Comparison of clinical oligonucleotides against HBV - what are crucial PK/PD features?

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PK/PD processes of ASOs and siRNAs

Targeting hepatocytes via GalNAc

SC Dose

Plasma exposure

Liver exposure

Nonspecific uptake + GalNAc targeting

GalNAc targeting

Hepatocyte exposure

Internalization

Endosomal escape

Nucleus*

Binding to targeted mRNA

RNAse H1 recruitment

Binding to targeted mRNA

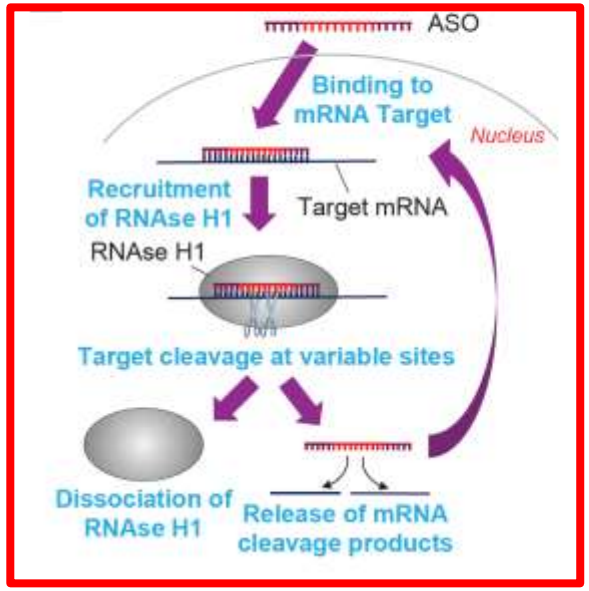
Reduction in mRNA levels

Reduction in protein levels

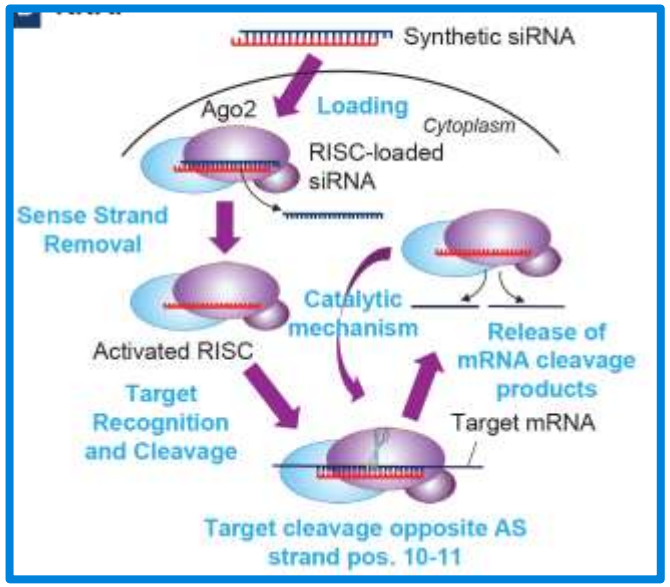
Cytosol*

Bind to Dicer, TRBP and Argonaute to form mature RISC

Antisense oligonucleotide (ASO)



siRNA



Chan et al., Poster, Alnylam Pharmaceuticals, Inc., Cambridge, MA, USA

* siRNAs and ASOs with different cellular location have also been described

PK/PD of oligonucleotides vs small and large molecules

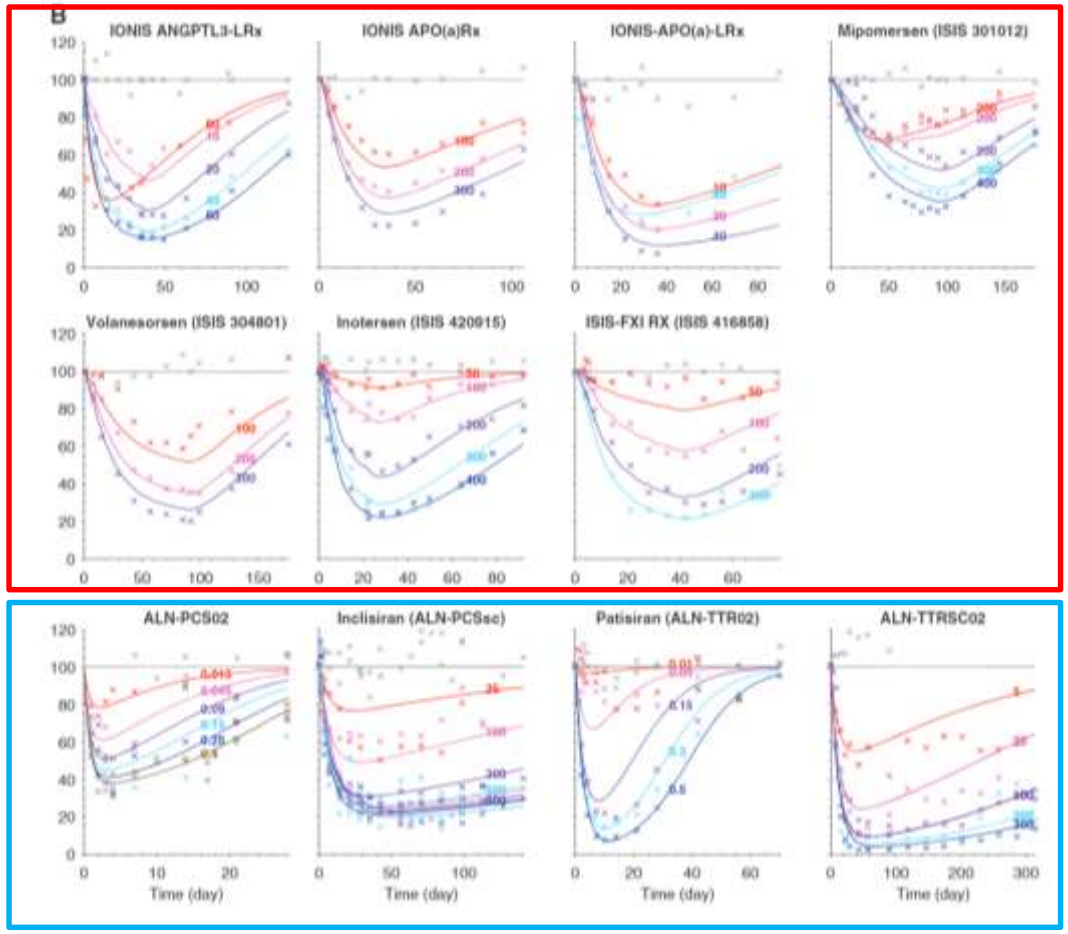
PK/PD	Small Molecule	Large Molecule	Oligonucleotide
Exposure	Relevant exposure is typically plasma PK	Relevant exposure is typically plasma PK	Relevant exposure is intact drug in tissue
Tissue penetration	good tissue penetration	Fair/low tissue penetration	High tissue penetration (when targeted)
PK $T_{1/2}$	hours-days	days-weeks	weeks-months
Scaling methods	Scaling from in-vitro to human established	Scaling from in-vivo to human established (if no TMDD)	Scaling on anticipated tissue concentrations

