IN VITRO EVALUATION OF PROTAC® DEGRADER ARV-110 (BAVDEGALUTAMIDE) 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-110 as a perpetrator to cause cytochrome P450 (CYP) and transporter-mediated drug-drug interactions (DDI), based on regulatory guidance (1, 2).

Key Findings

- ARV-110 at concentrations ranging from 0.01 to 3 μM did not induce mRNA of CYP1A2, 2B6, 2C8, 2C9, and 2C19 for all three lots of human hepatocytes. A slight induction of CYP3A4 mRNA was observed with a maximal 2.8-3.3-fold (1-2% of positive control response) at 0.1 μM and 0.03 μM across hepatocyte lots (**Table 1**).
- No direct or time-dependent inhibition was observed for any of the CYP isoforms tested after incubating human liver microsomes (HLM) with ARV-110 at concentrations of 0.013-15 μM except for a >2.5-fold shift after a 30 min-preincubation with an IC_{50} value of 6.0 μM for CYP2C8 (**Table 2**, **Figure 1**), but this time-dependent inhibition was reversible (**Figure 1**).
- ARV-110 exhibited low permeability in MDCK II cell monolayers and the inhibition of BCRP and Pgp was not observed in the MDCKII bidirectional assays up to 3 μM. In contrast, ARV-110 inhibited BCRP and Pgp in the vesicle assays in a concentration-dependent fashion, with IC_{50} values of 0.12 μM and 0.19 μM, respectively. ARV-110 also inhibited BSEP in the vesicle assays with IC_{50} of 0.10 μM (**Table 3**, **Figures 2-4**). ARV-110 did not inhibit any of the uptake transporters up to 15 μM tested (**Table 4**).

Conclusions

These data demonstrate that ARV-110 has a low potential to cause significant DDI via modulation of CYP enzymes or inhibition of uptake transporters. Clinical DDI studies with Pgp and BCRP substrates are under investigation.

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Background

- Prostate cancer is the second leading cause of cancer death in men in the US.
- ARV-110, also known as bavdegalutamide, is the first orally bioavailable PROTAC® degrader of androgen receptor (AR) and currently is being developed for the treatment of prostate cancer in a phase 2 clinical trial.
- ARV-110 has been generally well tolerated in human and may cause drug-drug interaction in patients taking concomitant rosuvastatin (3).
- The Potential of ARV-110 to cause drug-drug interaction via CYPs and transporters as a perpetrator *in vitro* has not been reported.

Methods

- **CYP Induction:** The induction potential of ARV-110 on CYP enzymes was assessed in cryopreserved human hepatocytes from three donors. Following treatment with ARV-110 at concentrations of 0.01-3 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 and concurrent with the induction assay.
- **CYP Inhibition:** The potential of ARV-110 to cause direct and time-dependent inhibition (TDI) of the activities of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 was evaluated in pooled human liver microsomes (HLM) at 0.015-15 μM. Prober substrate concentrations around Km were used for direction inhibition and initial TDI assays. A 30 min preincubation time in the presence of NADPH was conducted prior to the addition of probe substrates. A follow-up 10-fold dilution was performed to determine if the observed TDI for CYP2C8 is reversible. The assay was done with the probe substrate at 20 μM (the saturated concentration) and ARV-110 (0.05-30 μM).
- **Transporter Inhibition:**
  - Efflux transporters: The potential of ARV-110 to inhibit Pgp or BCRP was tested for bidirectional transport of the probe substrates in Pgp or BCRP-expressed MDCKII and control monolayers at ARV-110 concentrations of 0.004-3 μM. In addition, Pgp, BCR and BSEP inhibition were tested in inside-out membrane vesicles prepared from HEK293 overexpressing human Pgp, BCR and BSEP at 0.004-3 μM AVR-110 in the presence of 4 mM MgATP or MgAMP.
  - Uptake transporters: Uptake experiments were performed using MDCKII or HEK293 cells stably expressing the respective uptake transporters. Cells were preincubated with 1.5 and 15 μM ARV-110 for 30 min. After the preincubation, uptake was performed with the respective probe substrate and ARV-110.

Results

CYP Induction

- No significant decrease of hepatocyte viability was found in all three lots of hepatocytes at all ARV-110 test concentrations (0.01-3μM) after 2 days treatment in the concurrent MTT assay (data not shown).
- Positive control inducers behaved as expected.
- Maximal 2.8 and 3.3-fold induction in CYP3A4 mRNA were found at 0.1 and 0.03 μM in donor 1 and 3, respectively.

Table 1: Effect of ARV-110 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	-	46-62
CYP2B6	<2	-	<2	-	<2	-	11-15
CYP2C8	<2	-	<2	-	<2	-	1.9-11
CYP2C9	<2	-	<2	-	<2	-	1.9-4.4
CYP2C19	<2	-	<2	-	<2	-	0.75-1.8
CYP3A4	2.8	1	<2	-	3.3	2	18-145

*PC: positive control inducer, Omeprazole for CYP1A2, Phenobarbital for CYP2B6, Rifampicin for CYP2Cs and 3A4)

CYP Inhibition

- Positive control inhibitors demonstrated direct and TDI for all enzymes tested, with expected IC_{50} values and fold shift (data not shown).
- ARV-110 did not cause direct inhibition (<15% maximal inhibition) for all CYPs except for a slight direct inhibition (<25% maximal inhibition) and TDI for CYP2C8 with maximal 56% inhibition at 15 μM. The TDI was reversible when tested with a dilution method.

