Phase I/IIa study of TT-034, a DNA-directed RNA interference agent (ddRNAi) delivered as a single administration for the treatment of subjects with chronic hepatitis C virus (HCV)

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Abstract

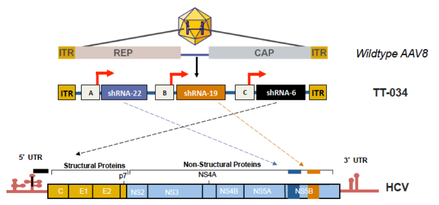
Background: Designed to treat HCV, TT-034 is an RNAi therapeutic comprised of a recombinant DNA that is delivered intravenously using an Adeno-Associated Virus capsid (AAV8) for transduction of hepatocytes. Once inside, TT-034 uses the cell's transcriptional machinery to drive long term expression of three independent short hairpin RNAs (shRNAs) to simultaneously target three well-conserved regions of the HCV RNA genome, including the 5' UTR (shRNA6) and NS5B (shRNA19 and shRNA22). In non-human primate (NHP) studies, clinically relevant doses of TT-034 transduced nearly 100% of hepatocytes and resulted in persistent shRNA expression for 180 days (the length of the study). Intended as a one-time treatment, the dosing with TT-034 is the first time a non-withdrawable RNAi therapeutic has been used in man.

Methods: This ongoing, first-in-man, Phase I/IIa open label dose-escalating trial is enrolling chronic HCV genotype 1 patients without cirrhosis. Patients receive a single intravenous infusion of TT-034 at one of 5 dose levels. A liver biopsy, collected 21 days post dosing, is used to assess hepatic TT-034 DNA levels and shRNA expression.

Results: To date, seven subjects have received a single dose of TT-034 at either 4.00E10, 1.25E11, or 4.00E11 vg/kg. Additional subjects will be enrolled in dose cohorts of 1.25E12 or 4.00E12 vg/kg. There have been no treatment-related SAEs in the study to date. Once administered, TT-034 clears from serum within the first week post dosing. No long term TT-034 shedding has been detected in the urine, stool, semen, or sputum. TT-034 DNA levels in liver biopsies are measured by QPCR and are similar to those reported in NHP models. Patients administered the lowest dose resulted in 0.01 or 0.02 copies of the TT-034 genome per cell, the equivalent of 1 or 2 % hepatocyte transduction. At a dose of 1.25E11 vg/kg, substantially higher levels were detected in the hepatic tissues from the three subjects, yielding 0.48, 3.65 and 10.44 copies of TT-034 DNA per cell respectively. The first subject dosed with 4.00E11 vg/kg demonstrated 17.74 copies per cell, indicating that a significant portion of the hepatocytes may have been transduced. QPCR analyses of RNA isolated from the biopsies confirmed concomitant, dose dependent expression of anti-HCV shRNAs. Copy numbers of shRNA6, shRNA19 and shRNA22 were measured at 66, 2032, and 99 copies per cell respectively in the subject dosed with 4.00E11 vg/kg.

Conclusion: Initial doses of TT-034 are well tolerated in human subjects infected with HCV. At higher doses, substantial portions of hepatocytes are transduced and result in concomitant dose-dependent expression of anti-HCV shRNAs.

Expression of Three anti-HCV shRNA From a Recombinant AAV Expression Cassette

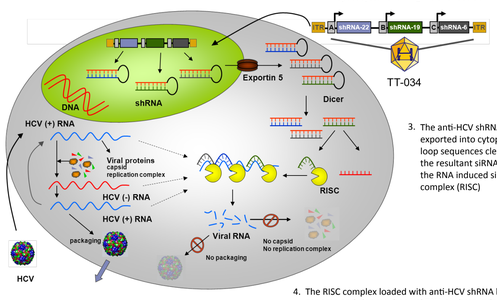


- TT-034 is delivered via intravenous infusion once, representing the sole treatment
- 3 independently transcribed short hairpin RNA (shRNA) elements target 3 separate, well-conserved regions of the HCV genome; helps prevent the generation of viral escape mutants
- Delivery uses capsid derived from adeno associated virus (AAV), a non-integrating, non-pathogenic virus used in over 117 clinical trials
- Sustained expression (potentially years based on other clinical studies using Factor IX) following a single injection
- Complete transduction of liver hepatocytes with serotype 8 (AAV8)

TT-034 Mechanism of Action – RNA Interference

2. TT-034 uses the cell's own transcriptional machinery to produce a constantly replenishing pool of anti-HCV shRNA at steady state levels.

1. TT-034 is comprised of an AAV8 capsid that delivers a non-integrating recombinant genome to hepatocytes following intravenous administration.



3. The anti-HCV shRNAs are exported into cytoplasm, the loop sequences cleaved and the resultant siRNAs enter the RNA induced silencing complex (RISC)

4. The RISC complex loaded with anti-HCV shRNA binds to the HCV genome and cleaves the viral RNA at three independent, well conserved regions rendering the RNA genome incapable of being packaged into nascent virions or serving as a template for the translation of viral proteins.

