

Capacity limits of asialoglycoprotein receptor-mediated liver targeting



Charlotte Bon¹, Thomas Hofer², Thierry Lavé¹, Ben-Fillippo Krippendorff¹, Nicolas Frances¹ and Hans Peter Grimm¹

¹ Roche Pharmaceutical Research and Early Development, Roche Innovation Center Basel, Basel, Switzerland
² Roche Pharmaceutical Research and Early Development, Roche Innovation Center Zurich, Zurich, Switzerland

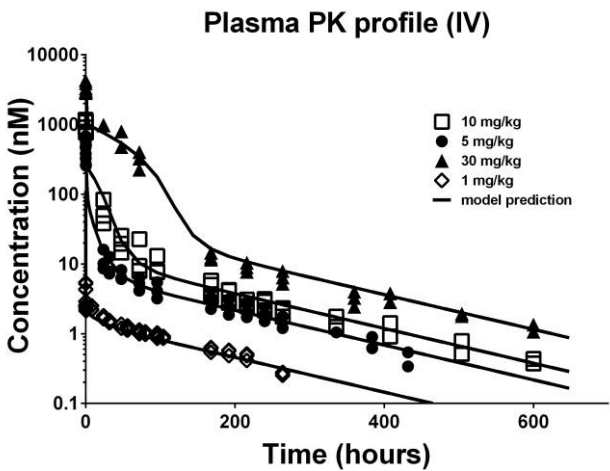
Introduction

- The abundant cell surface asialoglycoprotein receptor (ASGPR) is a highly selective receptor found on hepatocytes that potentially can be exploited as a selective shuttle for delivery [1]
- This receptor-mediated delivery mechanism can be saturated, which will likely result in reduced selectivity for the liver and therefore increase the likelihood for systemic adverse effects. Using a newly developed anti-ASGPR antibody (ASGPR Ab), we developed a TMDD model [2] to:
 - **Estimate the maximal capacity of the ASGP receptor and its kinetic of uptake**
 - **Provide a rational for dose optimization**
 - **Investigate the influence of the unspecific clearance of ASGPR targeting compounds on the outcome of the delivery strategy**

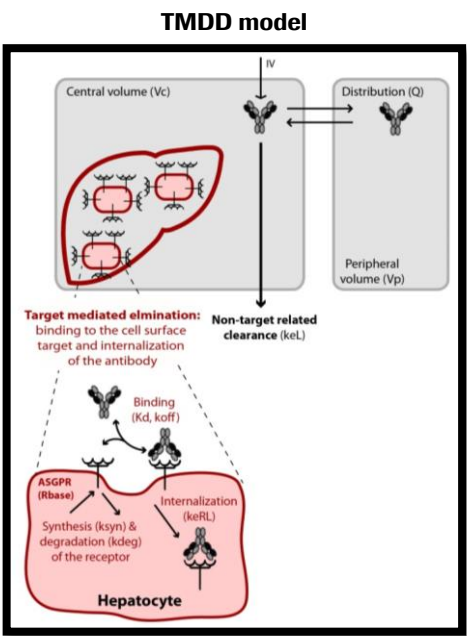
Method

1. A mouse PK study was designed to determine the capacity of the ASGPR shuttle using the ASGPR Ab (N=12, 3 observations/time point, 4 dose levels). A mechanistically based mathematical modeling approach was used for PK data analysis. The model was based on a generalized PK model for drugs exhibiting TMDD (Mager and Jusko J Pharmacokinetic Pharmacodyn 2001). Parameters were estimated using a NLME approach in MONOLIX 4.3.3.
2. A biodistribution study was then performed to assess if the ASGPR antibody distributes specifically to the liver and were not distributed in other organs (N=66, 3 animals/time point, 4 dose levels). However, the liver has been found to be a major elimination organ for mAbs [3]. Therefore, a radiolabeled non-targeting Ab (IL17 Ab) was used for comparison (N=30, 3 animals/time point, 1 dose level). Both Abs were radiolabeled with ¹¹¹Indium-DOTA complex, a residualizing radioactive marker accounting for the antibodies accumulation in tissue.

PK study: TMDD model and parameter estimation



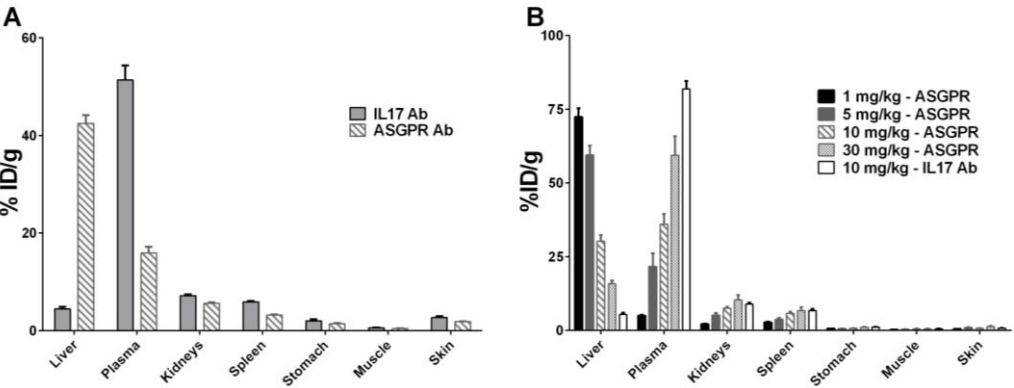
➔ PK profiles show **non-linear concentration time profiles** that are consistent with TMDD



