

Sodium Taurocholate Cotransporting Polypeptide Inhibitors ABI-6250 and Bulevirtide Reveal Shared Transcriptome and Cellular Pathway Profiles in Primary Human Hepatocytes

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Background

- Both hepatitis B virus (HBV) and hepatitis D virus (HDV) use sodium taurocholate cotransporting polypeptide (NTCP), a bile acid (BA) transporter expressed on hepatocytes, for viral entry¹⁻³
- Chronic HDV infection (cHDV), the most severe form of viral hepatitis, affects an estimated 12 million patients worldwide^{4,5}
- Patients with cHDV coinfectd with HBV have an increased risk of developing life-threatening liver conditions, such as fibrosis, cirrhosis, and hepatocellular carcinoma compared with patients with HBV alone⁶⁻⁸
- Bulevirtide (BLV), an HBV preS1 peptide inhibitor targeting NTCP, is the only drug currently approved for cHDV and requires daily injections^{2,9}
- ABI-6250 is a first-in-class, orally bioavailable, small-molecule NTCP inhibitor in clinical development to improve dosing convenience and long-term clinical outcomes in patients with cHDV. ABI-6250 has successfully completed a Phase 1a clinical study

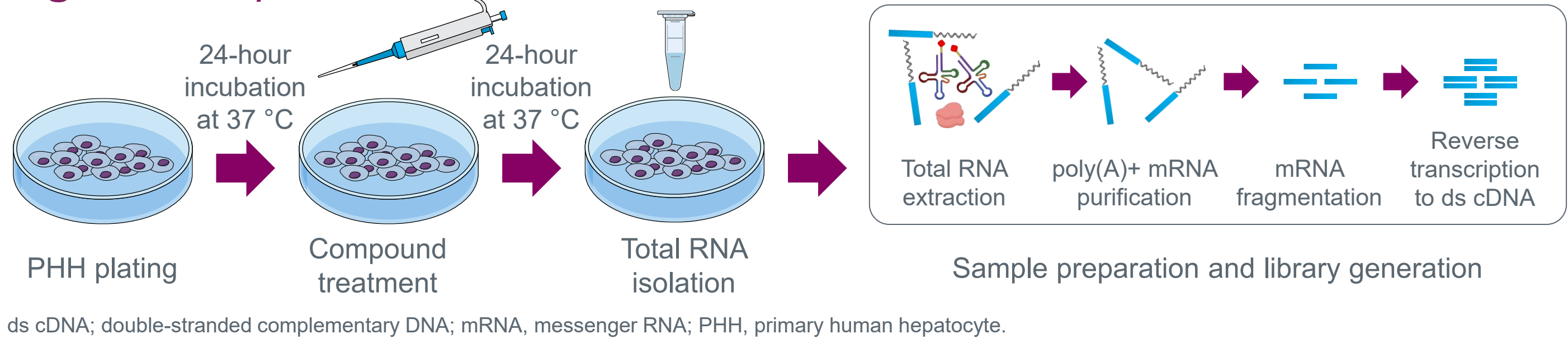
Objective

- To perform transcriptomic profiling of ABI-6250 and BLV in primary human hepatocytes (PHHs)

