YMTHE, Volume *25*

Supplemental Information

Clinical Proof of Concept for a Novel Hepatocyte-Targeting GalNAc-siRNA Conjugate

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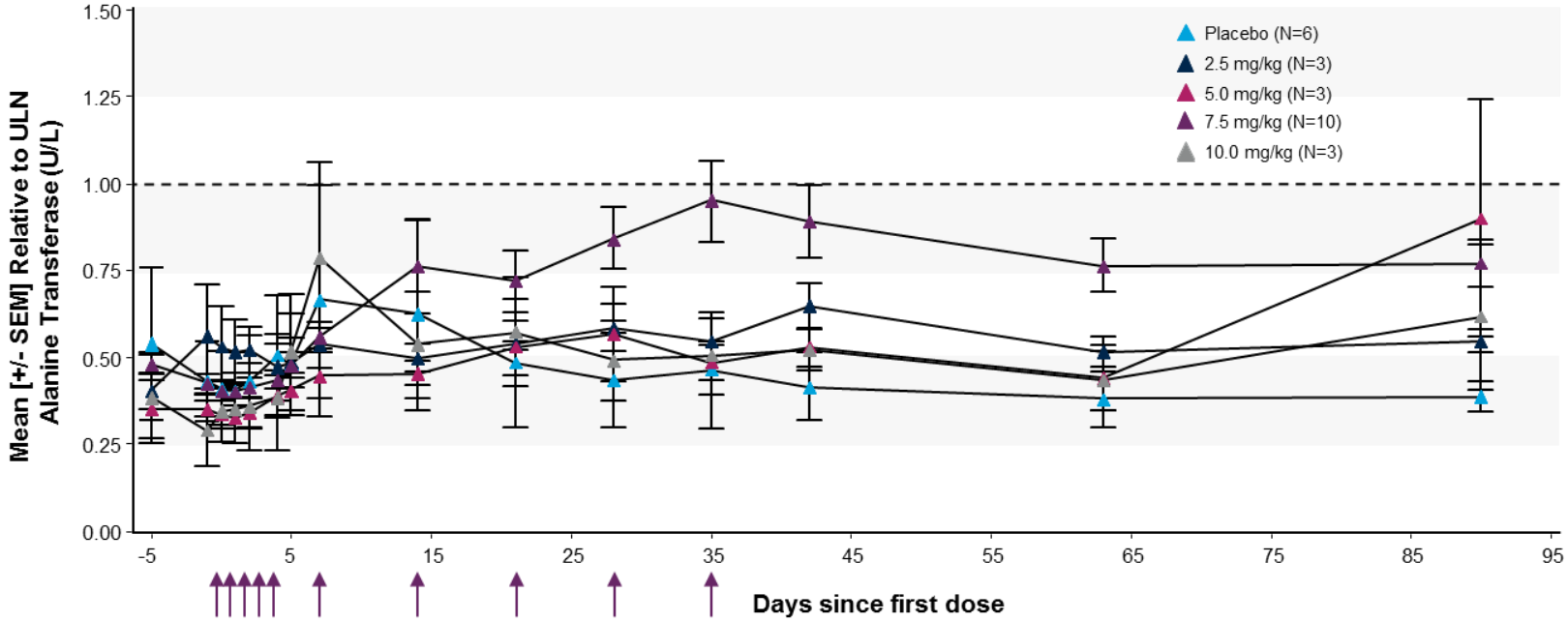
Supplemental Figures and Tables

Figure S1 Mean relative to upper limit of normal (a) alanine transferase and (b) aspartate transferase over time after multiple doses of revusiran. Symbols represent mean ± SEM. Dotted line corresponds to ULN. Arrows indicate days of dosing in the multidose cohorts at 2.5, 5, and 10 mg/kg (Days 0-4 then weekly from Days 7 to 35 [maintenance]). The combined 7.5 mg/kg MAD cohorts were dosed on Days 0-4 then either once weekly (N=7) or once every two weeks (N=3) during the maintenance phase.