- Oligonucleotides create new PK/PD challenges mostly due to their delivery and long effects.
- Mechanistic frameworks that could guide species translation and dose finding are often lacking so far.

PK in relevant tissue is often not measurable in the clinic

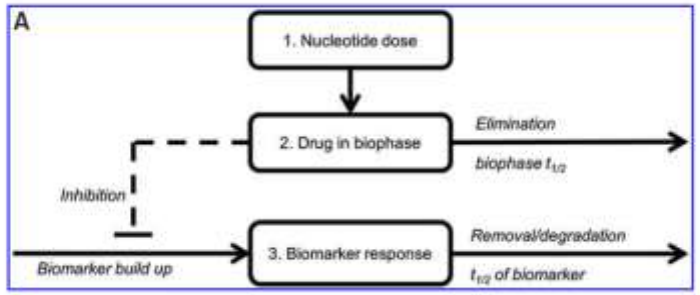
What can be done?

1. Relationship between tissue PK/ knock down/ biomarker kinetics established in preclinical species or in-vitro
2. Modeling often crucial to identify relevant PK/PD parameters
3. Species scaling based on anticipated tissue concentrations, turnover models
4. Biomarkers in the clinic used to infer crucial PK/PD parameters in relevant tissues



ASOs

siRNAs



Inferring Half-Lives at the Effect Site of Oligonucleotide Drugs
10.1089/nat.2018.0739

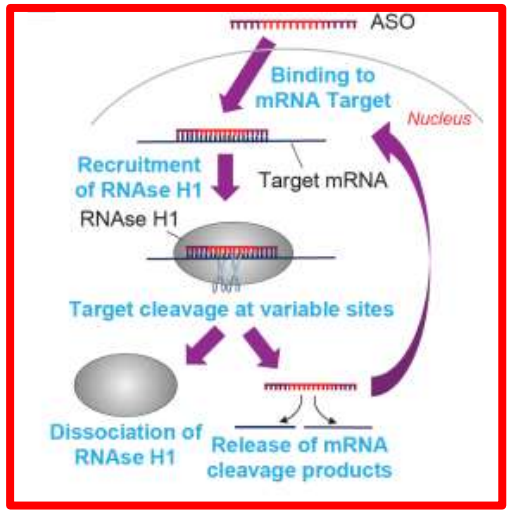
Rasmus Jansson-Löfmark and
Peter Gennemark, AZ

Oligonucleotides for blocking HBV protein synthesis

Multiple molecules now in the clinic that target viral RNA

ASO

RNAse H1

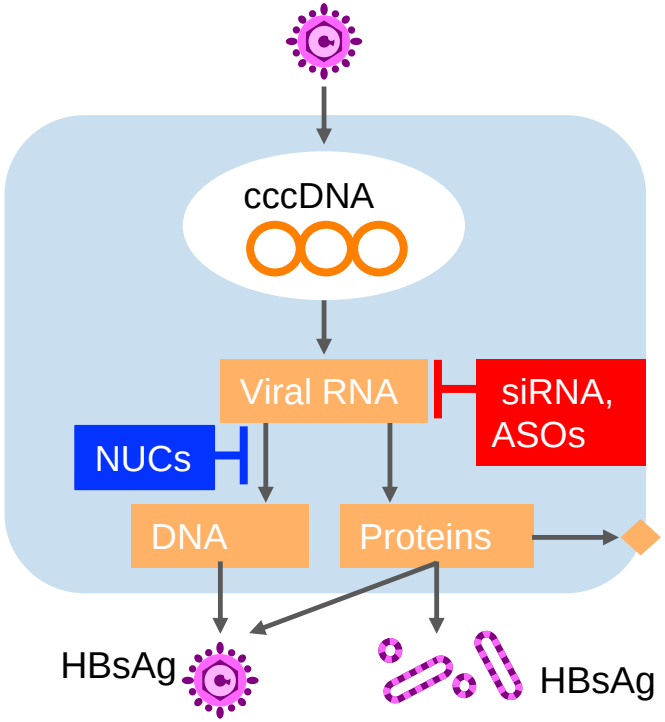


Naked (non-GalNAC)