Methods

- Cell culture (Figure 1):**
 - Cryopreserved PHHs from 10 independent donors were thawed, plated in collagen-coated 12-well plates (300,000 cells/well), and cultured overnight
 - Cells from each donor were treated in quadruplicate for 24 hours with ABI-6250 (1 μ M) or BLV (0.1 μ M), each at approximately 100-fold their respective half-maximal effective concentration values, or with 1% dimethyl sulfoxide (DMSO). Adherent cells were lysed in RNA lysis buffer containing dithiothreitol. Lysates from 2 wells were pooled to generate 2 biological replicates per condition per donor. Samples were submitted to SeqMatic for RNA isolation and RNA sequencing
- Total RNA isolation (Figure 1):**
 - RNA from samples was extracted using a manual column-based Qiagen RNeasy extraction method with RNA quality control (QC) conducted using an Agilent TapeStation RNA assay
- RNA seq (Figure 1):**
 - A sequence-ready library pool was prepared and normalized using DNase treatment and stranded poly(A) messenger RNA (mRNA) library preparation with data demultiplexing
 - FASTQ files were developed via cloud delivery after NovaSeq X sequencing (paired-end 2 $\times$ 150 base pairs)
- Statistical design, gene filtering, and clustering (Figure 1):**
 - Low-abundance genes were filtered, and inter-donor variability was regressed out
 - Differential gene expression analysis was performed using DESeq2. Gene expression profiles of BA transporters and other relevant transporters were visualized using heatmap analysis
 - Gene set enrichment analysis was conducted using the Hallmark gene sets
- PHH donor demographics:**
 - N=10 (A–J, all male); mean age, 31.2 years; mean body mass index, 27.7 kg/m²; HBV/hepatitis C virus, 100% negative

Figure 1. Experimental Procedure



ds cDNA; double-stranded complementary DNA; mRNA, messenger RNA; PHH, primary human hepatocyte.

Results

Table 1. ABI-6250 Potently Inhibits HDV and HBV in an NTCP-Dependent Manner

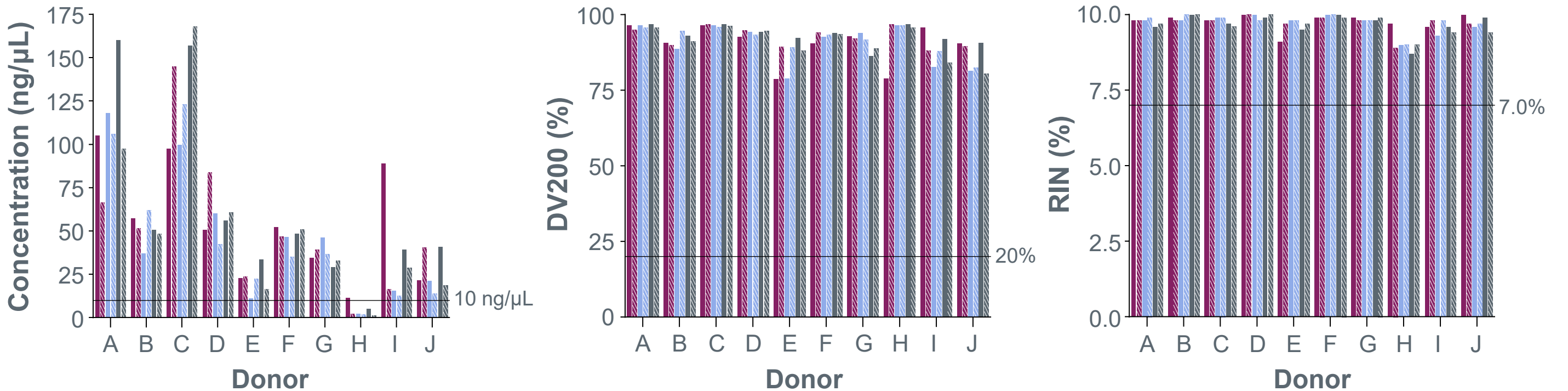
Compound	Antiviral Activity EC ₅₀ (nM)					Bile Acid Uptake Inhibition IC ₅₀ (nM)	
	HDV		HBV				
	Cell Culture Derived		Patient Isolates	Cell Culture Derived			
	PHH	HepG2-NTCP	HepG2-NTCP	PHH	HepG2-NTCP	PHH	Huh7-NTCP
ABI-6250	11 (GT-3B)	5 to 15 (GT-1,2,3,B,D)	21 ^a	14 (GT-A,C,D)	5 (GT-D)	3	8
Bulevirtide	0.6 (GT-3B)	0.5 (GT-3D)	-	0.2 (GT-D)	0.2 (GT-D)	2	5

^aMedian value of N=18 patient isolates. EC₅₀, half-maximal effective concentration; GT, genotype; HBV, hepatitis B virus; HDV, hepatitis D virus; IC₅₀, half-maximal inhibitory concentration; NTCP, sodium taurocholate cotransporting polypeptide; PHH, primary human hepatocyte.

