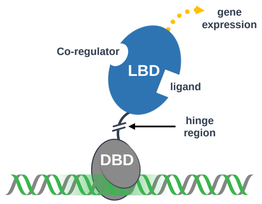
A Novel Steroidogenic Factor-1 Antagonist, OR-449, as a Targeted Therapy for Adrenocortical Cancer

Paul D. Crowe, PhD[1], Ray Fox, PhD[1], Haiyan Tao, PhD[1], Emily Eastwood, PhD[1], Neil Raheja, PhD[1], Raul Ribeiro, MD[2], Scott Thacher, PhD[1], [1]Orphagen Pharmaceuticals, San Diego, CA, USA, [2]St. Jude Children's Research Hospital, Memphis, TN.

Introduction

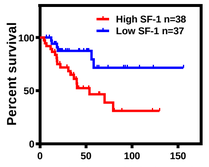
- Nuclear receptors (NRs) are a successful small molecule target class. NRs bind DNA & control tissue-specific gene transcription via small molecule ligand binding to a specific pocket in the ligand-binding domain (LBD) (**Figure 1**)
- The orphan nuclear receptor SF-1 (NR5A1) is required for adrenal differentiation[1]
- SF-1 is amplified at the chromosomal level in pediatric adrenocortical cancer (ACC) and SF-1 is identified as the major transcription factor in adult ACC[2,3]
- Relatively higher SF-1 expression (protein and mRNA) in an adult ACC tumor is correlated with worse patient survival[4] (**Figure 2**)
- SF-1 has a small molecule binding pocket that can be occupied by phospholipids. A coactivator peptide binding site is created simultaneously[5]
- We have identified small molecules that specifically block transcription through the SF-1 LBD
- Among these, OR-449 is potent, metabolically stable, orally bioavailable, and suitable for further development

Figure 1: NR control of gene expression



LBD: ligand-binding domain;
DBD: DNA-binding domain

Figure 2: SF-1 expression in ACC

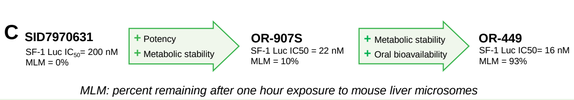


Kaplan-Meier analysis (Log-Rank, p < 0.001) of adult ACC patients expressing high or low levels of tumor SF-1 mRNA (from the Cancer Genome Atlas (TCGA))

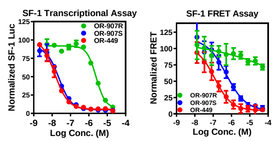
OR-449: a Specific Antagonist of SF-1

- Assay methodology: Transcription from SF-1 ligand-binding domain (**Figure 3A**) and coactivator recruitment assay based on FRET signal between terbium-anti-GST-labeled SF-1 LBD and streptavidin (SA)-D2-labeled co-regulator peptide (**Figure 3B**)
- Discovery of OR-449: Initial hits were identified by HTS.[6] Medicinal chemistry at Orphagen identified OR-907S, a potent antagonist with limited metabolic stability, and subsequently OR-449 (**Figure 3C, 3D**)
- Ligand pharmacology: OR-907R and OR-907S are enantiomers, one inactive, one active. Relative potencies are comparable in transcription and FRET assays (**Figure 3D**)
- OR-449 is inactive at a panel of nuclear receptor transcriptional assays (**Figure 3E**)

Figure 3: OR-449 Assay Methodology, Discovery, and Pharmacology



MLM: percent remaining after one hour exposure to mouse liver microsomes



E

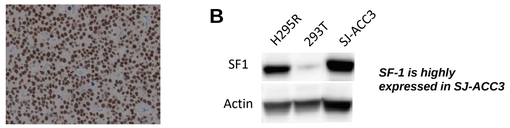
Receptor	OR-449 IC_{50} µM
LRH-1	>3.2
AR	>3.2
GR	>3.2
PPARα	>3.2
PPARβ	>3.2
PPARγ	>3.2
LXRα	>3.2
LXRβ	>3.2
FXR	>3.2

Receptor	OR-449 IC_{50} µM
RARα	>3.2
RARβ	>3.2
RARγ	>3.2
RORα	>3.2
RORβ	>3.2
RORγ	>3.2
RXRα	>3.2
ERRα	>3.2
ERRβ	>3.2

SF-1 Expression in SJ-ACC3 Patient-derived Xenograft (PDX)

Expression of SF-1 in SJ-ACC3 xenograft tumors[7] (from St. Jude, Memphis) was determined by IHC (**Figure 4A**) and immunoblotting (**Figure 4B**) using the N1665 anti-SF-1 antibody. H295R, an ACC cell line, and 293T cells are positive and negative controls, respectively.

