IN VITRO EVALUATION OF PROTAC® ANDROGEN RECEPTOR DEGRADER ARV-766 FOR CYTOCHROME P450- AND TRANSPORTER-MEDIATED DRUG-DRUG INTERACTION

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Objective

- The purpose of this *in vitro* study was to assess the potential of ARV-766 as a perpetrator and victim to cause cytochrome P450 (CYP) and transporter-mediated drug-drug interactions (DDI), based on regulatory guidance (1, 2).

Key Findings

- ARV-766 at concentrations ranging from 0.03 to 30 µM did not induce mRNA of CYP1A2, 2B6, 2C9, and 2C19 for all three donors of human hepatocytes. An induction of CYP3A4 mRNA was observed with a maximal 2.5−8.8-fold (5-6% of positive control response) at 0.1 µM and 0.3 µM across three donors. A 3.1-fold (28%) induction of CYP2C8 mRNA was found in one donor (**Table 1** and **Figure 1**).
- No direct or time-dependent inhibition was observed for any of the CYP isoforms tested after incubating human liver microsomes (HLM) with ARV-766 at concentrations of up to 15 µM (**Table 2**).
- ARV-766 was relatively stable in conventional incubations with HLM. An up to 23% loss of parent was seen in recombinant CYP3A5. Hydrolysis was the major metabolic pathway. Other minor pathways included oxidation, de-alkylation, and demethylation, which combined represent <2% of total abundance (**Table 3**).
- ARV-766 exhibited low permeability in Caco-2 cell monolayers and therefore ARV-766 as Pgp and BCRP substrate was not reliably determined. It inhibited Pgp in vesicle assays with an IC_{50} value of 0.23 µM but the inhibition was not observed in the MDCKII bidirectional assays. ARV-766 inhibited BCRP in both monolayer and vesicle assays with IC_{50} values of 0.21 µM and 1.55 µM, respectively (**Table 4** and **Figure 2**).
- ARV-766 was not a substrate for OATP1B1 and OATP1B3. It did not cause >50% inhibition for all the uptake transporters up to 3.75 µM tested except for a up to 52% inhibition of MATE1 with a EC_{50} value of 3.05 µM (**Table 5**).

Conclusions

- These data demonstrate that ARV-766 has a low potential to cause significant DDI via inhibition of CYP enzymes or DDI of uptake transporters.
- Clinical DDI studies with CYP3A inhibitors and inducers, and Pgp and BCRP substrates are being investigated.

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Background

- Prostate cancer is the second leading cause of cancer death in men in the US(3).
- ARV-766, is an orally bioavailable PROTAC® androgen receptor (AR) degrader and currently is being developed for the treatment of metastatic castration-resistant prostate cancer in a phase 1/2 clinical trial(4).
- The potential of ARV-766 to cause drug-drug interaction via CYPs and transporters *in vitro* has not been reported previously.

Methods

- **CYP Induction:** The induction potential of ARV-766 on CYP enzymes was assessed in cryopreserved human hepatocytes from three donors. Following treatment with ARV-766 at concentrations of 0.03-30 µM for 48 h, mRNA levels for CYP1A2, 2B6, 2C8, 2C9, 2C19, and 3A4 were determined by semiquantitative real-time PCR. In addition, cytotoxicity was tested prior to the induction assay. Test article concentrations during 24 hr incubation were determined.
- **CYP Inhibition:** The potential of ARV-766 to cause direct and time-dependent inhibition (TDI) of the activities of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 was evaluated in pooled HLM at 0.2-15 µM. Probe substrate concentrations around Km were used for direct inhibition and initial TDI assays. For TDI assay, ARV-766 was pre-incubated with pooled HLM with and without NADPH for 30 minutes prior to incubation with a single concentration of probe substrate.
- **Metabolism and CYP Reaction Phenotyping:** Metabolite profiling was conducted in incubations of ARV-766 (2 and 10 µM) with HLM (up to 60 min), human hepatocyte suspension (up to 240 min) and human plasma (up to 360 min). In addition, ARV-766 (2 µM) was incubated with recombinant human CYP enzymes CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5.
- **Efflux Transporter Inhibition:** The potential of ARV-766 to inhibit Pgp or BCRP was tested via measuring bidirectional transport of the respective probe substrates in Pgp or BCRP-expressed MDCKII and control cell monolayers at ARV-766 concentrations of 0.07-5 µM. In addition, Pgp and BCRP inhibition were tested in inside-out membrane vesicles prepared from HEK293 cells overexpressing human Pgp and BCRP at 0.01-9 µM ARV-766 in the presence of 4 mM MgATP or MgAMP.
- **Efflux Transporter Substrate:** Whether ARV-766 acts as a substrate for Pgp and BCRP was examined in Caco-2 cells at the concentrations of 0.075, 0.75, 3.75 and 7.5 µM in media containing 1% BSA. After 120 min incubation, the bidirectional permeability of ARV-766 was determined by LC-MS/MS.
- **Uptake Transporter Inhibition:** The potential of ARV-766 to inhibit uptake transporters in MDCKII or HEK293 cells stably expressing, individually, MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3 and OCT2 was tested at concentrations of 0.005-3.75 µM.
- **Uptake Transporter Substrate:** The potential involvement of ARV-766 as a substrate of OATP1B1 and OATP1B3 was assessed in uptake transporter substrate assays at 4 concentrations (0.1, 0.5, 1 and 5 µM). The ARV-766 concentration in cell lysates was determined by LC-MS/MS.