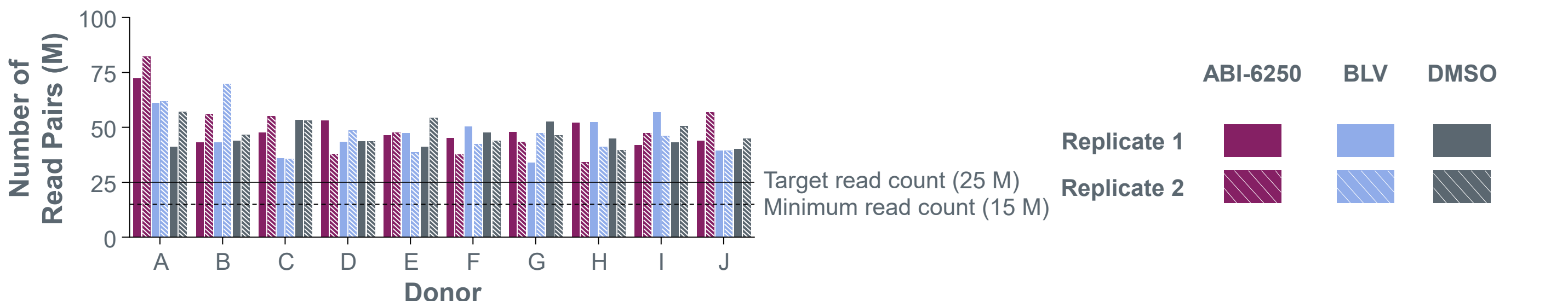
- ABI-6250 specifically inhibits HDV and HBV (Table 1)

Figure 2. QC Confirms Suitable Sequencing Reading Depths

A. RNA QC Metrics



B. Sequencing QC Metrics



BLV, bulevirtide; DMSO, dimethyl sulfoxide; M, million; QC, quality control; RIN, RNA integrity number.

- All samples passed sequencing depth and read-length QC (Figure 2)