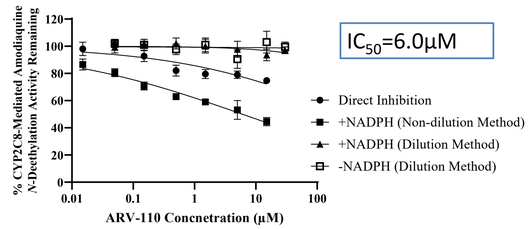
Table 2: Effect of ARV-110 on Direct and Time-dependent inhibition in Pooled Human Liver Microsomes

Enzyme	Substrate	IC_{50} Values (μM)		IC_{50} Shift
	μM	No Pre-incubation	30 min Pre-incubation	Fold
CYP1A2	Phenacetin 50	>15	>15	NA
CYP2B6	Bupropion 50	>15	>15	NA
CYP2C8	Amodiaquine 2	>15	6.0	>2.5
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

References
1. FDA guidance (2020) In vitro Drug Interaction Studies
2. EMA guideline (2013) on the Investigation of Drug Interactions
3. ARV-110 Phase ½ Dose Escalation: Interim Update (2020)

Figure 1: Direct and Time-dependent CYP2C8 Inhibition by ARV-110 in Human Liver Microsomes.



Data are the mean ± standard deviation from triplicate samples. Time dependent incubation was initially performed with a non-dilution method, followed by a 10-fold dilution method with the probe substrate set at ~5 fold >Km.

Efflux Transporter Inhibition

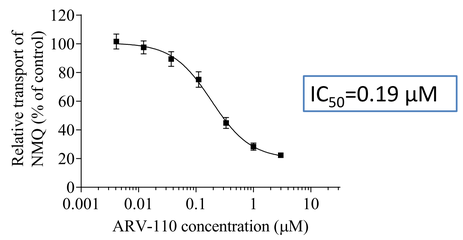
- Positive control probe substrates and inhibitors demonstrated functional assay systems (Data not shown).
- Monolayer assay indicated that ARV-110 exhibited low permeability (Data not shown).
- Concentration-dependent inhibition of Pgp, BCRP and BESEP was observed in the vesicle assays with IC_{50} values of 0.19, 0.12 and 0.10 μM.

Table 3: Effect of ARV-110 on Efflux Transporters in MDCKII or Vesicles Expressing Single Pgp, BCRP and BSEP

Transporter	Assay Type	Substrate (μM)	IC_{50} Values (μM)	
			Maximal % Inhibition	IC50 Values (μM)
Pgp	Monolayers	Prazosin (1)	<20	>3
	Vesicles	NMQ (1)	78 at 3 μM	0.19
BCRP	Monolayers	Digoxin (5)	<20	>3
	Vesicles	Rosuvastatin (1)	89 at 3 μM	0.12
BSEP	Monolayers	-	ND	ND
	Vesicles	Taurocholate (0.2)	88 at 3 μM	0.10

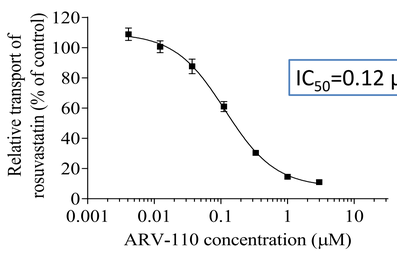
*ND: Not determined

Figure 2: Pgp Inhibition by ARV-110 in Vesicles Expressing Pgp



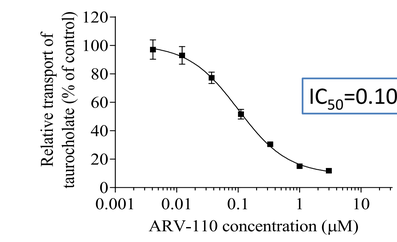
Data are the mean ± standard deviation from three triplicate samples
Abbreviations: NMQ: N-methyl quinidine

Figure 3: BCRP Inhibition by ARV-110 in Vesicles Expressing BCRP



Data are the mean ± standard deviation from triplicate samples

Figure 4: BSEP Inhibition by ARV-110 in Vesicles Expressing BSEP



Data are the mean ± standard deviation from triplicate samples

Uptake Transporter Inhibition

- Probe substrates and inhibitors demonstrated expected uptake activities and inhibitions for each transporter (data not shown)
- ARV-110 did not inhibit all uptake transporters up to 15 μM tested.

Table 4: Effect of ARV-110 on Uptake Transporters in MDCKII or HEK293 Expressing Single Uptake Transporters

Transporter	Substrate (μM)	IC_{50} Values (μM)	
		Maximal % Inhibition	IC50 Values (μM)
OATP1B1	Rosuvastatin (1)	4	>15
OATP1B3	Rosuvastatin (1)	31	>15
OAT1	Tenofovir (5)	20	>15
OAT3	E3S (1)	25	>15
OCT2	Metformin (10)	17	>15
MATE1	Metformin (10)	25	>15
MATE2-K	Metformin (10)	22	>15
NTCP	Rosuvastatin (2)	4	>15