Results

CYP Induction

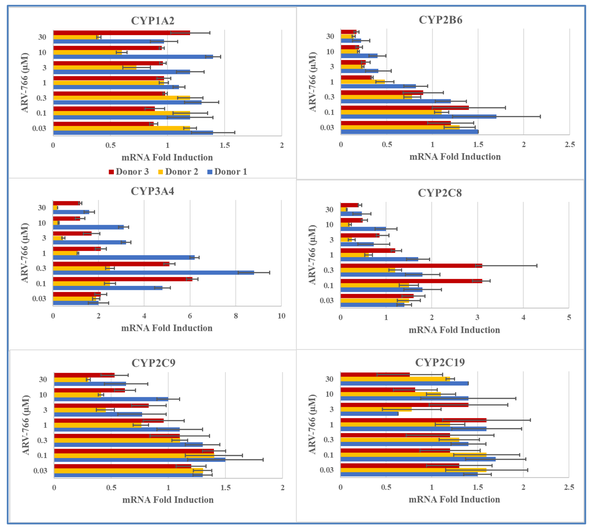
- No significant decrease of hepatocyte viability was found at ARV-766 concentrations tested (0.03-30µM) after 2 days treatment in the MTT assay (data not shown).
- Positive control inducers behaved as expected (Table 1).
- Maximal 2.5−8.8-fold induction in CYP3A4 mRNA was found at 0.1-0.3 µM in all three donors; a 3.1-fold induction of CYP2C8 mRNA was observed in donor 3 only at 0.1 µM. The induction response was attenuated at higher concentrations (Table 1, Figure 1).

Table 1: Effect of ARV-766 on CYP mRNA Expression in Human Hepatocytes

Enzyme	Donor 1		Donor 2		Donor 3		PC*
	Fold	%PC	Fold	%PC	Fold	%PC	Fold
CYP1A2	<2	-	<2	-	<2	-	77-88
CYP2B6	<2	-	<2	-	<2	-	14-25
CYP2C8	<2	-	<2	-	3.1	28	1.6-8.6
CYP2C9	<2	-	<2	-	<2	-	2.0-3.8
CYP2C19	<2	-	<2	-	<2	-	0.93-1.4
CYP3A4	8.8	5	2.5	6	3.3	5	26-149

*PC: positive control inducer, omeprazole for CYP1A2, phenobarbital for CYP2B6, rifampicin for CYP2Cs and 3A4)

Figure 1: Effect of ARV-766 on CYP mRNA Expression in Human Hepatocytes



CYP Inhibition

- Positive control inhibitors demonstrated direct inhibition and TDI for all enzymes tested, with expected IC_{50} values and fold shift (data not shown).
- ARV-766 did not cause direct inhibition (≤12% maximal inhibition) or TDI for all CYPs (≤15%) at concentrations up to 15 µM tested (Table 2).