SEM=standard error of the mean; ULN=upper limit of normal; U/L=Units/Liter

a



b

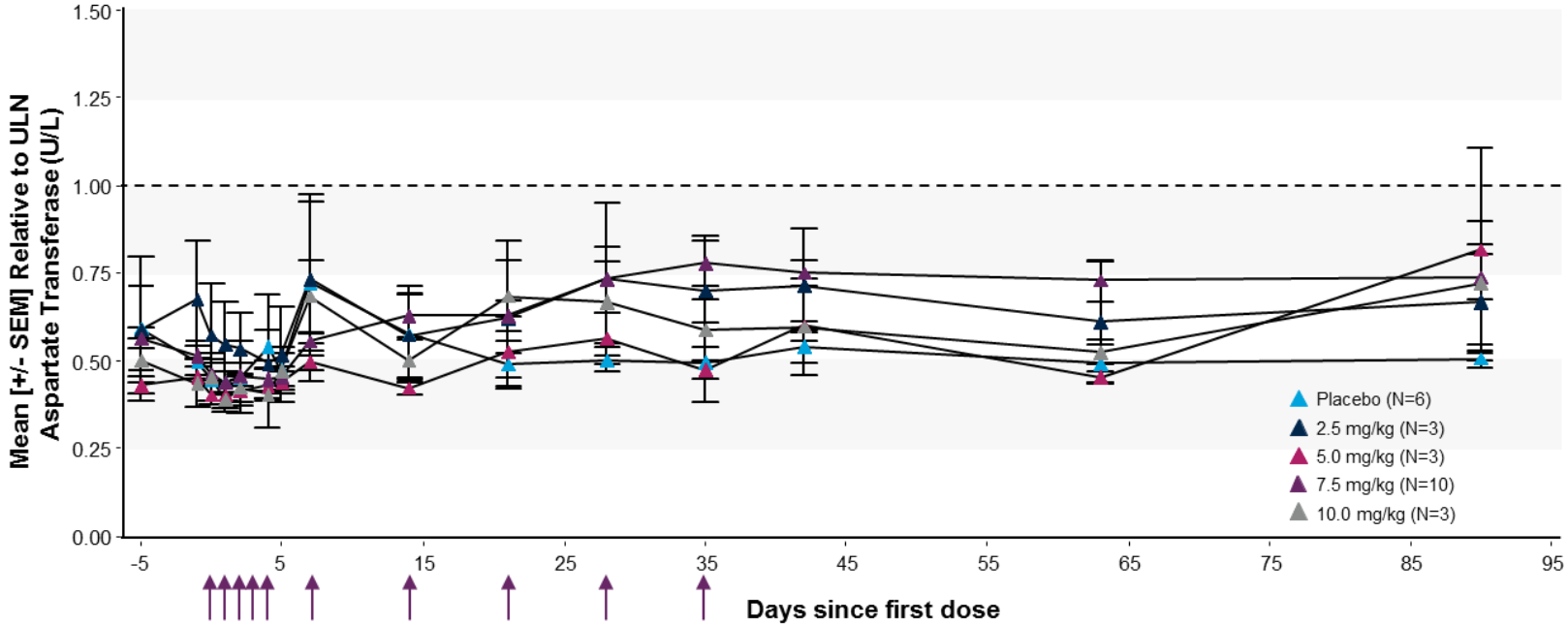

Mean [+/- SEM] Relative to ULN
Aspartate Transferase (U/L)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-5
5
15
25
35
45
55
65
75
85
95
Days since first dose
Placebo (N=6)
2.5 mg/kg (N=3)
5.0 mg/kg (N=3)
7.5 mg/kg (N=10)
10.0 mg/kg (N=3)

Figure S2 Correlation between human and non-human primate TTR knockdown after multiple doses of revusiran. Relationship between TTR KD in NHPs (x-axis) and humans (y-axis) per subject/timepoint at identical dose levels and paradigms. Symbols represent means ± SEMs. Line, R^2, and P value are associated with linear regression of human means on NHP means.

KD=knockdown; NHP=non-human primate (cynomolgus monkeys); SEM=standard error of the mean.

