A comparison of scAAV8-TT034 mediated transduction and shRNA expression in human liver biopsy samples versus a chimeric mouse model with humanized liver

[1]Shih-Chu Kao, [1]Tin Mao, [1]Terrie-Anne Cock, [1]Peter Roelvink and [1,2]David Suhy

[1]Benitec Biopharma, Hayward, CA. [2]Tacere Therapeutics, Hayward, CA

BENITEC BIOPHARMA silencing genes for life™

Abstract

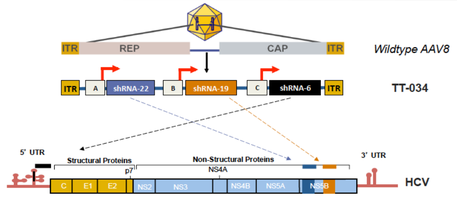
Background: TT-034 is DNA-directed RNA interference (ddRNAi) agent designed for the treatment of chronic HCV infection and is currently being tested in a phase I/IIa clinical study. TT-034 is comprised of a vector that expresses three independent short hairpin RNAs (shRNAs) simultaneously targeting three well-conserved regions of the HCV genome. The recombinant genome is packaged in a self-complementary adeno-associated virus serotype 8 (AAV8) capsid with tropism for hepatic tissues and delivered as a single dose intravenous (IV) infusion.

Methods: Chimeric mouse models in which human hepatocytes replace the majority of mouse hepatocytes are used to study human hepatic function. In order to assess validity of dose/transduction relationships in this murine model to those observed in a human clinical study, chimeric mice were infected with identical doses of TT-034 used in a phase I/IIa study using the same clinical lot of TT-034. In the clinical study, eight subjects have received a single IV infusion of TT-034 at 4.00E10, 1.25E11, 4.00E11 or 1.25E12 vg/kg. At 21 days post dosing, a liver biopsy was collected to assess TT-034 DNA (transduction) levels and shRNA expression by qPCR. PXB chimeric mice (Phoenix Bio) repopulated with a minimum of 80% human hepatocytes were dosed identically with the TT-034 drug product (5 groups, n=4). After 21-28 days, the livers of two mice in each group were removed and hand curated to purify human hepatic tissues (>93% purity). The liver tissues of the other two mice were dissociated and human hepatocytes were enriched to >99% using mouse hepatocyte-capturing Dynabeads. TT-034 transduction and shRNA expression were assessed by qPCR.

Results: In the human study, modest levels of TT-034 DNA copies were detected in the 3 subjects dosed at 1.25E11 vg/kg, yielding 0.48, 3.65 and 10.44 copies per cell respectively. Variability in transduction was noted at a higher dose of 4.00E11 vg/kg, with the two subjects yielding 17.74 and 1.01 copies per cell. qPCR analysis of the three anti-HCV shRNAs confirms concomitant, dose dependent expression. In the hand curated samples from the chimeric mouse model (>93% purity), a dose of 1.25E11 vg/kg yielded 1.1 or 1.3 DNA copies per cell while the 4.00E11 vg/kg dose resulted in 1.9 and 5.5 DNA copies per cell. Dynabead-enriched human hepatocytes (>99%) resulted in a considerable drop in DNA copy levels: a 1.25E11 vg/kg dose yielded average 0.35 copies per cell while the 4.00E11 vg/kg resulted in 0.85 DNA copies per cell. The lowered DNA levels in the chimeric mouse model led to a concomitant reduction in shRNA expressed in the hand curated tissues. Likewise, shRNA expression was reduced even further in enriched human hepatocytes.

Conclusions: Our data suggests that residual mouse hepatocytes present in the chimeric livers are transduced with the scAAV8 vector more efficiently than human hepatocytes and results in lower overall transduction as compared to human clinical samples. Thus, while these models can serve as a surrogate to assess the activity of gene therapy constructs against functions of normal human liver, the doses required for optimal activity may be modestly higher than required in the human clinical setting.

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

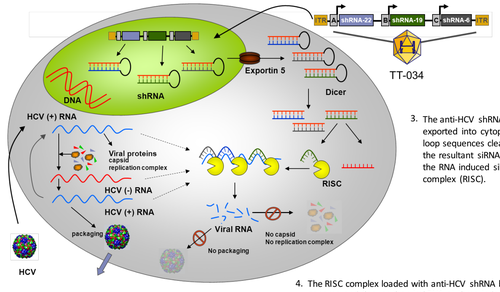


- TT-034 is delivered via intravenous infusion once, representing the sole treatment
- 3 independently transcribed short hairpin RNA (shRNA) elements target 3 separate, well-conserved regions of the HCV genome; helps prevent the generation of viral escape mutants
- Sustained expression, months to years, 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).