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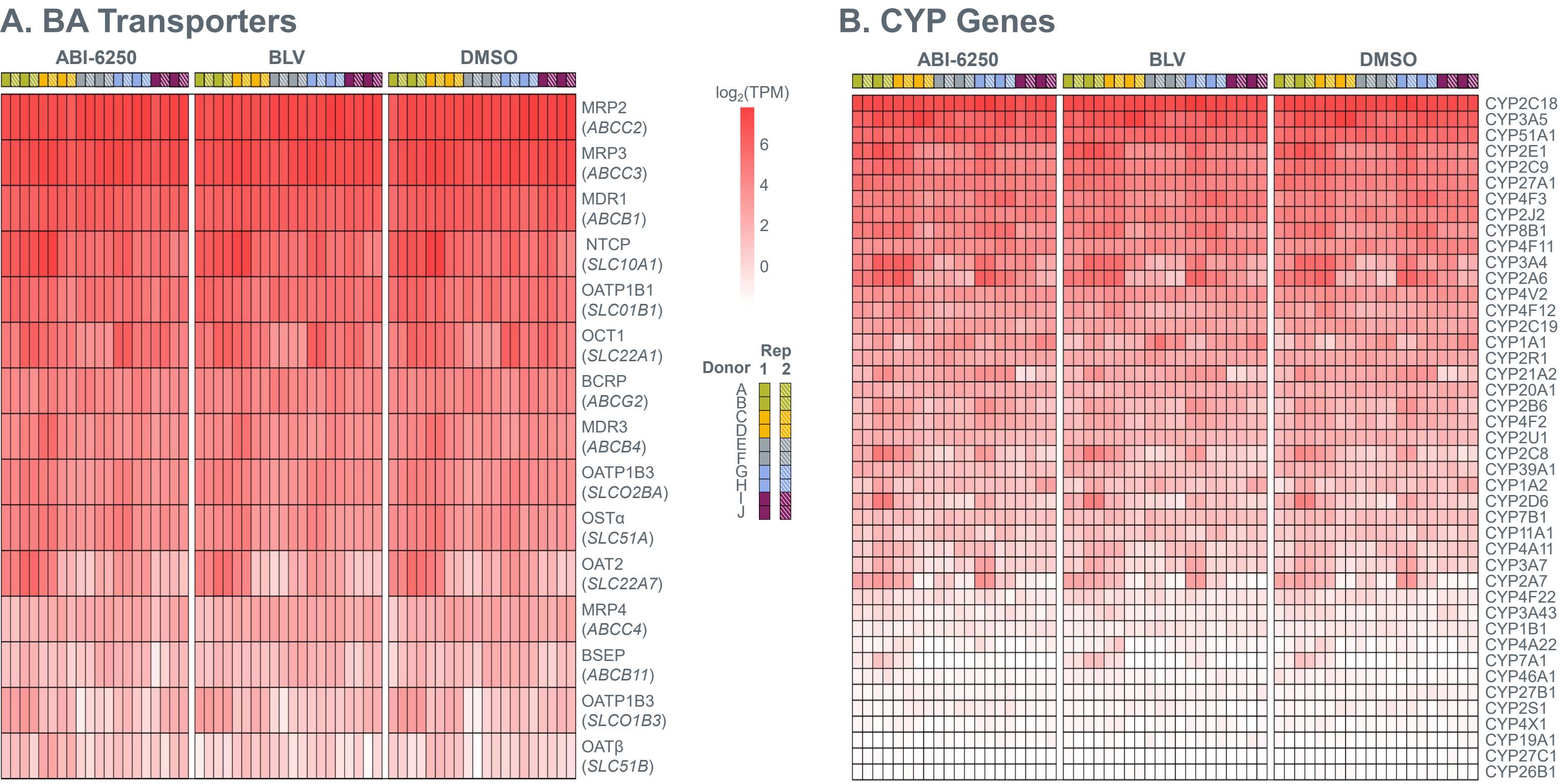
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DISCLOSURES

CL was an employee of Assembly Biosciences, Inc., at the time of the study. All remaining authors are employees of Assembly Biosciences, Inc., and may own stock.

Figure 3. ABI-6250 Treatment Does Not Alter Expression of BA Transporters or CYP Enzymes



For panel A, the protein name is listed first and the gene name is included in parentheses. For panel B, the CYP names are genes and proteins. BA, bile acid; BCRP, breast cancer resistance protein; BLV, bulevirtide; BSEP, bile salt export pump; CYP, cytochrome; DMSO, dimethyl sulfoxide; MDR, multidrug resistance; MRP, multidrug resistance–associated protein; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; OCT, octamer-binding transcription factor; OST, oligosaccharyltransferase; Rep, replicate; TPM, transcripts per million.

- The expression patterns of 15 BA transporter and 43 CYP genes are distinct for each donor (Figure 3)
- Inter-donor variability is more dominant than drug-induced variability

Table 2. ABI-6250 Is a Selective NTCP Inhibitor and Does Not Inhibit Major CYP450 Enzymes