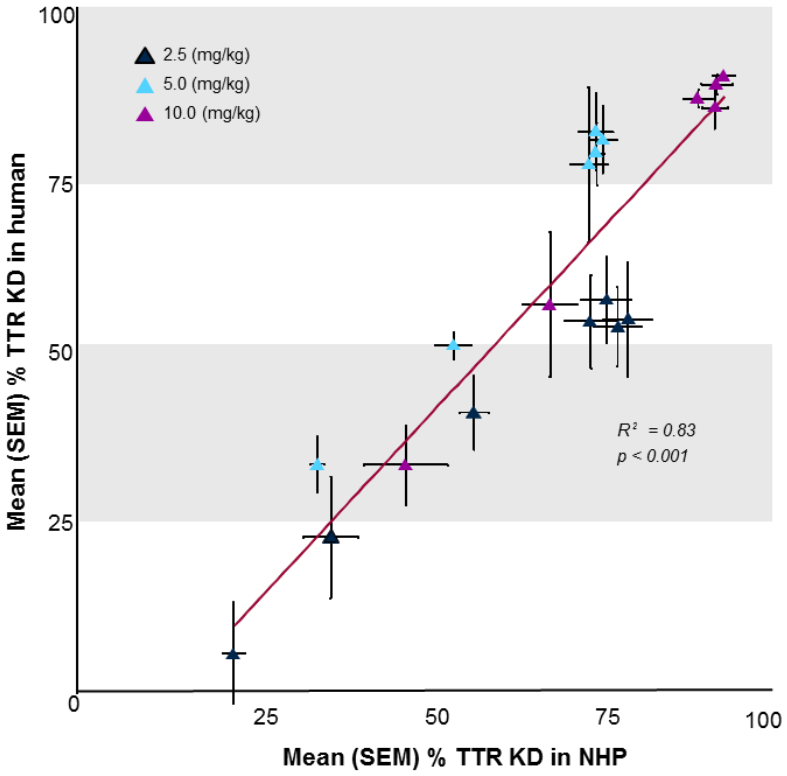


Figure S3 Correlation of changes in serum TTR with changes in RBP and vitamin A in the MAD phase.

Relationships between RBP and vitamin A relative to baseline (squares and triangles, respectively; y-axis), and TTR relative to baseline (x-axis), per subject/timepoint. Colours indicate treatment group (dose). RBP: $R^2 = 0.87$, $p<10^{-15}$; Vitamin A: $R^2 = 0.94$; $p<10^{-15}$ from linear regressions of log-transformed data.

MAD=multiple ascending dose; RBP=retinol binding protein; TTR=transthyretin.