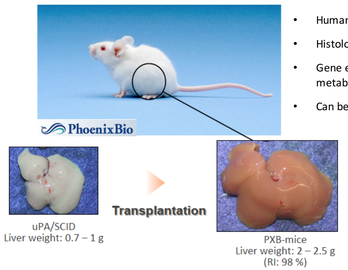
4. The RISC complex loaded with anti-HCV shRNA binds to the HCV genome and cleaves the viral RNA at three independent, well conserved regions rendering the RNA genome incapable of being packaged into nascent virions or serving as a template for the translation of viral proteins.

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.

Dosing Schema of The Human 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	2	Sequential and parallel (1+2)	10 weeks
5	4.00×10^{12}	0.5	N/A	Sequential and parallel (1+2)	Trial Discontinued Due to Commercial Considerations

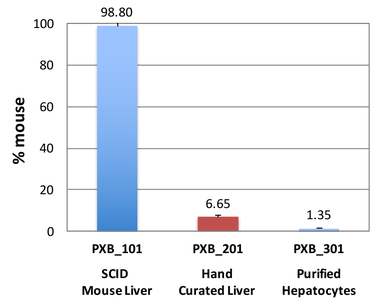
PXB Mice – A Chimeric Mouse Model with a Liver Highly Repopulated by Human Hepatocytes



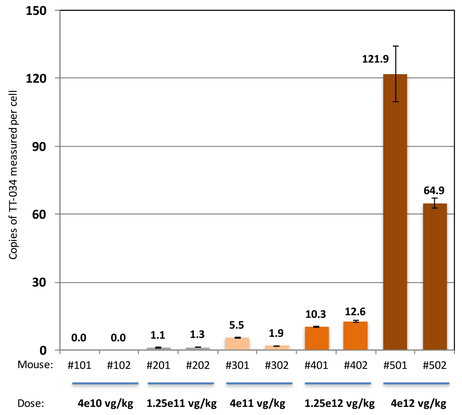
- Human hepatocytes proliferate in mouse liver
- Histologically normal liver constitution
- Gene expression and activities of human metabolic enzymes and transporters
- Can be infected with HCV and HBV

Relative Proportion of Mouse vs Human Hepatocytes Following Hand Curation vs Immunoisolation

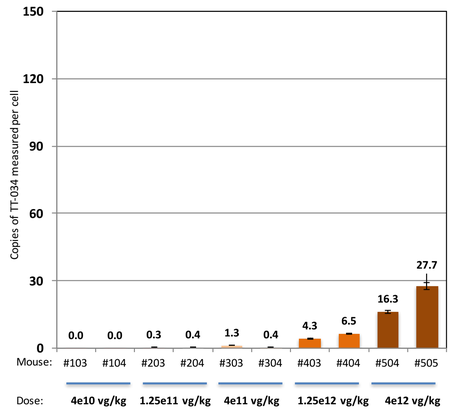
- The PXB-Mouse (Phoenix Bio, Japan) is a chimeric mouse with a humanized liver that is repopulated by human hepatocytes. PXB Chimeric Mice were generated and selected with a replacement index of > 87% human hepatocytes.
- PXB_201: The chimeric mice were sacrificed and the liver was harvested and hand curated to separate the human from mouse tissues.
- For PXB_301, The liver was harvested and treated with collagenase to separate into single cells. Anti-mouse antibodies (to a specific mouse surface marker) that coupled to magnetic beards were used to separate and eliminate the mouse cells from the human cells in the preparation.
- Assessment of mouse vs. human hepatocytes performed by QPCR analysis for mouse albumin genes



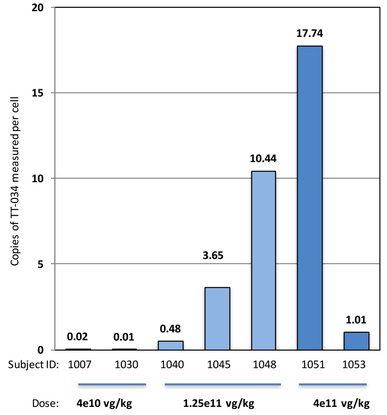
TT-034 DNA in Chimeric Mouse Livers Hand Curated for Human Tissues