Parameter estimates					
Parameter	Units	Population mean parameter		Intersubject variance	
		Estimate	RSE(%)	Estimate	RSE(%)
Vc	mL	1.12	5	0.115	26
Kd	nM	4.13	5	0.145	25
koff	1/day	12.31	2	0.0662	26
Rbase	nM	647	4	-	-
keRL	1/day	0.139	3	0.0599	39
kdeg	1/day	1.06	13	0.413	23
keL	1/day	0.449	13	0.302	37
Q	mL/day	20.28	13	0.296	40
Vp	mL	3.79	18	0.58	24
b	-	0.114	8	-	-

RSE: relative standard error, b: proportional error model

Biodistribution study: TMDD is located in liver

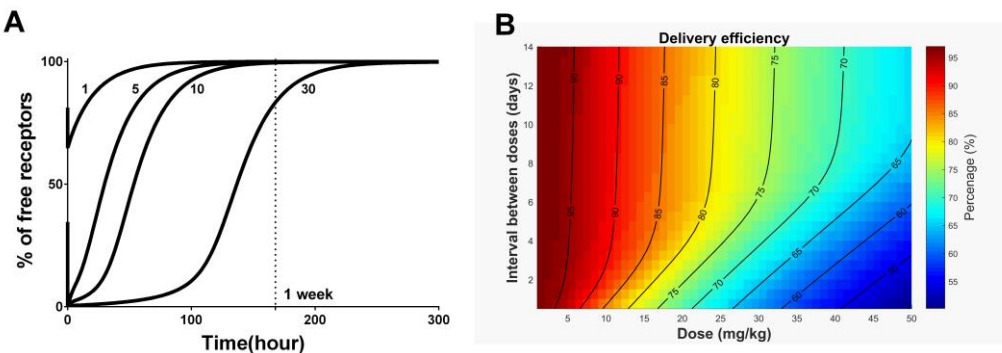


Tissue distribution:

- A: **Large accumulation of ¹¹¹In ASGPR in liver in comparison to other organs** 8h after a 10 mg/kg IV dose. In the contrary, the **IL17 Ab poorly distributes in tissues** and remains mostly in the plasma at the same dose.
- B: **Liver uptake decreases with dose increases** due to receptor saturation (0.5h after single IV doses). Non-specific distribution of IL17 Ab mimics a very high dose of ASGPR Ab where the receptor would be completely saturated.

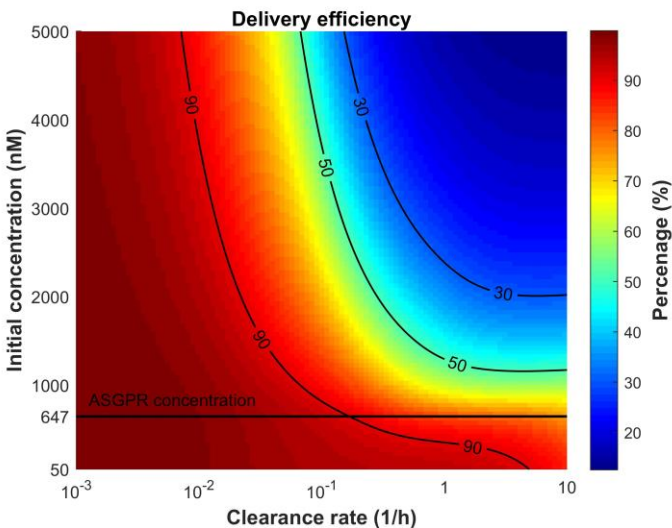
Simulation: optimization of administration protocol using TMDD model

- Once the model is fully parametrized, it can be used to optimize a dosing protocol in order to maximize the delivery efficiency, i.e., maximizing the internalization through the receptor, and therefore reducing the off-target distribution and unspecific clearance.



- A: Simulated free available receptor concentrations over time in mice after IV bolus for all tested doses (mg/kg). **The higher the dose the longer the receptor stays saturated.**
- B: Delivery efficiency (% of the dose internalized via the target-mediated clearance pathway) after two consecutive doses IV, varying the total amount and the interval between doses. **To maintain the same delivery efficiency with high dose levels requires to increase the interval between doses.**

Simulation: influence of plasma sustainability on delivery efficiency



The figure depicts that, for concentrations higher than the receptor baseline concentration, compounds with faster clearance show decreased delivery efficiency. Therefore, for compounds with high clearance rate such as small molecules, to sustain a high delivery efficiency special attention should be given to the dosing regimen.

Conclusions

- Using PK data and an in-silico TMDD model, we estimate an ASGPR concentration at baseline of 647 nM (36 nmol/kg). The half-life of the degradation of the receptor was found to be equal to 15 hours and the formed ligand-receptor complex is internalized with a half-life of 5 days.
- A biodistribution study was performed and confirmed the localization of the TMDD, i.e. in the liver. Therefore, assuming that the ASGPR is mainly expressed at the cell surface of hepatocytes and using the reported value of hepatocellularity number [4] the binding capacity can be translated into a surface expression of 1.8 million ASGPR/hepatocytes.
- The kinetics of the ASGPR shows that saturation of the shuttle at therapeutic concentrations is possible; however, simulation allows the dosing schedule to be optimized. The developed TMDD model can be used to support the development of therapies that use the ASGPR as a shuttle into hepatocytes.

References

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4. Sohlenius-Sternbeck, A.K., *Determination of the hepatocellularity number for human, dog, rabbit, rat and mouse livers from protein concentration measurements*. Toxicol In Vitro, 2006. 20(8): p. 1582-6.