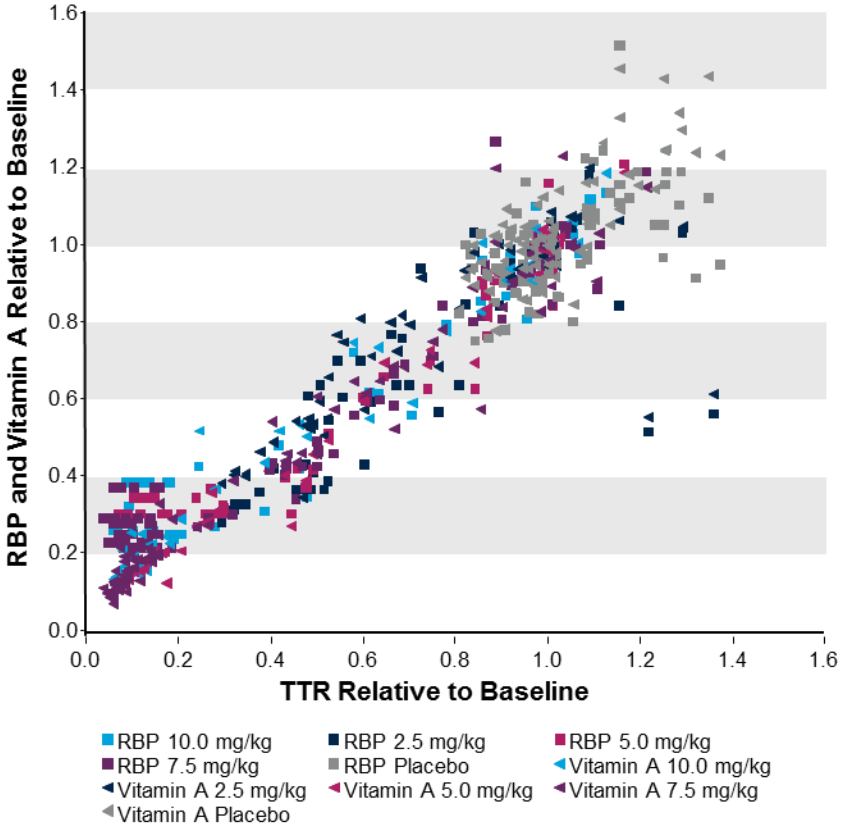


Table S1 Intensity of injection-site signs and symptoms[a]

Sign/symptom	Mild	Moderate	Severe
Pain[b]	Does not interfere with activity	Repeated use of non-narcotic pain reliever for >24 h or interferes with activity	Any use of narcotic pain reliever or prevents activity
Tenderness	Mild discomfort with strong palpation	Moderate discomfort with normal palpation	Severe discomfort with light palpation
Erythema	2.5–5 cm	5.1–10 cm	>10 cm
Induration/swelling	2.5–5 cm and does not interfere with ADL	5.1–10 cm or interferes with ADL	>10 cm or prevents ADL
Pruritus	Mild or localised; topical intervention indicated	Intense or widespread; intermittent; skin changes from scratching (eg, oedema, papulation, oozing/crusts); systemically administered drugs other than corticosteroids or other immunosuppressive drugs indicated	Intense or widespread; constant; limiting self-care ADL or sleep; systemically administered corticosteroid or immunosuppressive therapy indicated
Ulceration	Combined area of ulcers <1 cm; non-blanchable erythema of intact skin with associated warmth or edema	Combined area of ulcers 1–2 cm; partial thickness skin loss involving skin or subcutaneous fat	Combined areas of ulcers >2 cm; full thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to fascia
Pigmentation changes	Slight or localised; covering ≤10% of BSA	Marked or generalised; covering >10% of BSA	—
Other	Asymptomatic or mild	Moderate; minimal local or	Invasive or more significant

	symptoms; clinical or diagnostic observation only; intervention not indicated	non-invasive intervention indicated; limiting age-appropriate instrumental ADL	intervention indicated

ADL=activities of daily living; BSA=body surface area.

[a]Table adapted from guidance developed by the Food and Drug Administration for healthy volunteers enrolled in vaccine trials and Common Terminology Criteria for Adverse Events (version 4.0).[1]

[b]Pain is post-injection pain associated with the injection site but not associated with the needle stick.

Table S2 TEAEs occurring in >1 subject across the SAD and MAD phases

	TEAEs in >1 subject, n (%)											
	SAD phase					MAD phase						
		Revusiran (mg/kg)					Revusiran (mg/kg)					All
TEAE	Placebo (n=4)	1.25 (n=3)	2.5 (n=3)	5.0 (n=3)	10.0 (n=3)	Placebo (n=6)	2.5 QW (n=3)	5.0 QW (n=3)	7.5 QW (n=7)	7.5 Q2W (n=3)	10.0 QW (n=3)	revusiran (n=31)
Injection-site reaction	0	0	0	0	0	1 (17)	1 (33)	1 (33)	5 (71)	3 (100)	3 (100)	13 (42)
Injection-site erythema	0	0	0	0	0	0	2 (67)	1 (33)	2 (29)	3 (100)	2 (67)	10 (32)
Injection-site hematoma	0	0	0	0	1 (33)	2 (33)	3 (100)	1 (33)	4 (57)	0	0	9 (29)
Headache	1 (25)	0	1 (33)	1 (33)	0	2 (33)	0	0	2 (29)	0	1 (33)	5 (16)
Injection-site pain	0	0	0	0	1 (33)	0	0	0	2 (29)	2 (67)	0	5 (16)
Injection-site swelling	0	0	0	0	0	0	0	0	3 (43)	0	0	3 (10)
Dizziness	0	0	0	0	0	0	0	1 (33)	2 (29)	0	0	3 (10)
Nausea	0	0	0	0	0	0	0	1 (33)	1 (14)	0	0	2 (6)

SAD=single ascending dose; MAD=multiple ascending dose; TEAE=treatment-emergent adverse event; QW=every week; Q2W=every other week.