Table 2: Effect of ARV-766 on Direct and Time-dependent Inhibition of CYP Enzymes in Pooled Human Liver Microsomes

Enzyme	Substrate (µM)	IC_{50} Values (µM)		IC_{50} Shift
		No Pre-incubation	30 min Pre-incubation	Fold
CYP1A2	Phenacetin 50	>15	>15	NA
CYP2B6	Bupropion 50	>15	>15	NA
CYP2C8	Amodiaquine 2	>15	>15	NA
CYP2C9	Diclofenac 5	>15	>15	NA
CYP2C19	S-Mephenytoin 20	>15	>15	NA
CYP2D6	Bufuralol 5	>15	>15	NA
CYP3A	Midazolam 2	>15	>15	NA
CYP3A	Testosterone 50	>15	>15	NA

NA: Not applicable

Metabolism and CYP Reaction Phenotyping

- ARV-766 (2 µM) was stable in incubations with HLM (60 min) (data not shown).
- An up to 23% loss of parent was observed in recombinant CYP3A5 (data not shown). A total of 7 metabolites were detected and hydrolysis was the major metabolic pathway. Other minor pathways included oxidation, de-alkylation, and demethylation, which combined represent <2% of total abundance (Table 3).

Table 3: Metabolite Profile of ARV-766 in Human Liver Microsomes, Human Hepatocyte Suspensions and Human Plasma

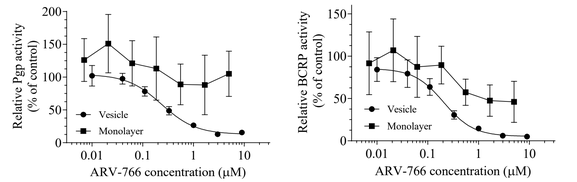
Metabolite Code	Reaction	% Parent at 0 Min		
		HLM at 60 min	HH at 240 min	HP at 360 min
ARV766 (Parent)	N.A.	92.6	86.9	77.0
M334/1	De-alkylation	0.24	0.28	-
M505/1	De-alkylation +Oxidation	0.65	1.23	-
M509/1	De-alkylation +Oxidation +Reduction +De-methylation	0.04	-	-
M521/1	De-alkylation +Oxidation	0.02	-	-
M793/1	De-methylation	0.05	-	-
M823/1	Oxidation	0.31	0.52	-
M825/1	Hydrolysis	-	-	42.3

*HLM: human liver microsomes; HH: human hepatocyte; HP: human plasma

Efflux Transporter Substrate and Inhibition

- Positive control probe substrates and inhibitors confirmed functional assay systems (data not shown).
- Caco-2 monolayer assay indicated that ARV-766 exhibited low permeability (data not shown). Due to its low permeability, ARV-766 as a substrate for Pgp and BCRP could not be reliably determined.
- ARV-766 inhibited Pgp in the vesicle assays with IC_{50} value of 0.21 µM but inhibition was not observed in the monolayer assays. ARV-766 exhibited BCRP inhibition with IC_{50} value of 0.21 and 1.55 µM in the vesicle and monolayer assays, respectively (Table 4, Figure 2).

Figure 2: Pgp and BCRP Inhibition by ARV-766 in MDCKII Cell Monolayer and Vesicles Expressing Pgp and BCRP



Data are the mean ± standard deviation from triplicate samples.

Table 4: Pgp and BCRP Inhibition by ARV-766

Transporter	Assay Type	Substrate (µM)	IC_{50} Values (µM)	
			Maximal % Inhibition	IC_{50} Values (µM)
Pgp	Monolayers	Digoxin (5)	<20	>5
	Vesicles	NMQ (1)	87	0.23
BCRP	Monolayers	Prazosin (1)	54	1.55
	Vesicles	Rosuvastatin (1)	95	0.21

*NMQ: N-methyl-quinidine

Uptake Transporter Substrate and Inhibition

- Probe substrates and inhibitors demonstrated expected uptake activity and inhibition for each transporter (data not shown)
- Accumulation of ARV-766 was similar in the OATP1B1/1B3-expressing and control cells (fold accumulations < 2), indicating no active accumulation of ARV-766 under the conditions tested (data not shown).
- ARV-766 did not cause >50% inhibition for all the uptake transporters up to 3.75 µM tested except for a up to 52% inhibition of MATE1 with a EC_{50} value of 3.05 µM (Table 5). The test with higher concentrations was limited by solubility.

Table 5: Uptake Transporter Inhibition by ARV-766

Transporter	Substrate (µM)	IC_{50} Values (µM)	
		Maximal % Inhibition	IC_{50} Values (µM)
OATP1B1	Rosuvastatin (1)	37	>3.75
OATP1B3	Rosuvastatin (1)	22	>3.75
OAT1	Tenofovir (5)	19	>3.75
OAT3	E3S (1)	13	>3.75
OCT2	Metformin (10)	28	>3.75
MATE1	Metformin (10)	52	3.05
MATE2-K	Metformin (10)	44	>3.75

*E3S: Estrone-3-sulfate

References

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