Characterization of OR-449, a Potent Antagonist to Steroidogenic Factor-1 (SF-1) and Clinical Candidate for Treatment of ACC

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Introduction 1

- Adrenocortical carcinoma (ACC) is frequently diagnosed at an advanced stage when prognosis is dismal (Stage III/IV 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.
- Steroidogenic factor-1 (SF-1, NR5A1), a nuclear receptor essential for the growth and development of the adrenal gland, has been described as the "master transcription factor" in adult ACC[1].
- SF-1 is very frequently amplified at the chromosomal level in pediatric ACC[2] suggesting that it is an oncogene or tumor driver in this cancer.
- OR-689, the first SF-1 antagonist suitable for *in vivo* studies, blocked growth of the rat R2C Leydig cell tumor at 60 & 100 mg/kg.
- OR-449 is a highly-specific small molecule antagonist to SF-1. OR-449 blocked the growth in two patient-derived tumor xenografts from pediatric cancers (SJ-ACC3 and SW1939)[3] as well as R2C tumor cell growth[4].
- We evaluated the responses of adult patient-derived xenografts to OR-449 in terms of growth, gene expression, and secretion of circulating steroids typical of ACC.
- OR-449 was evaluated in 28-day GLP safety studies in mouse and dog, and cortisol response to ACTH stimulation following daily OR-449 treatment was evaluated in dogs in a 21-day study.

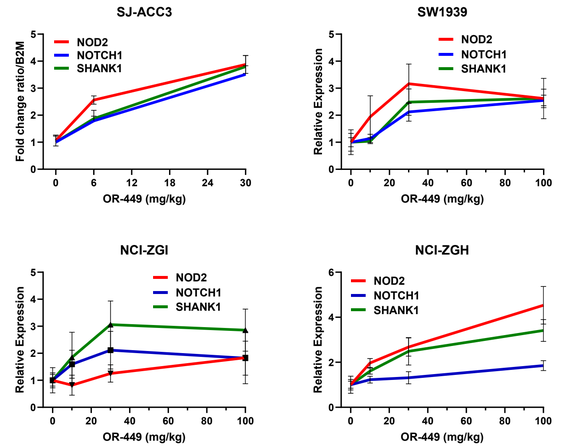
1. Corces, et al., Science 362:eaav1898, 2018
2. Pinto, et al., Nat Commun 6:6302, 2015
3. Crowe, et al., Journal of the Endocrine Society Vol. 5, Suppl. 1, A1010, 2021
4. Tao, et al., Abstract #7871, AACR 2022

Summary of OR-449 Activity in Experimental Tumors 3

Model	OR-449[1]	%TGI[2]	Gene exp[3]	Steroid response[4]	Patient info
R2C Rat Leydig tumor CDX	3 10 30	60 80 100	Yes	N.D.	Cell line available from ATCC
SJ-ACC3 Pediatric ACC PDX	30	105	Yes	N.D.	11 yo male; primary tumor; SF-1 locus amplification: yes; TP53: G245C
SW1939 Pediatric ACC PDX	10 30 100	43 65 74	Yes	Yes	13 yo male; primary tumor; SF-1 locus amplification: unknown; TP53: unknown
AD10272 Adult ACC PDX	30 100	27 32	Yes	Not signif.	50 yo female; primary tumor SF-1 locus amplification: unknown;TP53:R337P
NCI-ZGH Adult ACC PDX	30 100	Not significant	Yes	Yes	39 yo female; lung met; SF-1 locus amplification: unknown TP53: unknown
NCI-ZGI Adult ACC PDX	100	Not determined	Yes	Yes	66 yo female; aortocaval met SF-1 locus amplification: unknown;TP53: unknown

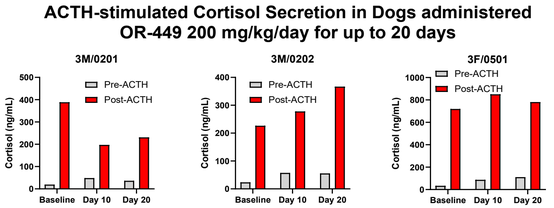
1. Dose, mg/kg/day
2. Percent tumor growth inhibition (TGI) relative to vehicle controls
3. A selection of SF-1 antagonist-regulated genes identified in SJ-ACC3 short-term cultures by RNAseq were selected for PDX studies. R2C target genes were identified in culture with the probe antagonists OR-907S & R.
4. Serum tumor-derived steroids identified by LC-MS/MS after 26-28 days dosing.

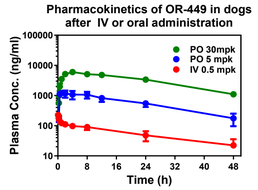
Comparison of OR-449 Gene Regulation Across Models 5



- Tumors were analyzed for SF-1 antagonist-responsive genes by qPCR after 7 days of dosing with OR-449.
- Genes that were upregulated by OR-449 in SJ-ACC3 tumors were also responsive to OR-449 in SW1939 and in two adult ACC PDXs (NCI-ZGI and NCI-ZGH).