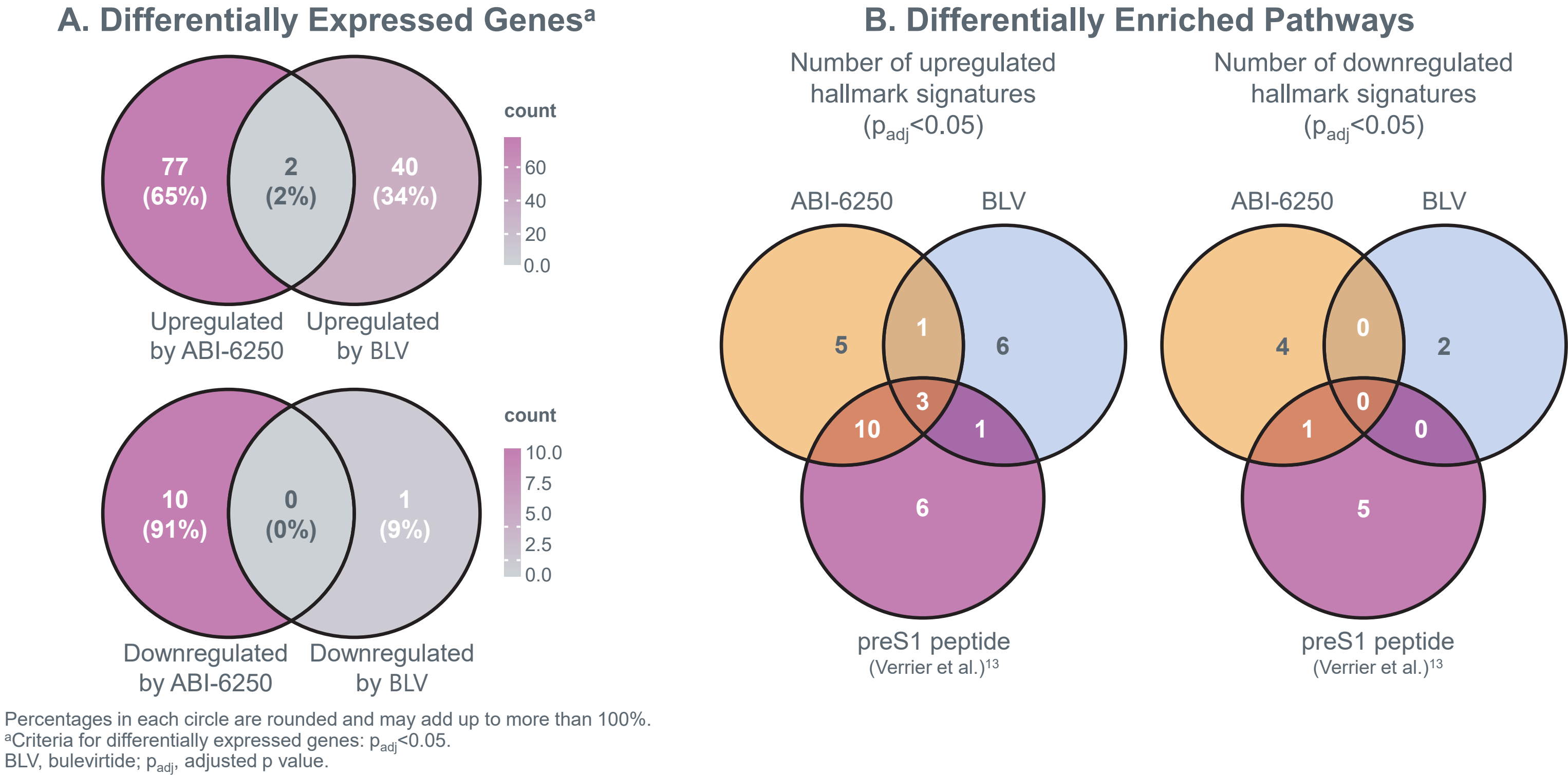
A. Transporters		B. CYP							
Transporter	ABI-6250 Fold Selectivity	ABI-6250 CYP IC ₅₀ (μM) ^a							
NTCP	1								
MRP2	>2400								
MATE1/2K	>1800								
OCT1/2	>1800								
OSTα/β	>1800								
MDR3	>1600								
OAT1	>1200								
OAT2	>1000								
MDR1	>1200								
BSEP	>1200								
MRP3/4	>800								
ASBT	>300								
BCRP	250								
OATP2B1	200								
OATP1B1	75								
OATP1B3	13								