5. Because the shRNA is produced continuously for months or even years following the single administration, the anti-shRNA produced from TT-034 may be able to protect the transduced hepatocytes from re-establishment of HCV infection long term.

Overview of Phase I/IIa Trial

Cohort	Dose (vg/kg)	Dose escalation step (log 10)	Anticipated Number of subjects	Dosing scheme for subjects	Subject Observation period between cohorts before dose escalation
1	4.00×10^{10}	Starting dose	2	Sequential (1+1)	6 weeks
2	1.25×10^{11}	0.5	3	Sequential and parallel (1+2)	6 weeks
3	4.00×10^{11}	0.5	2	Sequential and parallel (1+2)	6 weeks
4	1.25×10^{12}	0.5	3	Sequential and parallel (1+2)	10 weeks
5	4.00×10^{12}	0.5	3	Sequential and parallel (1+2)	10 weeks

Subjects Dosed To Date

Treatment:
- One time, intravenous infusion of TT-034 drug product

Follow-up:
- Active monitoring through 24 weeks post dosing

Subjects:
- Infected with Genotype I HCV
- Treatment experienced and naive

Primary Endpoints (Safety):
- Incidence of adverse events
- Changes in clinical parameters

Secondary Endpoints (Efficacy):
- Assessment of TT-034 levels in day 21 liver biopsy
- Assessment of shRNA expression in liver biopsy
- Sustained reduction in HCV viral load in the blood

Demographics of Subjects Enrolled and Dosed with TT-034

Cohort Demographics and Baseline Characteristics

	Total (N=7)	Cohort 1 $4.00e^{10}$ vg/kg (N=2)	Cohort 2 $1.25e^{11}$ vg/kg (N=3)	Cohort 3 $4.00e^{11}$ vg/kg (N=2)
Age (years) Mean (Min, Max)	47 (27, 64)	53 (51, 55)	44 (33,57)	45.5 (27,64)
Gender: Male (%)	86%	100%	67%	100%
BMI (kg/m^2), Mean	24.95	23.87	24.93	26.05
Race (%)				
Asian	0	0	0	0
Black or African-American	29%	50%	33%	0
White	71%	50%	67%	100%
Other	0	0	0	0

Data in database as of 28 October, 2015

Monitoring of TT-034 Persistence in Serum (PK) and Vector Shedding

cohort	Cohort 1		Cohort 2			Cohort 3
dose administered	4e10 vg/kg		1.25e11 vg/kg			1e10 vg/kg
Subject ID	1007	1030	1040	1045	1048	1051
collection time						
pre-dose	< LLD	< LLD	< LLD	< LLD	< LLD	< LLD
1 hour	91312275	> 1x10^6	> 1e6	> 1e6	> 1e6	> 1e6
2 hours	19712898	67962675	> 1e6	> 1e6	> 1e6	> 1e6
4 hours	1360428	19747845	> 1e6	> 1e6	> 1e6	> 1e6
					no sample collected	
6 hours	598070	7242366	> 1e6	> 1e6	> 1e6	> 1e6
10 hours	292686	2098437	> 1e6	> 1e6	> 1e6	> 1e6
24 hours	56512	384981	> 1e6	65636796	38413443	> 1e6
48 hours	7238	113031	20344580	7634516	3858041	13468426
120 hours	< LLD	> LLD, NQ	249154	13826	105085	27760
168 hours	< LLD	> LLD, NQ	131558	10116	> LLD, NQ	16597

*Samples collected for Subject 1053 (Cohort 3, Subject 2) – not yet analyzed
> LLD, NQ – greater than lower limit of detection, yet below linearity on standard curve thus not accurately quantified

- TT-034 levels spike rapidly following intravenous administration
- Vector is rapidly cleared from serum, with much of the vector cleared or transduced into tissues within 24 hours
- All collected samples from patients comprised of saliva, urine , stool and semen from male subjects revealed no quantifiable TT-034 vector shedding in those samples at Week 4, Week 8, Week 12, Week 16, Week 20 or at Week 24 .

TT-034 Safety and Tolerability Through First Three Cohorts

Summary of Adverse Events (Safety Population)

	Total (N=7) n (%)	Cohort 1 $4.00e^{10}$ vg/kg (N=2) n (%)	Cohort 2 $1.25e^{11}$ vg/kg (N=3) n (%)	Cohort 3 $4.00e^{11}$ vg/kg (N=2) n (%)
Number of patients who experienced ≥1				
TEAE	7 (100%)	2 (100%)	3 (100%)	2 (100%)
Mild TEAEs	7 (100%)	2 (100%)	3 (100%)	2 (100%)
Moderate TEAEs	3 (43%)	1 (50%)	1 (33%)	1 (50%)
Severe TEAEs	2 (29%)	0 (0%)	2 (67%)	0 (0%)
Drug-related TEAE	1 (14%)	1 (50%)	0 (0%)	0 (0%)
Drug-related moderate or severe TEAEs	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Treatment-emergent SAE	1 (14%)	0 (0%)	1 (33%)	0 (0%)
Drug-related treatment-emergent SAE	0 (0%)	0 (0%)	0 (0%)	0 (0%)