- ISIS505358/GSK836

GalNAC conjugated

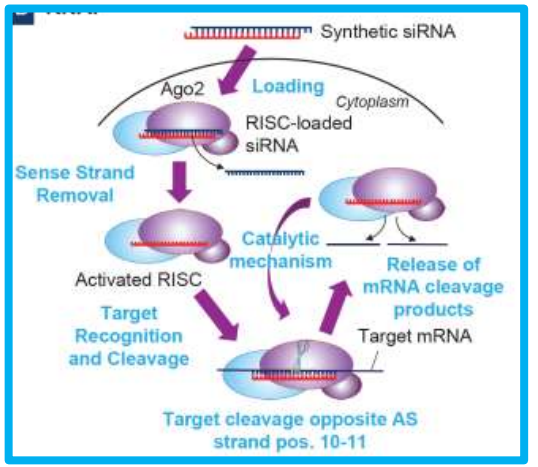
- RO7062931
- GSK3389404



Virions and SVPs
Ratio 1:10,000

siRNA

RISC



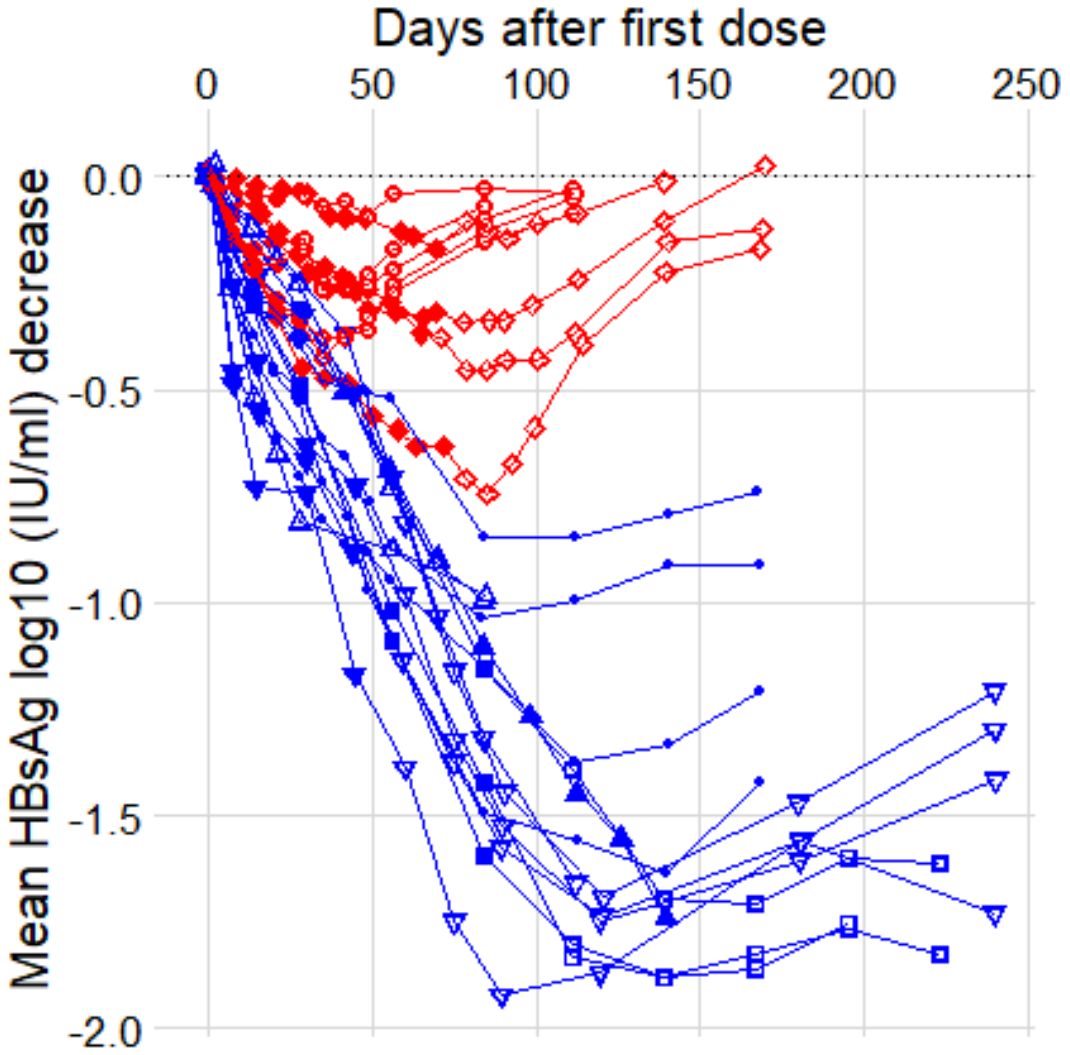
GalNAC conjugated

- AB-729
- ARO-B/JNJ-3989
- DCR-HBVS/RG-6346
- ALN HBV02/VIR-2218

All clinical trials for these molecules have HBsAg response as an endpoint, which creates unique opportunity to compare ASOs and siRNAs

siRNAs show greater reduction in HBsAg in patients compared to ASOs

Data analysis of GalNAc-targeted molecules in the clinic against HBsAg



Digitized data of GalNAc-targeted anti-HBV oligonucleotides. Data shows different cohorts investigated per molecule until max day 250 after first dose. Open symbols show the response after stop of dosing (off-treatment).