Figure 4: SF-1 Expression in SJ-ACC3 PDX

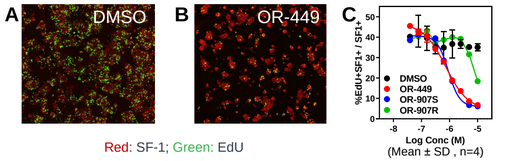


OR-449 Inhibits ACC Cell Proliferation

SJ-ACC3 cells were cultured with 0.1% DMSO (**Figure 5A**) or 2.5 µM OR-449 (**Figure 5B**) for 5 d. Proliferating cells were labeled with EdU (green) and anti-SF-1 (red).

SF-1+EdU+ cells were quantified by high-content imaging, expressed as the percentage of SF-1+ cells that are also EdU+ (**Figure 5C**). OR-449 and OR-907S are equally active, as in **Figure 3D**.

Figure 5: OR-449 vs. DMSO in SJ-ACC3 Cells



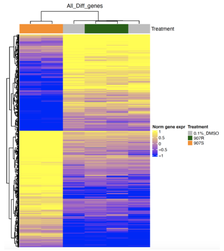
Red: SF-1; Green: EdU

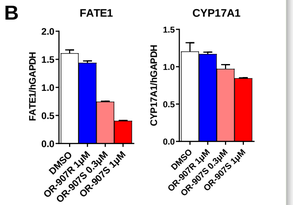
(Mean ± SD , n=4)

Regulation of Gene Expression by SF-1 Antagonist In Vitro

- SJ-ACC3 cells were cultured with 0.1% DMSO, 1 µM OR-907S, or 1 µM OR-907R for 3 days (N=2/group). Gene expression was determined by RNA sequencing. Heatmap analysis (**Figure 6A**) showed that gene expression patterns for DMSO and 1 µM OR-907R (inactive enantiomer) are similar and differ significantly from OR-907S (active enantiomer)
- Gene pathway analysis (not shown) demonstrates that OR-907S treatment affects multiple cancer-related pathways (p<0.001), such as extracellular matrix organization, epithelial-mesenchymal transition (EMT); interferon signaling; and hypoxia signaling
- qPCR results showing dose-dependent regulation of SF-1 target genes FATE1 (left) and CYP17A1 (right) by OR-907S but not OR-907R (**Figure 6B**). FATE1 encodes a tumor-associated cancer/testis antigen that has been implicated in apoptosis and response to chemotherapy in ACC cells. CYP17A1 encodes a key steroidogenic enzyme.

Figure 6: OR-907R, OR-907S and DMSO SJ-ACC3 gene expression





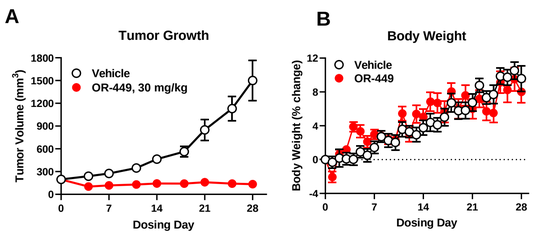
Heatmap of SJ-ACC3 gene expression Z score for 862 genes up- or down-regulated by 1 µM OR-907S), relative to the mean for all samples, based on RNAseq after 3 d exposure in culture.

OR-449 Inhibits ACC Tumor Growth In Vivo

SJ-ACC3 cells (5x10[5]) were resuspended in HBSS:Matrigel (1:1, v/v) and implanted subcutaneously in the right flank of male CB.17 SCID mice. Mice were randomly assigned to groups of 12 bearing similarly sized tumors. The mean tumor volume at the start of dosing (23 days post implantation) was 200mm[3]. Mice were dosed with OR-449 or vehicle by daily oral gavage for 28 days.

OR-449 (30 mg/kg) completely suppressed tumor growth compared to vehicle-treated mice (**Figure 7A**). OR-449 was well-tolerated as demonstrated by similar increase in body weight compared to vehicle mice (**Figure 7B**)

Figure 7: ACC Tumor Growth In Vivo



Tumor volume was calculated as: (length x width2) x 0.5. Mean tumor volume at the start of dosing (23 days post implantation) was 200mm[3]. Data shown are mean ± SEM. (n=12/group)

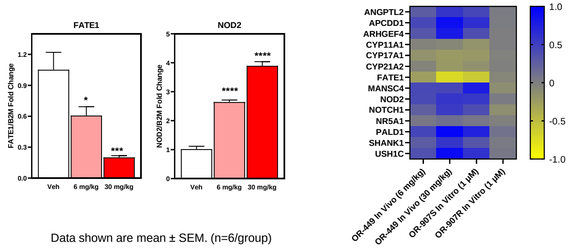
Regulation of Gene Expression by OR-449 In Vivo

SJ-ACC3 tumor-bearing mice were randomly assigned to groups of 5 bearing similarly sized tumors. The mean tumor volume at the start of dosing (41 days post implantation) was 400mm[3]. Mice were dosed with OR-449 (6 or 30 mg/kg, PO) for 7 days. Tumor mRNA was isolated on Day 8.