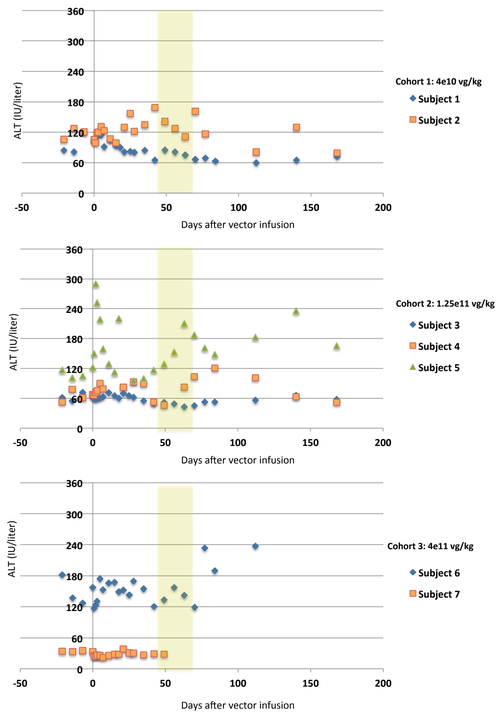
AE = adverse events
n = number of patients with an observation
N = number of patients in the safety population
SAE = serious adverse events
TEAE = treatment-emergent adverse events.

The most common AE, headache, was reported in 3 subjects. Contusion, nasopharyngitis, URI and nausea were each reported in 2 subjects.

Summary of Adverse Events Graded as Moderate or Severe (Safety Population)

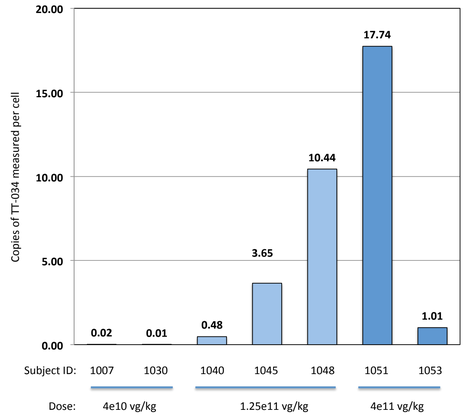
	Grade	Relationship to Study Drug
Preferred Term		
1007		
Decreased appetite	Grade 2 (Moderate)	Not related
Pain in extremity	Grade 2 (Moderate)	Unlikely related
1045		
Rib fracture	Grade 3 (Severe)	Not related
Pulmonary embolism	Grade 3 (Severe)	Unlikely related
Encephalopathy	Grade 2 (Moderate)	Unlikely related
Tooth fracture	Grade 2 (Moderate)	Not related
Aspartate aminotransferase increased	Grade 3 (Severe)	Unlikely related
1048		
Syncope	Grade 3 (Severe)	Unlikely related
Subarn	Grade 2 (Moderate)	Not related

No Evidence for T-Cell Capsid Response Post Dosing



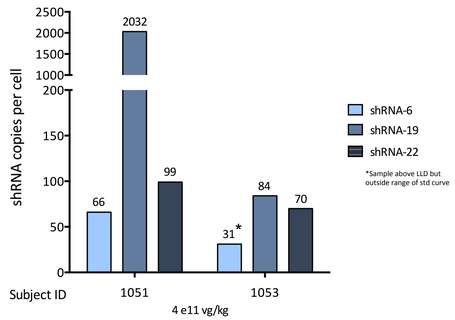
- Previous systemic administration of AAV8 vectors results in a T-cell capsid response in transduced hepatocytes (as characterized by a > 4X sudden increase in ALT) between weeks 7 and 10 with a product that expresses Factor IX for the treatment of Hemophilia. (N Engl J Med. 2014 Nov 20;371(21):1994-2004).
- No evidence has been demonstrated for a T-cell response at the doses of TT-034 that have been administered

Measurement of TT-034 DNA in Liver Biopsies



- In order to assess DNA transduction expression, DNA was isolated from a section of the liver biopsy collected 21 days post dosing. QPCR assays was used to assess DNA levels
- Assumes 6.6 pg of genomic DNA per cell for copy / cell analyses

Measurement of anti-HCV shRNAs in Liver Biopsies From Cohort 3



- In order to assess shRNA expression, RNA was isolated from a section of the liver biopsy collected 21 days post dosing. Customized QPCR assays designed to detect each small RNA species was used to quantify the level of each shRNA.
- 1 ng of RNA interrogated / assumes 50pg of RNA per cell for copy / cell analyses

Summary and Next Steps

TT-034 is a promising, first in man RNAi therapeutic for the treatment of subjects infected with HCV

- The majority of TT-034 not taken up by tissues is cleared from serum within 1 week
- No quantifiable TT-034 shedding has been noted in urine, sputum, semen or stool
- TT-034 is well tolerated:
  No treatment-related SAEs
  Only one mild treatment-related adverse event
- No evidence of T-cell capsid response in hepatic tissues at any dose administered
- Dose dependent transduction of TT-034 DNA in hepatic tissues is observed
- Concomitant, dose-dependent increases in anti-HCV shRNA expression are also observed
- Dosing into first subject of Cohort 4 anticipated Q4 2015