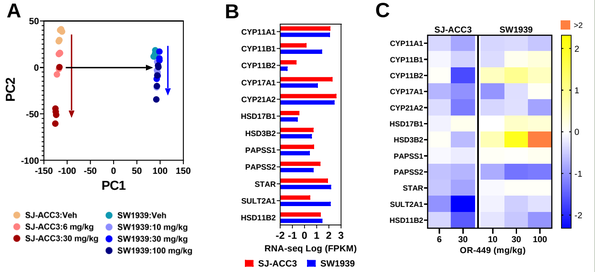
C

Pregnenolone-S	1903 ng/ml	17OH-Pregnenolone-S	393 ng/ml	DHEA-S	312 ng/ml
Pregnenolone	N.A.	17OH-Pregnenolone	N.A.	DHEA	N.A.
Progesterone	3.8 ng/ml	17OH-Progesterone	4.3 ng/ml	Androstenedione	911 pg/ml
11-DOC	1.2 ng/ml	11-Deoxycortisol	6.3 ng/ml	11OH-Adione	585 pg/ml
Corticosterone	9.2 ng/ml	Cortisol	17.2 ng/ml	Androstenediol-S	14.2 ng/ml
18OH-Corticosterone	269 pg/ml	18OH-Cortisol	96 pg/ml	Androstenediol	pending
Aldosterone	107 pg/ml	Cortisone	4 ng/ml	Testosterone	854 pg/ml

OR-449 Regulates Steroidogenic Gene Expression *In Vivo* 6

- SJ-ACC3 or SW1939 tumor-bearing mice were treated with vehicle or OR-449 when tumors reached 500 mm[3]. Tumor gene expression was assessed by mRNA sequencing (RNA-seq) after 7 days of dosing.
- Principal component analysis (PCA) of tumor RNA-seq data demonstrating differential gene expression in SJ-ACC3 and SW1939 (PC1, black arrow) and similar gene regulation with increasing dose of OR-449 (PC2, brown and blue arrows) in these two models (**Fig 5A**).
- Absolute expression (log10 scale) of adrenal steroidogenic genes in SJ-ACC3 and SW1939 is shown in **Fig 5B**.
- Heat map (**Fig 5C**) showing fold change in adrenal steroidogenic gene expression (log2 scale) in OR-449-treated SJ-ACC3 or SW1939 tumor-bearing mice compared to vehicle-treated controls.

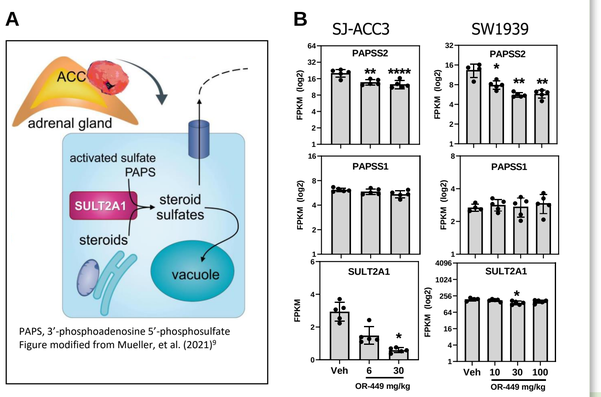
Figure 5: RNA-seq analysis of pediatric ACC models following OR-449 treatment



OR-449 Inhibits Steroid Sulfation Gene Expression *In Vivo* 7

- OR-449 dose-dependently inhibits expression of the activated sulfate donor enzyme PAPS Synthase 2 (PAPSS2) in SJ-ACC3 and SW1939 tumor-bearing mice, whereas PAPSS1, which is elevated in ACC compared to benign adrenal adenomas[9], is not regulated by OR-449 (**Fig 6A**).
- Expression of SULT2A1, the major adrenal steroid sulfotransferase and a known SF-1 target gene, is markedly suppressed by OR-449 in SJ-ACC and, to a lesser extent, in SW1939 indicating that sulfation pathway genes may be differentially regulated by OR-449 in ACC (**Fig 6B**).

Figure 6: Steroid sulfation gene regulation in OR-449-treated tumor-bearing mice



Conclusions 8

- Serum steroids and steroid precursors are abundant in mice harboring pediatric and adult ACC patient-derived tumor xenografts.
- Sulfated steroids are markedly suppressed in SW1939 tumor-bearing mice following administration of the SF-1 antagonist OR-449.
- Steroid sulfation pathway genes SULT2A1 and PAPSS2 are down-regulated in SW1939 and SJ-ACC3 PDX tumors following administration of OR-449.
- Serum steroid profiling in ACC patients may be a useful clinical pharmacodynamic biomarker for demonstrating OR-449 activity independent of tumor growth effects.

References & Acknowledgements 9

1. Altieri, et al., Best Pract Res Clin Endocrinol Metab 34:101434, 2020.
2. Corces, et al., Science 362:eaav1898, 2018.
3. Sbiera, et al., J Clin Endocrinol Metab 95:E161-71, 2010.
4. Pinto, et al., Nat Commun 6:6302, 2015.
5. Pinto, et al., Clin Cancer Res 19:1740-7, 2013.
6. Crowe, et al., Journal of the Endocrine Society Vol. 5, Suppl. 1, A1010, 2021.
7. Tao, et al., Abstract #7871, AACR 2022.
8. Rege, et al., J Steroid Biochem Mol Biol 190:273-280, 2019.
9. Mueller, et al., J Clin Endocrinol Metab 106:3385-3397, 2021.

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