TT-034 DNA in Chimeric Mouse Livers Immunoisolation of Human Cells

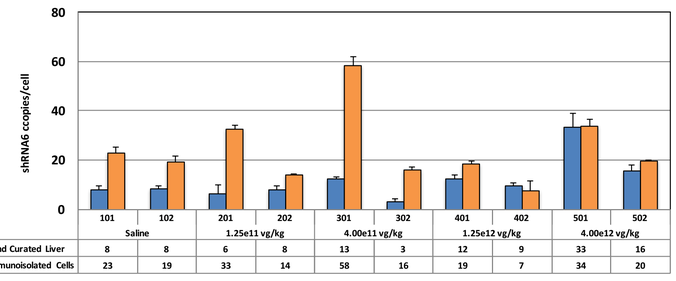


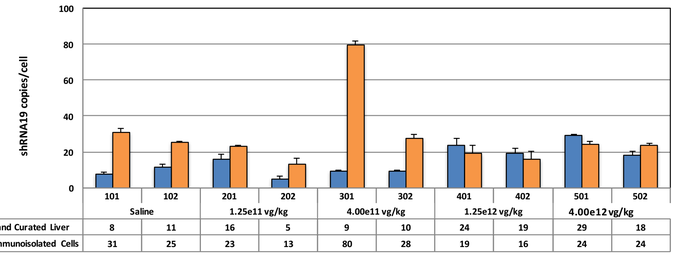
TT-034 DNA in Human Liver Biopsies

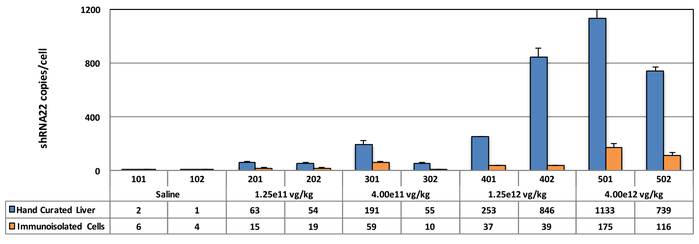


- In order to assess DNA transduction expression, DNA was isolated from a section of the liver biopsy collected 21 days post dosing. QPCR assays was used to assess DNA levels
- Assumes 6.6 pg of genomic DNA per cell for copy / cell analyses

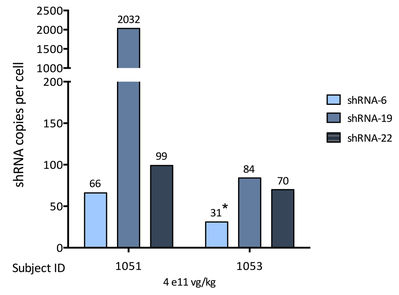
Anti-HCV shRNAs Produced in Chimeric Mouse Livers







Anti-HCV shRNAs Produced in Human Liver Biopsies From Cohort 3 Subjects



- 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

- The data presented suggest that the mouse hepatocytes present in the chimeric livers are transduced with the scAAV8 vector more efficiently than human hepatocytes. This is consistent with a recently published paper by Vercauteren et al. (*Mol. Ther.* 2016 AOP) which claim mouse hepatocytes have transduction rates 19-fold higher than human hepatocytes in a mouse/human *fah -/-* chimeric model.
- Using the exact same clinical material administered in the human trial, equivalent doses of TT-034 resulted in lower DNA levels in the chimeric mouse model in which samples had been hand curated versus levels measured in biopsies from the treated human subjects. Using antibody-based isolation techniques, purified human hepatocytes from the chimeric model had even less DNA copies per cell, strongly suggesting mouse hepatocytes are preferentially transduced.
- Correspondingly low levels of shRNA were noted in the chimeric model.
- Interestingly, the relative distribution of shRNA species differs between human hepatocytes from the chimeric model (shRNA22> shRNA19=shRNA6) versus the human biopsies taken from treated subjects (shRNA 19>shRNA22=shRNA6).
- While chimeric mouse models can serve as a surrogate to assess the activity of gene therapy constructs against functions of normal human liver, the doses required for optimal activity for pre-clinical efficacy studies may be modestly higher than required in the human clinical setting.