Pharmacokinetics of OR-449 & Effects on Cortisol Release 7



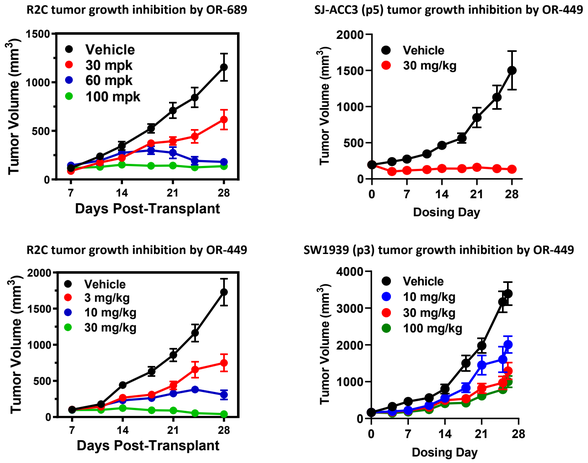


Cortisol release: Dogs were treated daily with 200 mg/kg **OR-449** for 21 days by oral gavage. On day -1, day 10 and day 20, cortisol levels were measured before and 1 h after injection of 5 μg/kg (i.v.) of $ACTH_{1-24}$. Treatment with OR-449 did not significantly change the level of cortisol release.

Pharmacokinetics: The half-life of **OR-449** in dogs is >18 h and bioavailability is ~100% at 30 mg/kg.

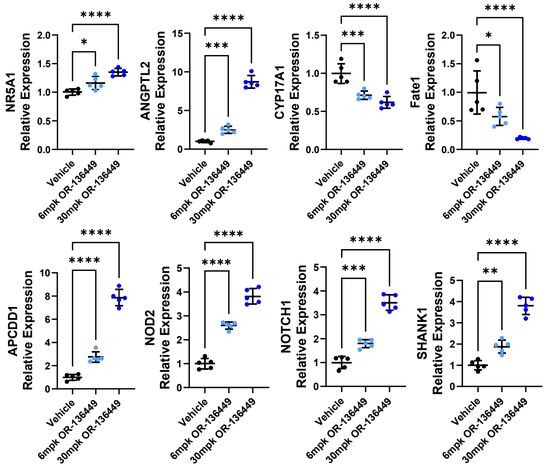
We predict that OR-449 can be dosed once daily in clinical studies and that the effects on cortisol release by the adrenal gland may be limited.

Regulation of Tumor Growth by SF-1 Antagonists 2



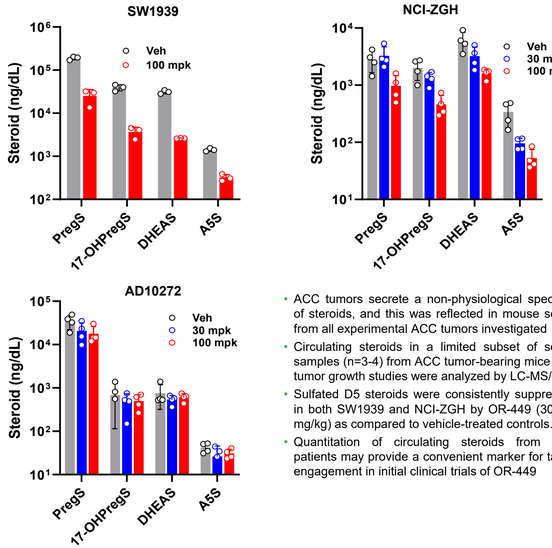
Daily oral dosing of OR-689 or OR-449 in immunocompromised mice implanted subcutaneously with dissociated cells from early passage tumors (SJ-ACC3 p5 or SW1939 p3) or cultured cells (R2C) in Matrigel was initiated when average tumor size was in the range of 100-300 mm^3. No significant change in body weight or appearance occurred in treated mice in the SJ-ACC3 or SW1939 studies relative to vehicle controls. In separate studies (not shown), we observed that OR-449 inhibition of SJ-ACC3 DNA synthesis in dissociated cultures declined very significantly with increasing passage number beyond passage 5.

OR-449 Regulates Gene Expression in SJ-ACC3 Tumors 4



- SF-1 antagonist-regulated genes were initially identified in short term cultures of dissociated SJ-ACC3 cells by RNAseq after 3 days of treatment with 1 μM OR-907S vs its inactive enantiomer, OR-907R.
- The most highly differentially regulated genes (both up and down) were selected to assess target engagement in SJ-ACC3 tumor-bearing mice after 7 days of dosing with OR-449 (6 or 30 mg/kg/day).
- Tumors were collected 24 hours after the last dose and gene expression was evaluated by qPCR using B2M as a reference gene. Data shown are mean±SD, N = 5/group.

OR-449 Regulates Tumor Steroid Secretion *In Vivo* 6



- ACC tumors secrete a non-physiological spectrum of steroids, and this was reflected in mouse serum from all experimental ACC tumors investigated
- Circulating steroids in a limited subset of serum samples (n=3-4) from ACC tumor-bearing mice from tumor growth studies were analyzed by LC-MS/MS
- Sulfated D5 steroids were consistently suppressed in both SW1939 and NCI-ZGH by OR-449 (30-100 mg/kg) as compared to vehicle-treated controls.
- Quantitation of circulating steroids from ACC patients may provide a convenient marker for target engagement in initial clinical trials of OR-449

Summary 8

- SF-1 antagonists block tumor growth in a rat Leydig cancer cell line and two PDXs derived from pediatric ACC at early passage in mouse.
- There was evidence of OR-449 tumor target engagement (gene expression and/or steroid secretion) in all tumors tested regardless of growth response.
- OR-449 fails to inhibit cortisol release in response to ACTH stimulation in dogs.
- IND-enabling studies for OR-449 are nearing completion.

Favorable Safety Profile

- No serious adverse events in 28-day GLP tox studies up to 200 mg/kg/day
- No evidence of adrenal insufficiency
- Anticipated clinical starting dose: 100 mg QD

Small Molecule CMC & PK

- Clinical solid oral dosage form: 50 & 200 mg immediate release tablets
- Long plasma half-life in preclinical species
- Predicted human PK consistent with once-daily dosing

Acknowledgements

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