Concentration Range (μM)	ABI-6250 CYP IC ₅₀ (μM) ^a							
	1A2	2B6	2C8	2C9	2C19	2D6	3A4	3A4
0.25 to 50	36.7	19.1	42.5	43.5	>50	>50	42.8	>50

^aIsoform-selective probe substrates are phenacetin (1A2), paxitaxel (2C8), diclofenac (2C9), S-mephenytoin (2C19), bufuralol (2D6), midazolam (3A4), and testosterone (3A4). ASBT, apical sodium-dependent bile acid transporter; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CYP, cytochrome; IC₅₀, half-maximal inhibitory concentration; MATE, multidrug and toxic compound extrusion; MDR, multidrug resistance protein; MRP, multidrug resistance–associated protein; NTCP, sodium taurocholate cotransporting polypeptide; OAT, ornithine aminotransferase; OATP, organic anion transporting polypeptide; OCT, octamer-binding transcription factor; OST, oligosaccharyltransferase.

- ABI-6250 selectively targets NTCP among diverse BA and non-BA transporters (Table 2A)
- ABI-6250 shows limited inhibition of major CYP450 enzymes (Table 2B)

Figure 4. ABI-6250 and BLV Exert Similar Effects on Gene Expression and Modulate Similar Cellular Pathways



Percentages in each circle are rounded and may add up to more than 100%.

^aCriteria for differentially expressed genes: p_{adj}<0.05. BLV, bulevirtide; p_{adj}, adjusted p value.

Table 3. Modest Gene Expression Changes Are Induced by ABI-6250 and BLV

Comparison	Mean (Fold Change)	Max (Fold Change)	Min (Fold Change)
Upregulated by ABI-6250	1.20	1.91	1.05
Downregulated by ABI-6250	0.91	0.96	0.87
Upregulated by BLV	1.31	1.81	1.08
Downregulated by BLV	0.89	0.89	0.89

BLV, bulevirtide; max, maximum; min, minimum.

- Overall, 19 and 11 hallmark pathways in ABI-6250– and BLV-treated PHHs, respectively, are significantly upregulated, with 4 pathways common for both treatments (Figure 4B, left panel). Furthermore, 5 and 2 hallmark pathways in ABI-6250– and BLV-treated PHHs, respectively, are significantly downregulated, with no overlap between the two treatments (Figure 4B, right panel)
- BLV has shown favorable safety, tolerability, and efficacy in patients with cHDV for up to 4 years.¹⁰ ABI-6250 has demonstrated a favorable safety and tolerability profile in preclinical and Phase 1a studies^{11,12}
- Verrier et al performed a hallmark gene set analysis in Huh-7 cells treated for 48 hours with 0.2 μ M HBV preS1 peptide.¹³ In that study, 20 hallmark pathways in HBV preS1 peptide–treated Huh-7 cells were significantly upregulated, 13 of which overlapped with ABI-6250 in PHHs, and 6 hallmark pathways were significantly downregulated by the HBV preS1 peptide, one of which overlapped with ABI-6250 (Figure 4)

Conclusions

- ABI-6250 is a highly potent, specific, orally bioavailable, small-molecule NTCP inhibitor
- At the given experimental conditions, ABI-6250 and BLV exert similar *in vitro* effects on gene expression and cellular pathways, with no large-scale changes in overall gene expression
- ABI-6250 is expected to enter Phase 2 clinical development for cHDV by the end of 2026, followed by Phase 2 studies in chronic autoimmune cholestatic liver diseases, specifically primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), in the first quarter of 2027