A translational platform quantitative systems pharmacology (QSP) model for preclinical to clinical translation of in-vivo CRISPR-Cas9 therapy

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BACKGROUND

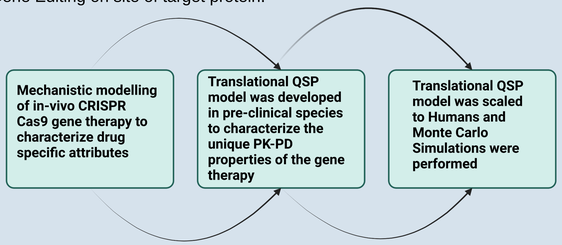
In-vivo CRISPR Cas genome editing is a complex therapy including three components: 1) Lipid Nano Particle (LNP); 2) Messenger RNA (mRNA); and 3) Single guide RNA (sgRNA). This next generation of gene editing comes into a class of novel modalities where little is known about their dose-exposure relationship and biodistribution[1,2]. Within this work we have built a QSP model to characterize, predict, and translate the Pharmacokinetics / Pharmacodynamics (PK/PD) of CRISPR therapies from preclinical species (mouse, NHP) to clinic (humans) using two case studies.

METHODS

- PK/PD data of LNP, mRNA and sgRNA were obtained from the literature for both case studies for transthyretin amyloidosis and reduction of LDL-cholestrol[1,3].

The QSP model includes the following mechanisms upon IV injection:

- Binding of LNP to opsions in the liver vasculature
- Internalization of LNP into Mononuclear Phagocytotic System (MPS)
- Internalization of LNP in Interstitial layer of liver via endocytosis and binding to LDL receptors via receptor mediated endocytosis as well as via macropinocytosis
- Internalization of the LNP into cellular layer of liver and release of transgene product i.e. mRNA and sgRNA;
- Disposition of mRNA and sgRNA into the systems by including exocytosis as well as clathrin-mediated endocytosis and macropinocytosis;
- Elimination of LNP and sgRNA via renal elimination;
- Exonuclease degradation of sgRNA and mRNA;
- Translation of mRNA into Cas9 and binding with sgRNA to form RNP complex;
- Gene Editing on site of target protein.



RESULTS

- Both the mechanistic and translational QSP model were able to well characterize the disposition of LNP and the transgene product (sgRNA and mRNA), by predicting it's response across species
- Step-wise parameter estimation analysis using our developed model indicates that the differences in PK at a lower dose level is due to it's binding with opsions in liver described by kass and kdis in the model. A low IC50 is reported in humans as the RNP complex indicative, that the production of target protein will be saturated at a low concentration
- The model structure and corresponding parameters were able to successfully describe dose-exposure-response across species

"Translational QSP model for in-vivo CRISPR-Cas9 gene therapy helps identify key determinants for biodistribution, release kinetics of transgene product, interaction between sgRNA and Cas9 protein and gene editing for the target of interest"

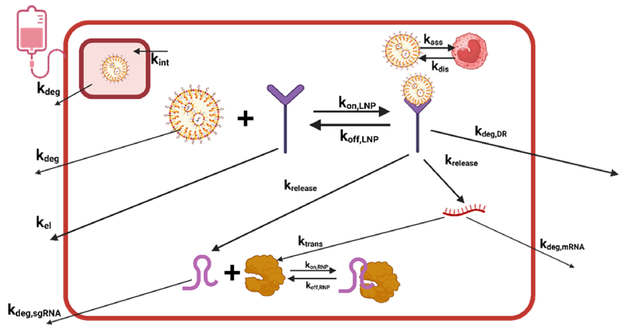


Figure 1. Mechanistic model to quantify the drug specific attributes

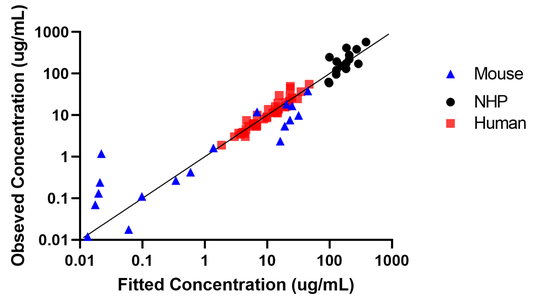


Figure 2. Model performance of the mechanistic model across species when fitted simultaneously