[b]An ISR was defined as ≥2 mild signs or symptoms, or one moderate or severe sign or symptom (Supplemental Table S1).

Table S3 PK parameter estimates at comparable dose levels following a single dose (SAD cohort) and 5 and 10 doses (MAD cohorts) of revusiran

	Revusiran 2.5 mg/kg			Revusiran 5.0 mg/kg			Revusiran 10.0 mg/kg		
PK parameter	**SAD (n=3)**	**MAD Dose 5 (n=3)**	**MAD Dose 10 (n=3)**	**SAD (n=3)**	**MAD Dose 5 (n=3)**	**MAD Dose 10 (n=3)**	**SAD (n=3)**	**MAD Dose 5 (n=3)**	**MAD Dose 10 (n=3)**
AUC_{0-last} (µg h/ml)	3.60	2.96	3.45	6.13	9.65	8.00	17.01	22.50	22.74
	(14.1)	(9.64)	(21.73)	(27.3)	(18.79)	(20.54)	(8.2)	(28.14)	(14.72)
C_{max} (µg/l)	0.24	0.27	0.31	0.50	0.65	0.53	1.05	1.84	1.80
	(9.9)	(27.99)	(38.16)	(7.0)	(25.33)	(2.09)	(15)	(35.02)	(23.43)
T_{max} (h)	6.00	3.33	4.67	2.00	6.00	4.00	6.02	1.83	2.17
	(6.0–12.0)	(34.64)	(24.74)	(2.0–2.0)	(0.00)	(50.00)	(0.50–8.0)	(103.25)	(81.04)
$t_{1/2\beta}$	7.37	7.29	5.75	6.02	6.41	10.98	9.59	7.87	6.01
	(NC)	(15.99)	(17.46)	(NC)	(29.52)	(47.66)	(NC)	(61.54)	(43.07)
CL/F (l/h/kg)	0.65	0.69	0.70	0.67	0.48	0.50	0.56	0.41	0.41
	(NC)	(18.85)	(19.61)	(NC)	(12.74)	(25.82)	(NC)	(36.01)	(7.14)
Vz/F (l/kg)	6.80	7.37	5.88	5.85	4.47	7.51	7.82	4.42	3.58
	(NC)	(32.62)	(36.18)	(NC)	(36.66)	(35.40)	(NC)	(47.77)	(46.78)

$AUC_{0\text{-last}}$=area under the plasma concentration–time curve from zero to the last measurable time point; CL/F=apparent clearance; $C_{max=}$observed maximum plasma concentration; CV=coefficient of variation; NC=not calculated because ≤2 subjects had data; PK=pharmacokinetic; $t_{1/2\beta}$=beta half-life; T_{max}=time of observed maximum plasma concentration; V_z/F=apparent volume of distribution.

Values are mean (CV%) except for T_{max} in the SAD cohort, which is median (range)

Table S4 Mean maximal KD and individual maximum KD for RBP and vitamin A by dose cohort, relative to baseline values