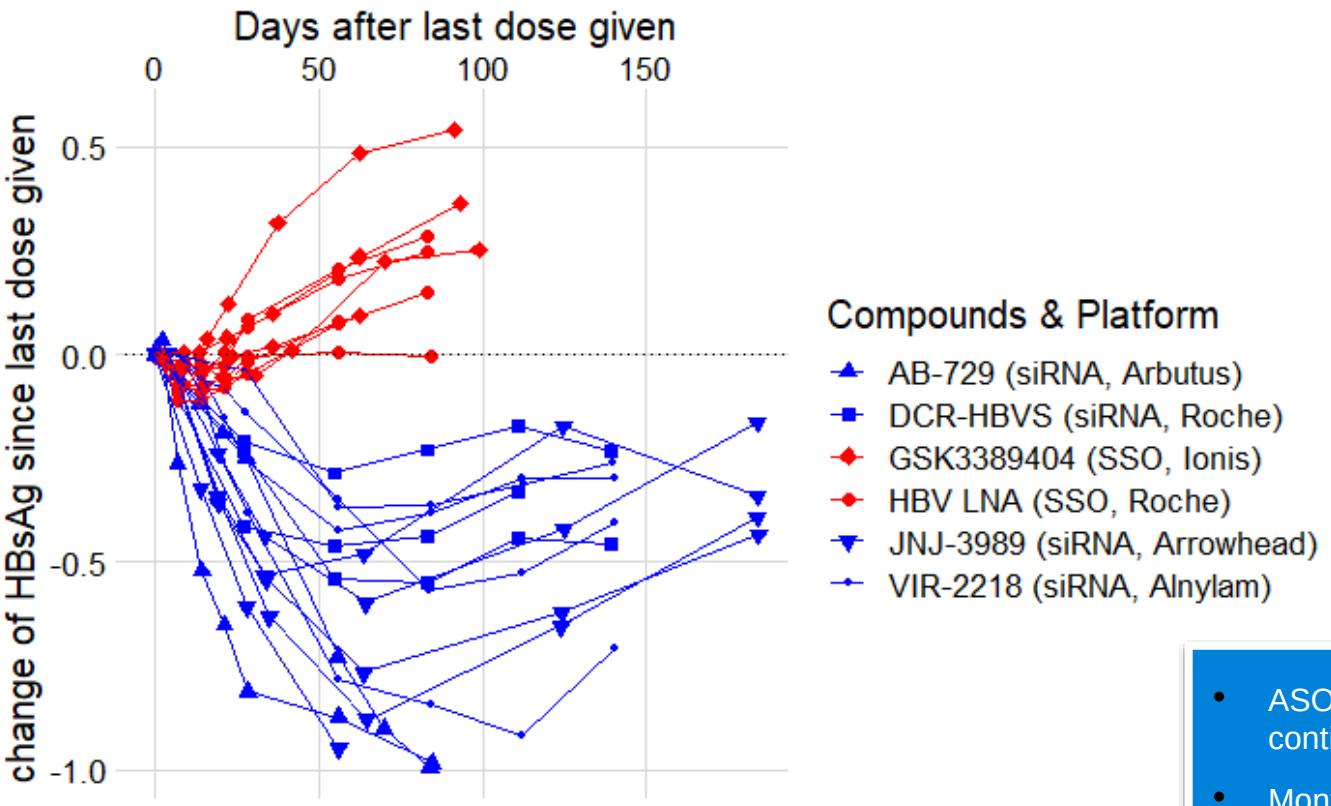
Compounds & Platform

- AB-729 (siRNA, Arbutus)
- DCR-HBVS (siRNA, Roche)
- GSK3389404 (SSO, Ionis)
- HBV LNA (SSO, Roche)
- JNJ-3989 (siRNA, Arrowhead)
- VIR-2218 (siRNA, Alnylam)

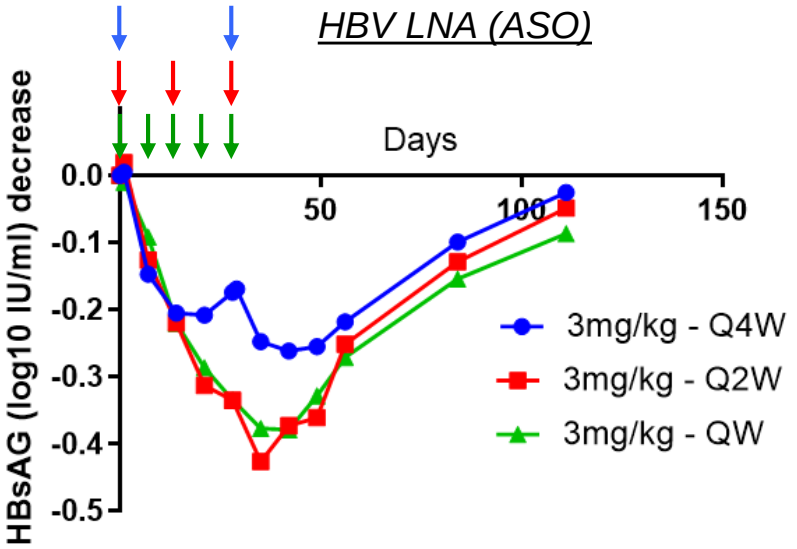
- Analysis excludes non-GalNAc ASOs
- SSOs were dosed multiple time per month (up to every 3rd day), while dosing of siRNA was monthly.
- Limitations of comparison: Dosing frequency, doses & number of doses used are different for SSOs & siRNAs
- Four different GalNAc-siRNAs (blue) show a stronger inhibition of HBsAg than two GalNAc-ASOs (red).