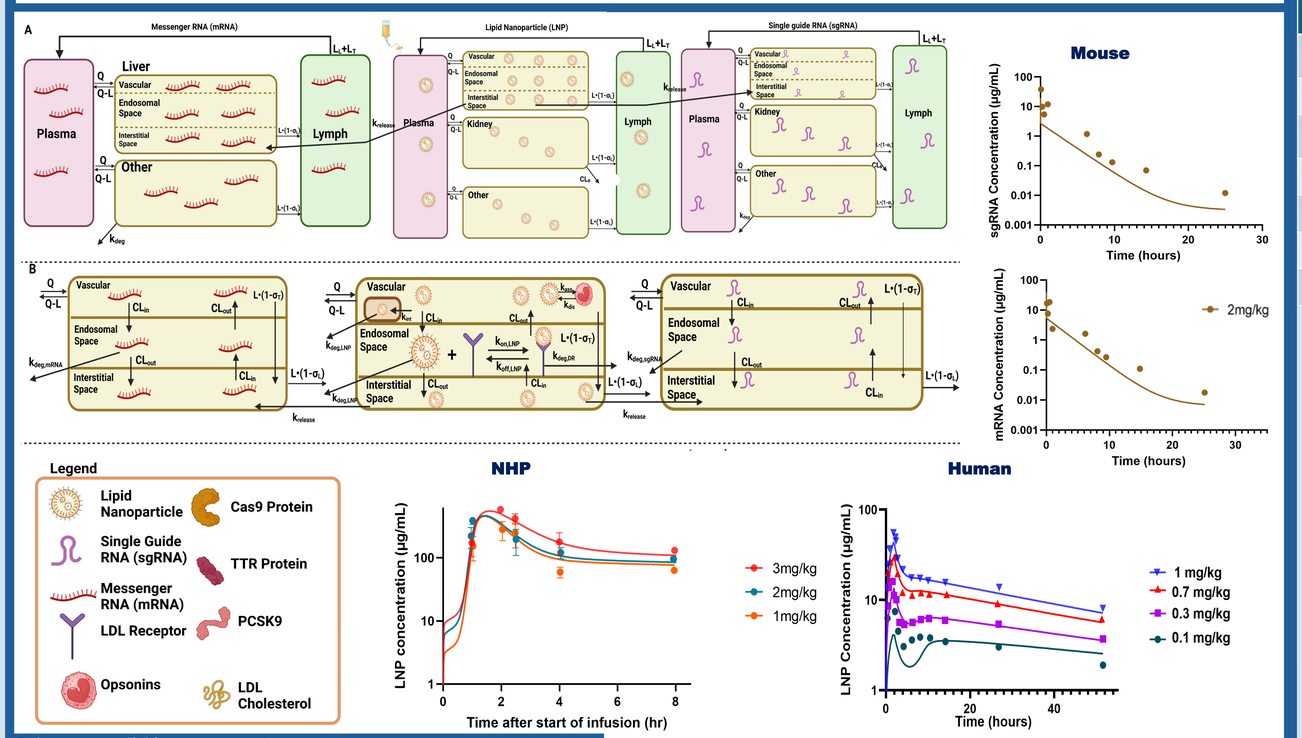


Figure 3. Model Structure of translational QSP model (A) Body-Level model structure in-vivo CRISPR therapy, (B) Organ-level structure. Model Fittings for mouse, NHP and Human

References available upon request

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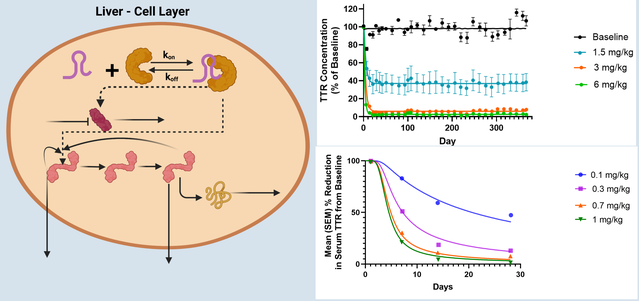

Figure 4. Model Structure of QSP model characterizing binding of sgRNA and Cas9 protein to enable gene editing (upper left panel), reduction of TTR proteins in NHP and Humans (upper right panel), Reduction of PCSK9 and LDL-cholestrol in NHP (below panel).

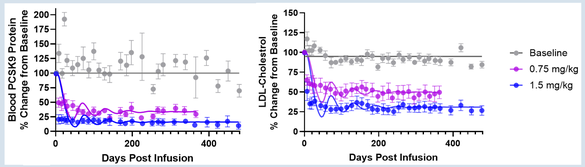

Table 2. Tabular summary of pharmacodynamic parameter estimates

Parameter	Description	NHP (Final Estimate and RSE%)	Human (Final Estimate and RSE%)
IC50 (ug/mL)	Concentration at 50% of effect for TTR	4.77 (11.5%)	0.3 (0.02%)
k_{out} (1/d)	Rate of degradation for TTR	0.493 (15%)	0.247 (17.5%)
IC50 (ug/mL)	Concentration at 50% of effect for PCSK9	21.5 (0.994%)	-
Imax	Maximum inhibition for PCSK9	0.771 (14.2%)	-
MTT (d)	Mean transit time for PCSK9	14.5 (0.563%)	-
k_{out} (1/d)	Rate of degradation for LDL-cholesterol	4.66 (67.4%)	-
γ	Gamma coefficient for LDL-cholesterol	0.672 (18.5%)	-

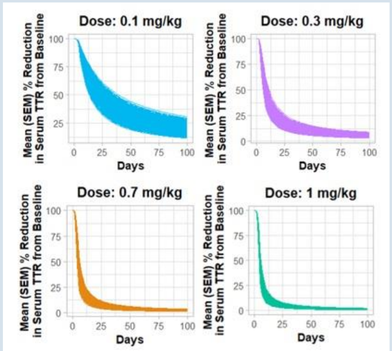

Figure 5. Monte-Carlo Simulations performed for 1000 subjects to characterize the Reduction of serum TTR across different doses in humans.

LIMITATIONS

- Modelling process based on digitized dataset
- Identifiability issue for several parameters
- **Very first conceptual application**

►Validation datasets required