Supplemental Material

Drug Metabolism and Disposition

In Vitro Drug-Drug Interaction Evaluation of GalNAc Conjugated siRNAs Against CYP450 Enzymes and Transporters

Author List: Diane Ramsden, Jing-Tao Wu, Brad Zerler, Sajida Iqbal, Jim Jiang, Valerie Clausen, Krishna Aluri, Yongli Gu, Sean Dennin, Joohwan Kim and Saeho Chong

Alnylam Pharmaceuticals, Inc., Cambridge, Massachusetts (DR, JTW, JJ, VC, KA, YG, SD, JK and SC)

The Medicines Company, Parsippany, New Jersey (BZ)

Sanofi, Waltham, Massachusetts (SI)

Supplemental Table 1. rCYP incubation concentrations

Compound	rCYP stock (mg/mL)								[Final incubation] mg/mL
	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP3A5	
revusiran	5.1	6.4	7.9	1.8	3.1	7.1	4.7	9.5	0.5
HBV01	5.1	6.4	7.9	1.8	3.1	7.1	4.7	9.5	0.5
AAT01	5.1	6.4	7.9	1.8	3.1	7.1	4.7	9.5	0.5
fitusiran	5.1	6.4	7.9	1.8	3.1	7.1	4.7	9.5	0.5
cemdisiran	5.1	6.4	7.8	1.8	4.7	5.6	9.2	12	0.6
vutrisiran	5.1	6.4	7.8	1.8	4.7	5.6	9.2	12	0.6
lumasiran	5.1	6.4	7.9	1.8	3.1	7.1	4.7	9.5	0.5

Supplemental Table 2. Donor demographics for induction studies

Lot #	Age	Race	Sex	BMI	Post-thaw viability (%)
QHuf14016	66	Caucasian	Female	NA	93, 96, 93, 87
QHuf14024	55	Caucasian	Female	29	90, 92, 80, 90, 87
QHu0030	30	Caucasian	Male	20.9	94, 86.3
QHum15041	32	Caucasian	Male	23.4	84.2
QHum15015	54	Caucasian	Male	28	83
BHuf16068	61	Caucasian	Female	26.5	90
PHum15065	20	NA	Male	NA	88
QHuf15061	58	Caucasian	Female	25.5	82
QHum15027	43	Caucasian	Male	34	85

Supplemental Table 3. Transporter assay details

Assay type	Transporter	Protein content/well (µg) or cell #/well	Incubation time (min)	Probe Substrate (µM)	Reference inhibitor (µM)
Vesicular inhibition	BCRP	25	1	E3S (1)	Ko134 (1)
	BSEP	50	5	TC (2)	CSA (20)
	P-gp	50	3	NMQ (2)	Verapamil (100)
Uptake	MATE1	1×10^5	15	Metformin (10)	Pyrimethamine (1)
	MATE2-K	1×10^5	15	Metformin (10)	Pyrimethamine (1)
	OATP1B1	1×10^5	3	E217βG (1)	Rifampicin (50)
	OATP1B3	1×10^5	10	CCK-8 (0.1)	Rifampicin (50)
	OAT1	1×10^5	10	tenofovir (5)	Probenecid (200)
	OAT3	1×10^5	3	E3S (1)	Probenecid (200)
	OCT1	1×10^5	20	Metformin (10)	Verapamil (100)
	OCT2	1×10^5	10	Metformin (10)	Verapamil (100)

Supplemental Table 4. rCYP incubation percent of probe substrate remaining after 30 min incubation

Compound	CYP isoform (% remaining)							
	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP3A5
revusiran	14	73	53	BLQ	28	BLQ	0.34	0.062
HBV01	22	61	50	BLQ	35	BLQ	0.071	0.091
AAT01	16	68	55	BLQ	26	BLQ	0.082	0.070
fitusiran	37	68	64	BLQ	36	0.377	0.131	0.104
cemdisiran	42	73	78	BLQ	48	BLQ	0.046	0.139
vutrisiran	16	68	74	BLQ	41	BLQ	0.090	0.134
lumasiran	14	72	74	BLQ	26	BLQ	2.26	0.058

BLQ = below limit of quantitation

Supplemental Table 5. Positive control data from CYP direct inhibition studies

GalNAc-siRNA	IC50 (μM)								
	CYP1A2	CYP2A6	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4/5 (MDZ)	CYP3A4/5 (TST)
	furafylline	tranylcypromine		montelukast	sulfaphenazole	benzynirvanol	quinidine	itraconazole	
cemdisiran	7.3	ND	2.5	0.28	0.19	0.17	0.04	0.04	0.01
HBV01	4.2	ND	3.2	0.22	0.26	0.32	0.05	0.26	0.06
vutrisiran	3.9	ND	3.8	0.25	0.30	0.43	0.05	0.26	0.07
AAT	5.5	ND	3.1	0.36	0.24	0.32	0.05	0.51	0.11
fitusiran	1.4	0.08	2.9	0.46	0.14	15.5	0.07	0.33	0.39
givosiran	5.1	ND	2.2	0.08	0.17	0.18	0.04	0.09	0.03
revusiran	1.8	ND	ND	ND	0.10	11.9	0.07	0.27	0.20
lumasiran	4.3	ND	2.9	0.41	0.28	0.31	0.04	0.37	0.12
inclisiran	4.6	ND	3.0	0.40	0.27	0.32	0.04	0.29	0.18
AGT01	2.5	ND	2.0	0.21	0.09	0.35	0.03	0.74	0.20
AAT02	3.3	ND	1.0	0.20	0.05	0.18	0.02	0.17	0.08
HBV02	9.1	ND	1.9	0.14	0.06	0.24	0.03	0.51	0.23

ND = not determined, isoform not evaluated during the study

Supplemental Table 6. Positive control data from CYP time-dependent inhibition studies

GalNAc-siRNA		IC50 shift						
	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4/5 (MDZ)	CYP3A4/5 (TST)
	furafylline	ThioTEPA	gemfibrozil glucuronide	tienilic acid	ticlopidine	paroxetine	azamulin	
AAT01	3.9	2.8	2.1	1.6	1.9	1.6	2.0	2.0
HBV01	4.1	3.2	3.1	1.7	2.7	1.5	1.6	1.5
givosiran	2.4	5.1	3.3	3.3	2.1	1.6	1.9	2.1
lumasiran	4.5	3.4	3.3	1.7	3.4	1.5	2.1	2.1
cemdisiran	2.2	4.6	2.7	3.2	2.0	1.9	2.2	3.1
vutrisiran	4.1	3.0	2.9	2.0	3.2	1.5	1.7	1.7
inclisiran	4.1	2.8	2.5	1.7	1.9	1.5	1.9	1.5
AGT01	7.8	`11	4.6	3.5	4.4	6.7	2.8	14
HBV02	6.8	12	11	6.4	2.7	5.4	2.3	13
AAT02	6.3	10	11	4.5	2.7	6.7	2.4	12