siRNAs show greater reduction in HBsAg in patients compared to ASOs

Longer duration of action could be a key factor



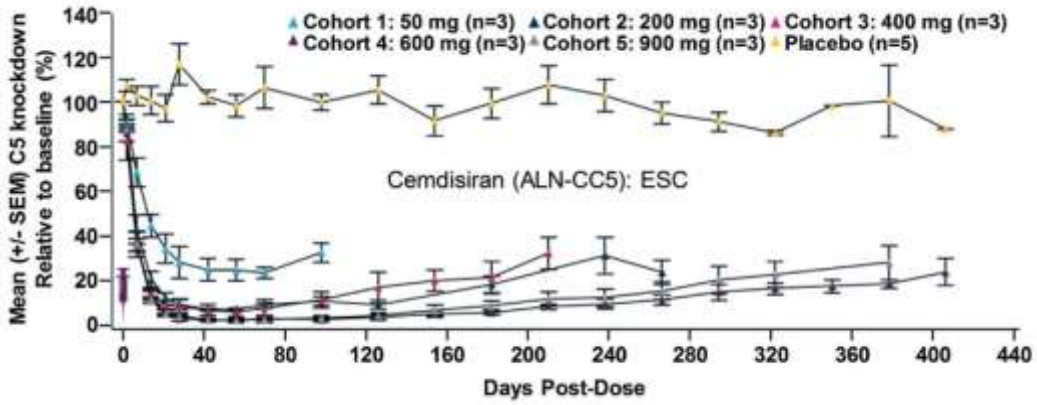
Digitized data of GalNAc-targeted anti-HBV oligonucleotides. Digitized data of GalNAc-targeted anti-HBV oligonucleotides. Data shows different cohorts investigated per molecule from day of last dose to max day 250 after first dose.

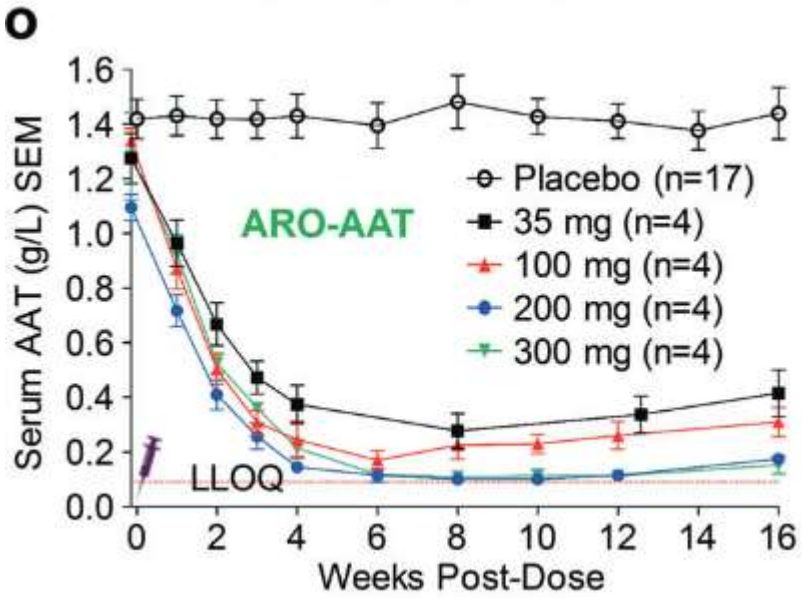


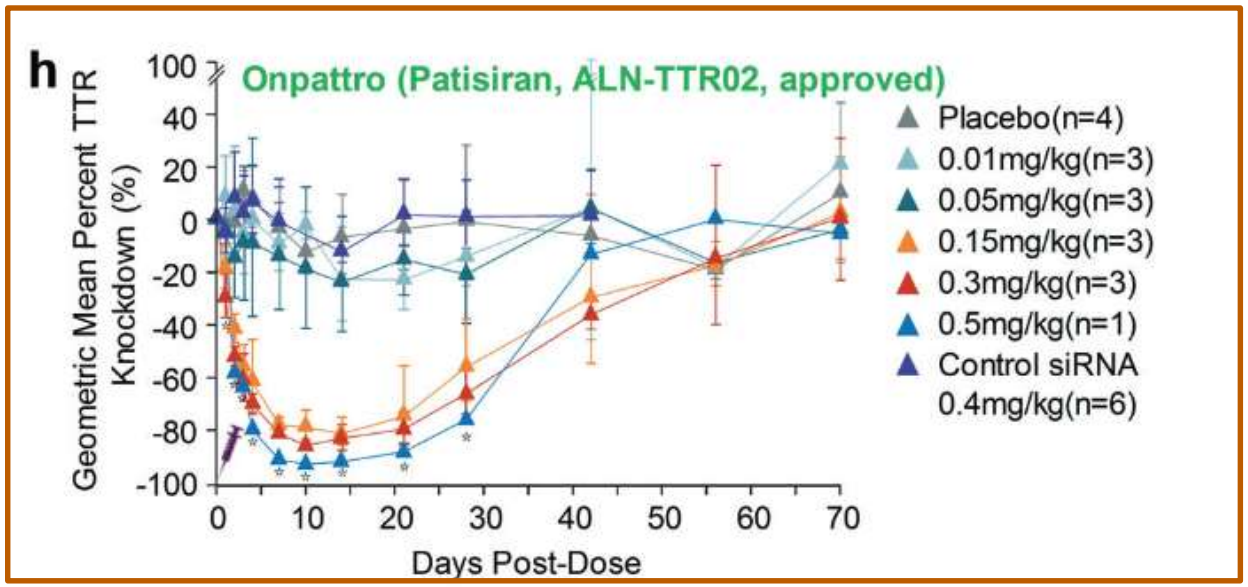
- ASOs reductions start to rebound quickly during off-treatment, while siRNAs continue to decline
- Monthly dosing of ASO in HBV patients leads to rebound of HBsAg between doses while Q2W and QW did not.
- Longer duration of action of siRNAs seems to drive their strong HBsAg effect.
- HBsAg shows a slow decrease in plasma, therefore long acting agents seem to have a distinct advantage.

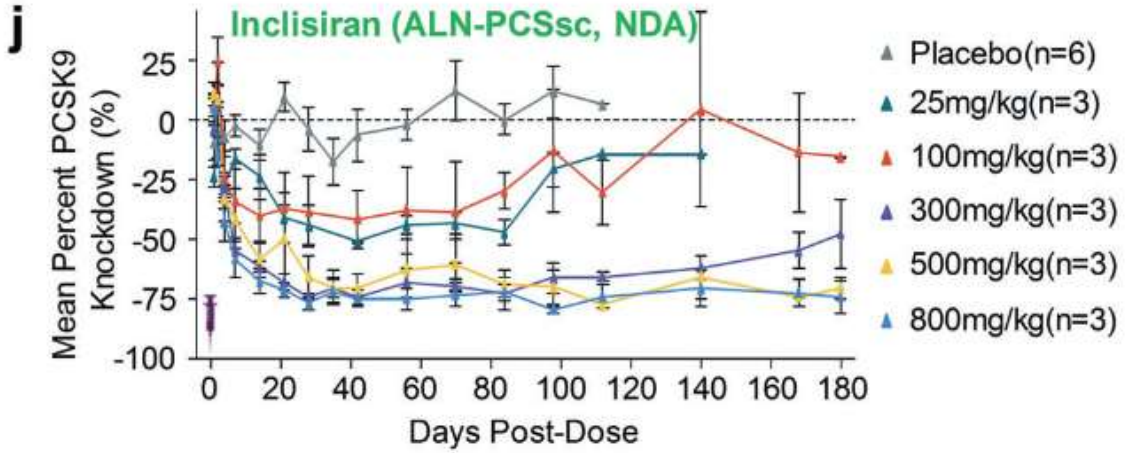
Comparison with non-HBV siRNAs in the clinic

Long effect seems universal feature of GalNAc-targeted siRNAs









No GalNAc-targeting, early chemistry

Hu, B. et al. Therapeutic siRNA: state of the art. Signal Transduct Target Ther 5, 101 (2020).

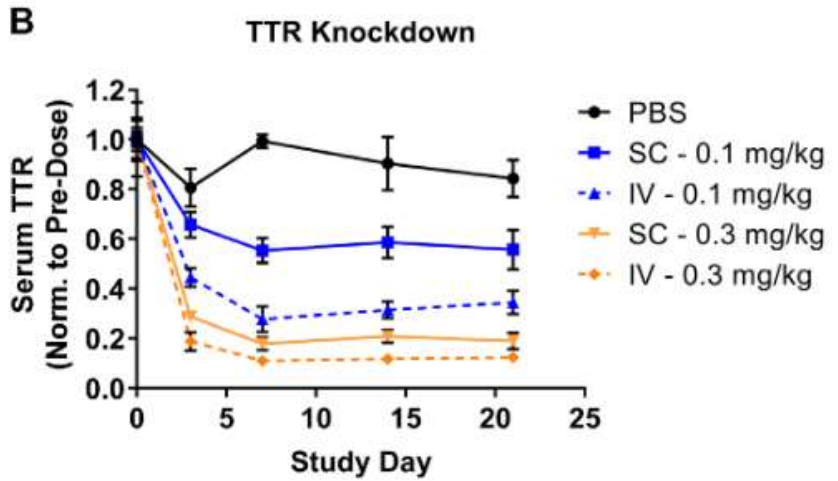
But what is driving the long duration of action of siRNAs?

PK/PD cascade of siRNA

What is driving the long activity of GalNAc-siRNAs?

Slow release of compound from the injection site?

Investigating the pharmacodynamic durability of GalNAc–siRNA conjugates, Nucleic Acids Research, 2020, doi: 10.1093/nar/gkaa670



SC Dose

Plasma exposure

Liver exposure

Hepatocyte exposure

Internalization

Endosomal escape

Cytosol*

Bind to Dicer, TRBP and Argonaute to form mature RISC

Binding to targeted mRNA

Reduction in mRNA levels

Reduction in protein levels

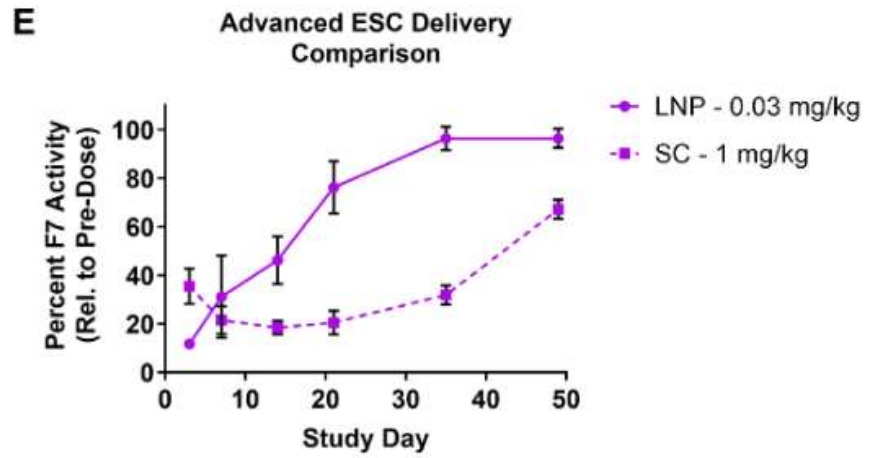
SC and IV administration leads to strong knock-down and long duration of action.

PK/PD cascade of siRNA

What is driving the long activity of GalNAc-siRNAs?

Could the functional RISC complex have a long half-life?

Investigating the pharmacodynamic durability of GalNAc–siRNA conjugates, Nucleic Acids Research, 2020, doi: 10.1093/nar/gkaa670



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RISC loaded antisense levels at the time of maximum target knockdown were similar for SC and LNP delivery, but duration was different. Hence, RISC-loaded siRNA has a finite half-life that cannot support prolonged target knockdown without being replenished

PK/PD cascade of siRNA

What is driving the long activity of GalNAc-siRNAs?

Is an intracellular depot driving the duration of action of siRNAs?

Investigating the pharmacodynamic durability of GalNAc–siRNA conjugates, Nucleic Acids Research, 2020, doi: 10.1093/nar/gkaa670

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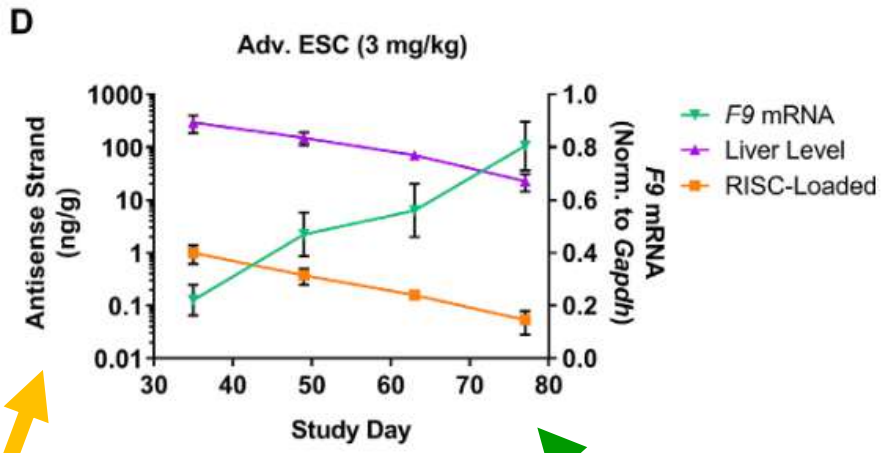
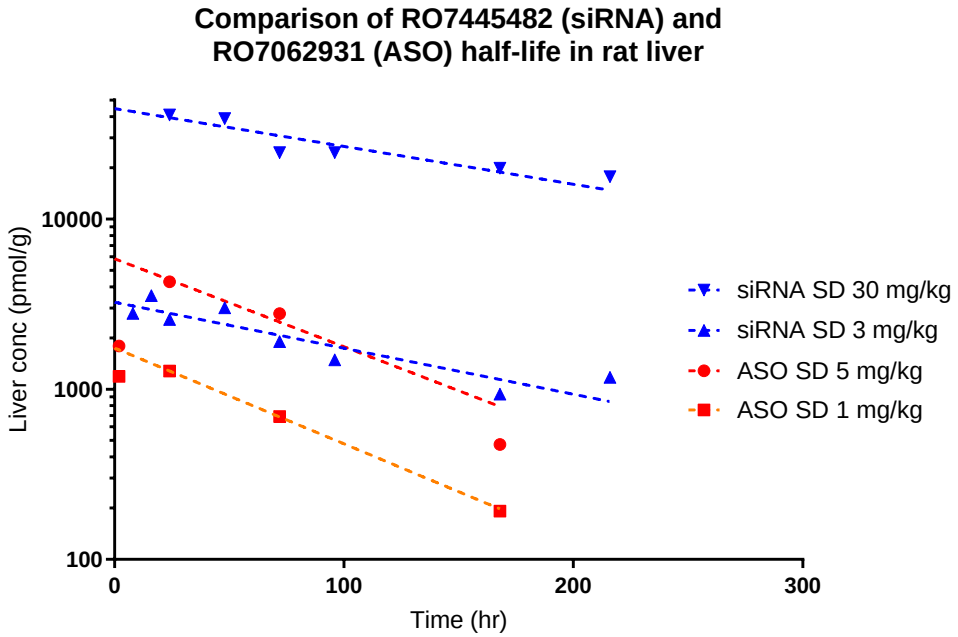


Paper suggests total liver concentration is driving RISC-loading and therefore duration of effect. Hence normal PK/PD considerations as for other modalities can be applied.

Learning about PK/PD of siRNAs from preclinical experiments

Half-life in rat liver



siRNA (RO7445482)

Dose	T1/2
3 mg/kg	111 h
30 mg/kg	134 h
300 mg/kg	147 h

~140h half life in rat liver

ASO/LNA (RO7062931)

Dose	T1/2
0.3 mg/kg	79 h
1 mg/kg	78 h
5 mg/kg	85 h

RO7062931 ~80h half life in rat liver

- RO7445482 (siRNA) shows a longer (~1.8x) half-life in rat liver than RO7062931 (ASO)
- In NHP, no SD liver PK data exists so far - but initial data suggest difference in monkey even bigger (~3x).
- Preclinical liver stability data important to judge on likely duration of effect in humans.
- Turnover of target is important to link compound half-life in liver with anticipated duration of effect.

PK/PD cascade of siRNA

Time delay between liver exposure, RISC loading and mRNA/protein knockdown

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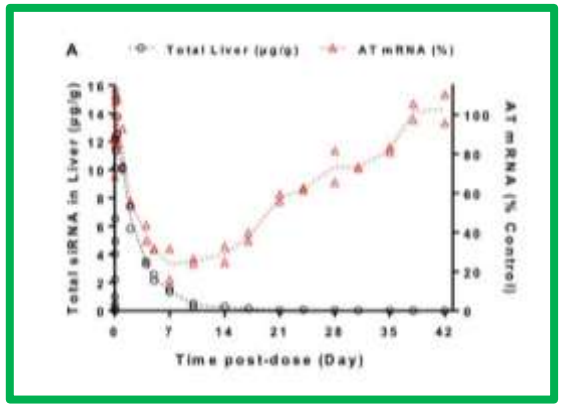
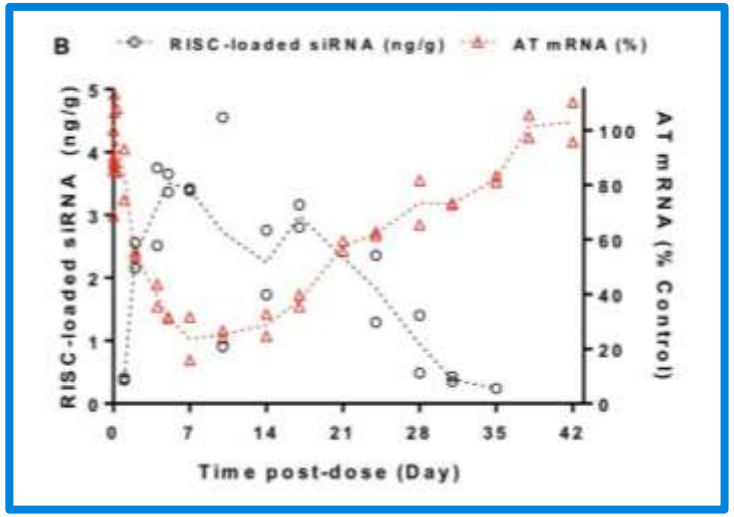
Alnylam data



Half life of total siRNA in liver ~1 days (mice)

Alnylam data

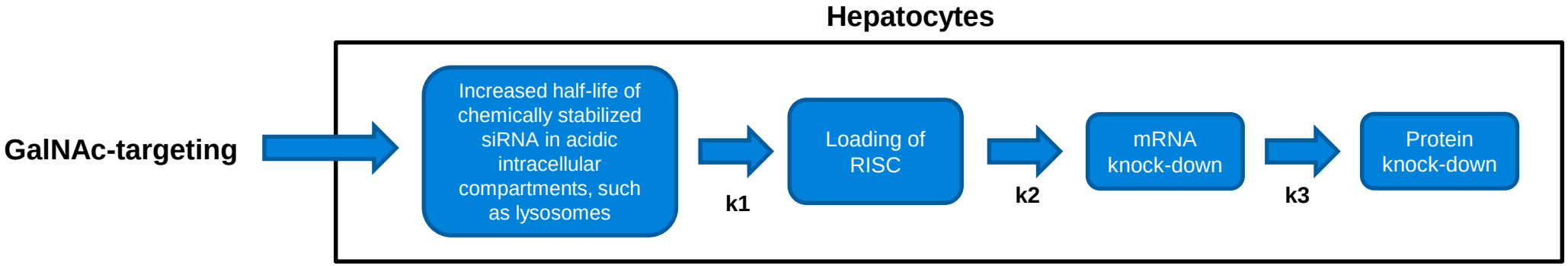


Potency and duration of effect of siRNA depends on different dynamic processes (tissue half-life, RISC loading, mRNA dynamics and protein dynamics) that are coupled like as a usual coupled indirect-response models.

Nair, J. K. et al. Impact of enhanced metabolic stability on pharmacokinetics and pharmacodynamics of GalNAc–siRNA conjugates. Nucleic Acids Research 45, 10969–10977 (2017).

PK/PD cascade of siRNA

Relationship between liver exposure and mRNA/protein knockdown



For siRNA: k1 is slow, k2 usually is fast, k3 depends on the turnover of the protein

- Longer tissue half-life (possibly a functional intracellular reservoir that can sustain RISC loading), together with indirect response model, driven by mRNA and protein turnover, could be responsible for longer effect of siRNAs vs ASOs.

- Other processes could also contribute to the long effect of siRNAs, but this is poorly understood right now.

- Possible further processes of ASO and siRNAs are contributing to the different duration of action:

1. *Cellular location of action (nucleus vs cytoplasm)*
2. *Continuous occupation of Ago2 protein by the guide strand vs the transient interaction of RNAse H with ASO/mRNA duplexes*
3. *More nonspecific binding of ASOs due to higher percentage of PS modifications.*
4. *Different cellular processes that influence potency and therefore allow to maintain efficacious tissue levels for longer*

Conclusions

- Advances in siRNA design, primarily through **chemical modification for increased metabolic stability**, have led to steady improvements in potency and duration of GalNAc– siRNA conjugates.
- Long duration of action of siRNA is likely due to **efficient loading** of the liver/hepatocyte and the **high stability** of siRNAs in tissues, but other processes could contribute.
- Better understanding of contributing processes would benefit molecular design and inform the anticipated duration of actions in humans.
- Long duration of action has been demonstrated for GalNAc-siRNAs for other disease areas.
- Similar duration of action of siRNA for i) tissues other than the liver and ii) delivery technologies other than GalNAc targeting remains to be fully confirmed.

Thank you to Dicerna & Roche colleagues.....

Henrik Müller, Søren Ottosen, Cynthia Wat, Sabine Lohmann, Ved Pavlovic, Bernadette Surujbally, John Young, Martin Bopst, Nelson Guerreiro, Simon Buatois, Yann Tessier, Miriam Triyatni and many more.

Bob Brown, Wendy Cyr, Jeffrey Foy, Amber Beaudry, Mark Pirner, and Andy Amrite

And thanks to you for the attention!

Doing now what patients need next