Gene expression (**Figure 8A**) and a heat map (**Figure 8B**) of up- and down-regulated genes in SJ-ACC3 tumors in comparison with selected genes regulated by 1 µM OR-907S in SJ-ACC3 tumor cells *in vitro*. Genes were selected from the *in vitro* analysis (Figure 6A).

Gene regulation by OR-449 in SJ-ACC3 tumors *in vivo* follows a similar pattern as gene regulation by OR-907S treatment in SJ-ACC3 cells *in vitro*, supporting the hypothesis that OR-449 is regulating tumor gene expression through SF-1.

Figure 8: Gene Regulation by OR-449 In Vivo

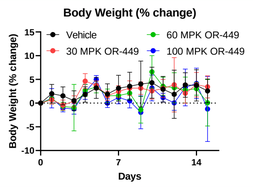


Data shown are mean ± SEM. (n=6/group)

OR-449 is Well-tolerated in Mice

- Male C57BL/6J mice, n=6/group
- OR-449 (30, 60 or 100 mg/kg) or vehicle, PO for 14 days
- Body weight, general health – normal (**Figure 9**)
- Clinical chemistry (Complete blood count, electrolytes, liver enzymes) – normal
- Terminal organ weights (incl. prostate, seminal vesicles, epididymis) – normal
- Histology – normal

Figure 9: Body Weight Maintained



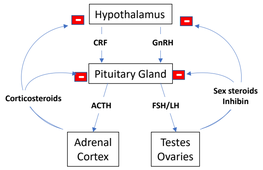
OR-449 Does Not Affect Adrenal or Gonadal Axes

Male Sprague-Dawley rats, n=6/group were dosed with vehicle or OR-449 at 60 mg/kg, PO for 14 days. On Day 14, basal (evening) ACTH, LH and FSH were measured by ELISA. On Day 15 rats were challenged with ACTH (0.3 µg/g, IP). Plasma collected 1.5 hr after ACTH challenge was assayed for corticosterone by ELISA (**Figure 10**).

Figure 10: ACC Tumor Growth In Vivo

	Vehicle	OR-449	P<0.05
Basal			
ACTH, ng/mL	0.7 ± 0.4	0.8 ± 0.3	No
LH, ng/mL	126 ± 11	130 ± 11	No
FSH, ng/mL	10 ± 3	11 ± 2	No
ACTH-stimulated Corticosterone, ng/mL	1008 ± 166	1006 ± 163	No

Data are mean ± SD (n=5-6)



Conclusions

- OR-449, a specific antagonist of SF-1, inhibits proliferation of SJ-ACC3 tumor-derived cells in culture and completely blocks SJ-ACC3 growth as a xenograft
- OR-907S responsive genes identified in SJ-ACC3 cells in culture track with OR-449 responsive genes in SJ-ACC3 tumors at doses that produce a tumor growth response, consistent with SF-1 acting as the drug target for OR-449-mediated control of growth
- OR-449 is generally safe in rodents at 2-3x its efficacious dose of 30 mg/kg. Adrenal and gonadal function are not inhibited
- OR-449 is a potential oral therapy for ACC . Medical therapy for ACC is limited. About two-thirds of patients become metastatic and their 5-year survival is <15%[8]

References & Acknowledgements

1. Schimmer & White, Mol. Endocrinol. 24:1322, 2010
2. Pianovski, et al., Eur. J. Cancer 42:1040, 2006
3. Corces, et al., Science 362:eaav1898, 2018
4. Sbiera, et al., J. Clin. Endo. Metab. 95:E161, 2010
5. Blind, et al., Proc Natl Acad Sci USA 111:15054, 2014
6. Madoux et al; Mol. Pharmacol. 73:1776, 2008
7. Pinto, et al., Clin. Cancer Res. 19:1740, 2013
8. Ayala-Ramirez, et al., Eur J Endocrinol 169:891-9, 2013

The authors acknowledge the support of the following NIH grants: R43 DK 102221, R43 CA 150540, R43 HD 068078, R43 CA 099875 and generous advice from Emilia Pinto and Christopher Morton at St. Jude on growth of the SJ-ACC3 PDX.