	SAD phase					MAD phase				
		Revusiran (mg/kg)					Revusiran (mg/kg)			
	Placebo (n=4)	1.25 (n=3)	2.5 (n=3)	5.0 (n=3)	10.0 (n=3)	Placebo (n=6)	2.5 QW (n=3)	5 QW (n=3)	7.5 QW (n=7)	7.5 Q2W (n=3)	10.0 QW (n=3)
RBP											
Maximum RBP KD, %	28.8	30.5	47.2	43.9	57.1	25.0	71.9	69.8	78.3	75.0	75.0
RBP KD at nadir (mean ± SD), %	24.3 ± 4.01	24.1 ± 8.88	38.9 ± 8.08	42.5 ± 1.44	49.6 ± 9.96	20.0 ± 6.58	60.3 ± 13.52	68.4 ± 2.3	70.6 ± 7.66	72.2 ± 2.64	70.3 ± 7.37
Vitamin A											
Maximum KD, %	32.0	33.2	37.3	55.7	55.1	33.4	65.6	89.1	92.9	87.6	86.8
KD at nadir (mean ± SD), %	24.2 ± 5.56	20.6 ± 11.32	31.7 ± 7.29	46.4 ± 8.88	46.1 ± 10.74	15.3 ± 12.76	56.1 ± 13.51	84.4 ± 4.89	85.6 ± 13.89	81.3 ± 5.55	83.7 ± 4.08

KD=knockdown; SAD=single ascending dose; MAD=multiple ascending dose; RBP=retinol binding protein; SD=standard deviation; QW=every week; Q2W=every other week.

Supplemental Methods

Pharmacodynamics

Serum samples for pharmacodynamic assessments including TTR concentrations and secondary markers Vitamin A and RBP were taken at screening, admission (Day –1), and pre-dose on Day 0 for both SAD and MAD cohorts. Additionally, serum samples were collected up to Day 56 in the SAD cohorts (Days 1, 2, 4, 7, 10, 14, 21, 28, 42 and 56) and up to Day 90 in the MAD cohorts (Days 3, 5, 7, 14, 18, 21, 28, 31, 35, 36, 42, 49, 56, 63 and 90). Serum draws on a dosing day were taken prior to dosing in the MAD cohorts (Days 3–35).

Pharmacokinetics

The concentration of TTR siRNA (revusiran) was evaluated in plasma samples using the validated hybridisation-based ATTO-Probe-HPLC assay (Tandem laboratories, Salt Lake City, UT, USA). In the SAD cohorts, comprehensive sampling plasma PK was performed on Day 0 (5 [±1], 10 [±1], and 30 [±2] minutes pre-dose, and 1, 2, 4, 6, 8 and 12 h [all ±5 minutes] post-dose); samples were also taken on Days 1, 2, 4, 7, 10, 14, 21 and 28. For the MAD cohorts, plasma samples were taken on Days 0–5 and up to Day 90 in the maintenance phase (Days 7, 14, 21, 28, 35, 36, 42, 49, 56, 63 and 90). On Days 0, 4 and 35, samples were taken Day 0 (5 [±1], 10 [±1], and 30 [±2] minutes pre-dose and 1, 2, 4, 6, 8 and 12 h [all ±5 minutes] post-dose); on all other dosing days, plasma was collected approximately 1 h prior to dose and 2 h after dosing. Noncompartmental and/or compartmental models were used to estimate PK parameters.

Anti-Drug Antibody

Anti-drug antibody production was assessed using a validated ELISA. In brief, ~1 ng of revusiran was coupled to each well of an ELISA plate as a modified 5′-phosphate duplex (5′-phosphate on sense strand). Serum samples from subjects were then tested for binding to the revusiran duplex in this sandwich ELISA with detection by anti-immunoglobulin G/M coupled with horseradish peroxidase. A rabbit anti- keyhole limpet haemocyanin-revusiran polyclonal antibody was used as a positive control. Serum samples were first screened for potentially positive samples. A potentially positive sample was defined as a sample that produced mean $A_{450nm} \geq$ the assay plate specific cut-point (PSCP). All serum samples considered potentially positive (Mean $A_{450nm} \geq$ PSCP) during the screening analysis were evaluated in a confirmatory assay. To be considered as a confirmed positive sample, the %signal inhibition between the spiked (spiked with revusiran)

and the unspiked sample had to be ≥ the confirmatory cut point. All serum samples from the multiple dose cohorts were negative in the screening assay (A_{450nm} < PSCP) and therefore negative for anti-drug antibodies (data not shown).

References

1. Food and Drug Administration (2007). Guidance for industry. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials.