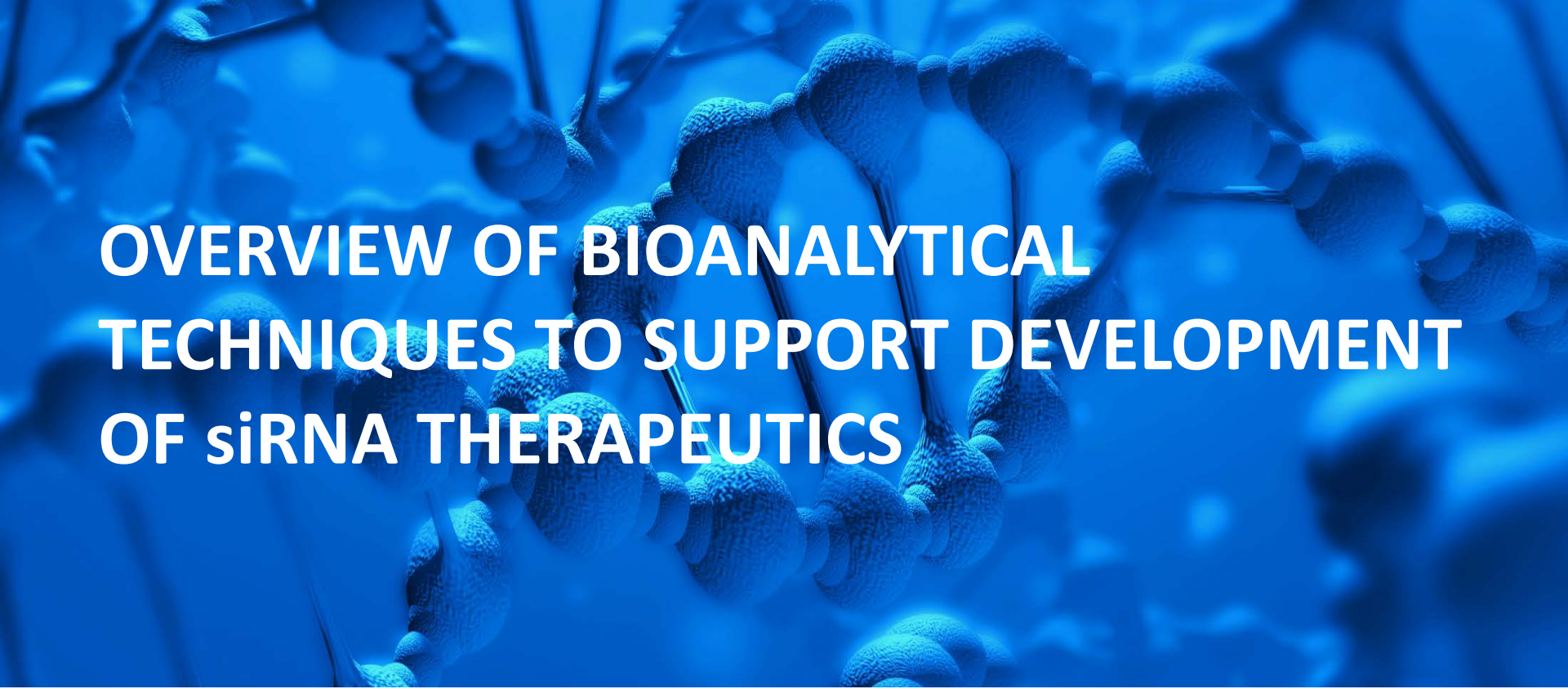

OVERVIEW OF BIOANALYTICAL TECHNIQUES TO SUPPORT DEVELOPMENT OF siRNA THERAPEUTICS

BROOKE ROCK, TRANSLATIONAL SCIENCES

ASCPT, 2018

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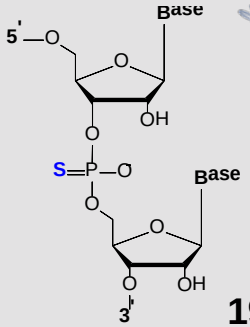
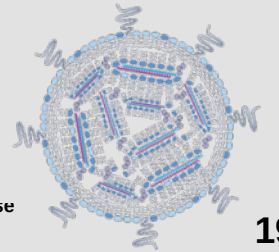
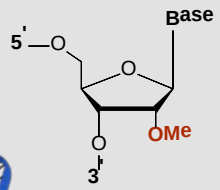
OVERVIEW OF PRESENTATION ON BIOANALYTICAL TECHNIQUES TO MEASURE OLIGONUCLEOTIDE THERAPEUTICS (siRNA)

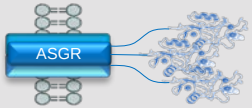
- **Brief overview of RNAi mechanism and history of siRNA development**
- **Comparison of siRNA modality to small molecule and large molecule therapeutics**
- **Discussion on bioanalytical measurements to inform translational of preclinical data to clinical dose**
  - **Focus of GalNac-siRNA constructs targeted to the liver**
- **Consideration for delivery to other tissues**

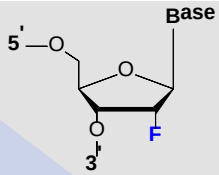
HISTORY TO THE DISCOVERY OF siRNA: CHEMICAL MODIFICATIONS & ASGPR HAVE DRIVEN siRNA EVOLUTION











1971
Clearance of asialoglycoproteins (Ashwell receptor)

1984
Phosphorothioate induced stabilization and increased protein binding
W. Stec, G. Zon, & W. Egan

1990s
Lipid nanoparticle delivery (ASO) with poorly stabilized RNAs.
First utilization of ASGR1 ligands

1998
Genetic interference and siRNA 21-nucleotide duplexes (Mello 1998 & Tuschl, 2001)

2003-2006
2’ Ribose stabilization and reduced off-target (TLR) via 2′-O-methyl modification

2011-13
Enhanced stabilization via alternating 2′-OMe & 2′-fluoro (F)
ASGPR delivery for siRNA GalNAc-siRNA conjugates

Pending first approval for siRNA Feb 2018:
https://www.nature.com/articles/nrd.2018.20

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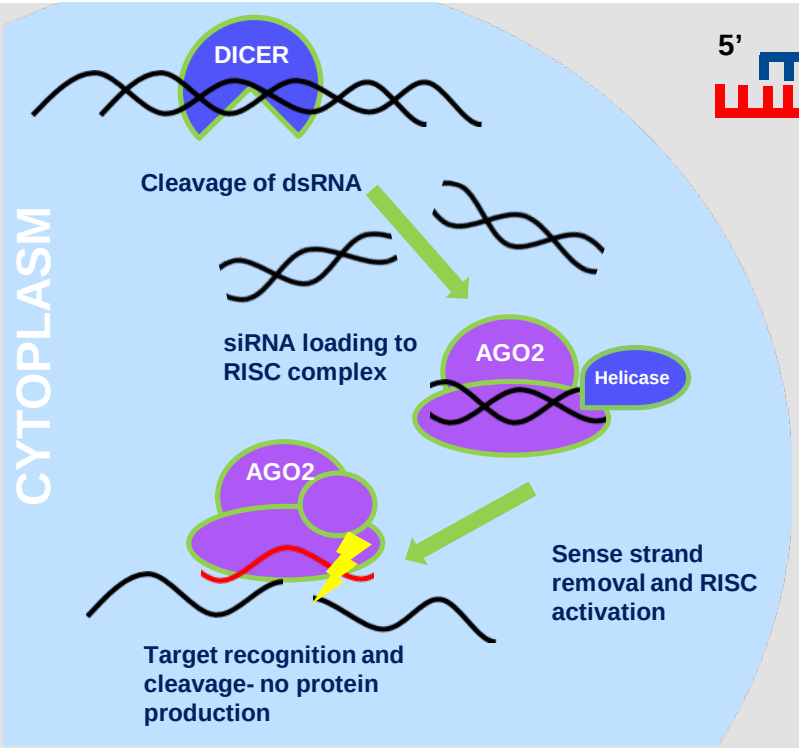
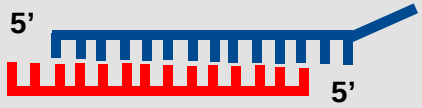
NATURAL MECHANISM OF RNA INTERFERENCE:

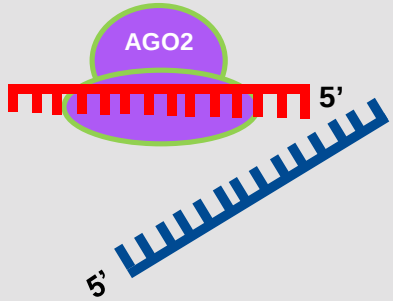
RNA interference has an important role in defending cells against parasitic nucleotide sequences, and influences development



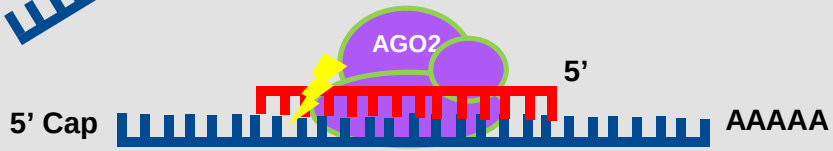


Therapeutic Intervention

Synthetic siRNA duplex



Disassociation of passenger strand (by Helicase)



Target mRNA Degradation and catalytic release of mRNA product

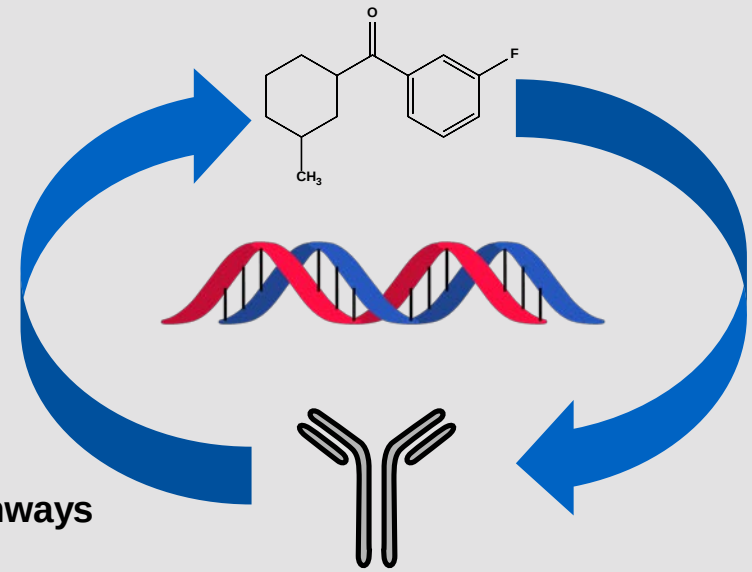
SIRNA IN COMPARISON TO CLASSIC SMALL MOLECULE AND LARGE MOLECULES THERAPEUTICS

Typical small molecule Bioanalytical Techniques

LC/MS for drug Measurements and Metabolite ID

Potential for DDI (co-mediations)

Phenotyping to understand Magnitude of clearance pathways



Typical large molecule Bioanalytical Techniques

Immunoassay based Assays for drug measurements

Immunogenicity assays

Role of target in clearance And efficacy

All modalities rely on multiple measures to aid in translate of preclinical results into clinical setting

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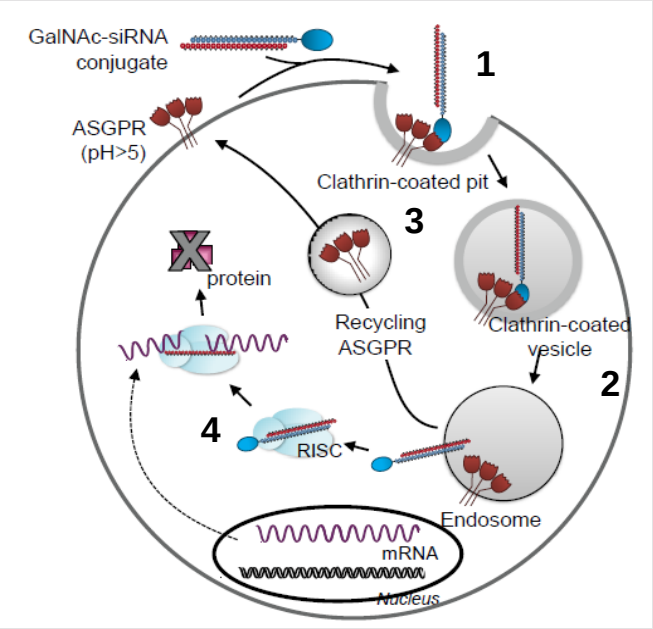
BIOPHYSICAL CHARACTERISTIC DIFFERENCE OF EACH MODALITY: WHAT ARE THE CRITICAL PIECES FOR siRNA?

Property	Small Molecule	Large Molecule	siRNA
Common Administration Route	Oral	SC; IV	SC; IV
Physical Chemical Properties	MW<600 LogP>2	MW>150 kDa pI= 5-9	MW~ 15kDa Negative charge
Distribution/Clearance	Equilibration of unbound drug allow activity intracellular	Can be driven by target antigen; FcRn recycling	Can be driven by delivery vehicle or target ligand
Metabolism	Hepatic metabolism, transporters	Catabolism to amino acids Limited metabolism	Nuclease activity (blood, at target); metabolism of target ligand
Pharmacodynamics	Direct relationship to plasma kinetics	Indirect or direct models link PD to serum kinetics	Tissue and subcellular kinetics are more relevant

MODELING SIRNA MECHANISM OF ACTION: A CLOSER LOOK AT LIVER TARGETED DELIVERY



1. Saturation of the ASGR occurs at concentrations > 35 mg, the # of receptors per cell ~500,000

Spiess, M. (1990). *Biochemistry*,*29*(43), 10009–10018

2. Release from the endosome is rate-limiting step (best case 2% dose)

Gilleron, J et al. Nature Biotechnology 31: 7 (2013)

3. The mean recylcing time of ASGR is 5-13 mins

Harrison, N et al. JBC 14:1 (1981)

4.siRNA binding to RISC (RNA induced silencing complex) in the cytosol cause unwinding of the duplex and binding to mRNA target to silence/knockdown protein

Figure presented by Alnylam at DIA/FDA Oligonucleotide Based Therapeutic Conference, 2015

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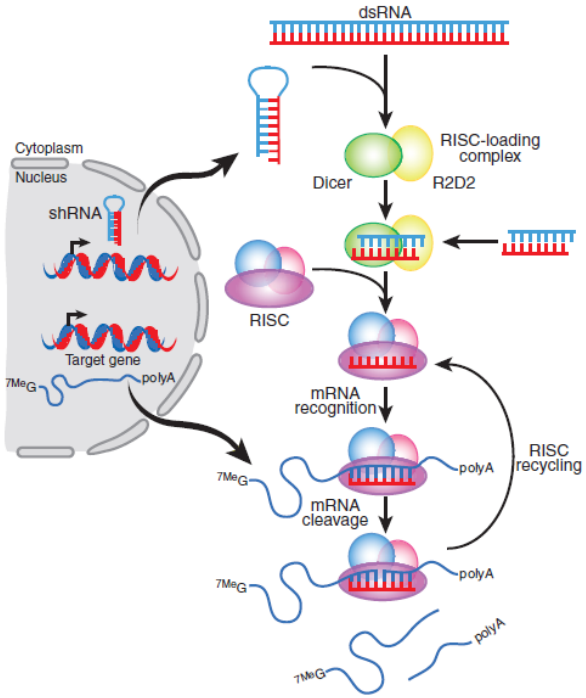
WHAT IS RISC?

- **RISC = RNA-induced silencing complex, mediates post-transcriptional gene silencing**
  - **Dicer – RNase III family member**
  - **TRBP (HIV transactivating response RNA-binding protein) – orientates siRNA loading**
  - **Ago2 – endonuclease, catalytic core of RISC**

- **Phase I: Programming – siRNA loading into Ago2**
- **Phase II: Execution – cleavage of mRNA target complimentary to antisense (guide) strand of siRNA**
- **Repeat: Catalytic Process**



Journal of Cell Science 123:1183-1189 (2010)

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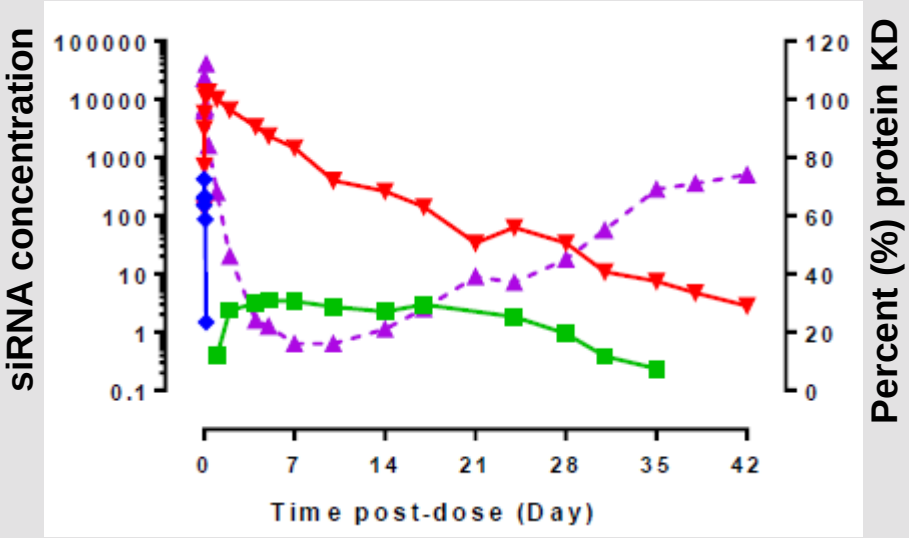
A CLOSER LOOK AT THE MECHANISM OF ACTION FOR LIVER-TARGETED SIRNA MOLECULES: SIMPLIFYING TO RATE CONSTANTS

- **Blood Pharmacokinetics is not sufficient to describe the PK-PD of siRNA mechanism**
- **Two compartment modeling of liver pharmacokinetics can describes the long duration of effect**



Symbol	Description
▲	Percent (%) Target knockdown
▼	Liver siRNA drug concentrations
◆	Plasma siRNA drug concentrations
■	Amount of siRNA bound to RISC

Lag time is observed between peak liver concentration and maximum KD

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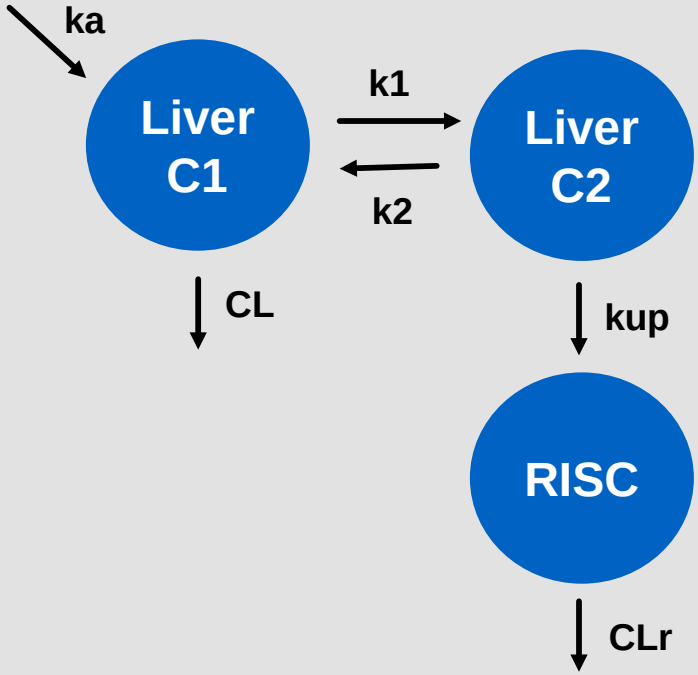
A CLOSER LOOK AT THE MECHANISM OF ACTION FOR LIVER-TARGETED SIRNA MOLECULES: SIMPLIFYING TO RATE CONSTANTS



Establishing the link between liver kinetics and protein knockdown in preclinical species

<u>Assumes:</u> serum surrogate marker for protein KD In humans

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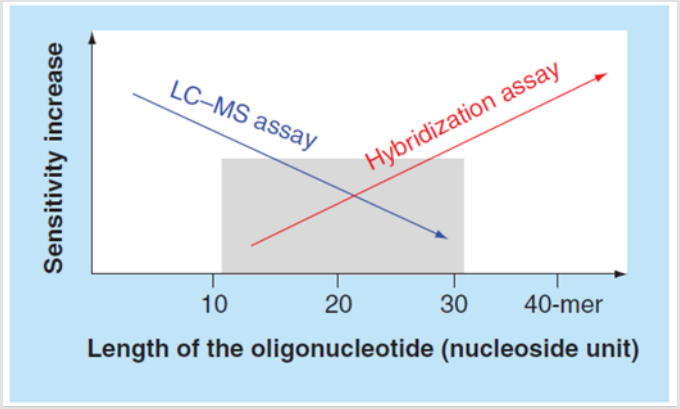
WHAT TOOLS ARE AVAILABLE TO INFORM MODELING: OVERVIEW OF BIOANALYTICAL ASSAY PLATFORMS

- **Hybridization-based assays**
  - **Watson-Crick base pairing mechanism**
  - **Enzyme involved**
  - **qPCR and digital PCR**
  - **Hybridization-ELISA**

- **Chromatographic-based assays**
  - **HPLC-UV**
  - **Hybridization LC-fluorescence**
  - **LC-HRAM or LC-MS/MS**
  - **Gel chromatography**

Sensitivity

Specificity



Bioanalysis, 2016, 8(2), 143-155

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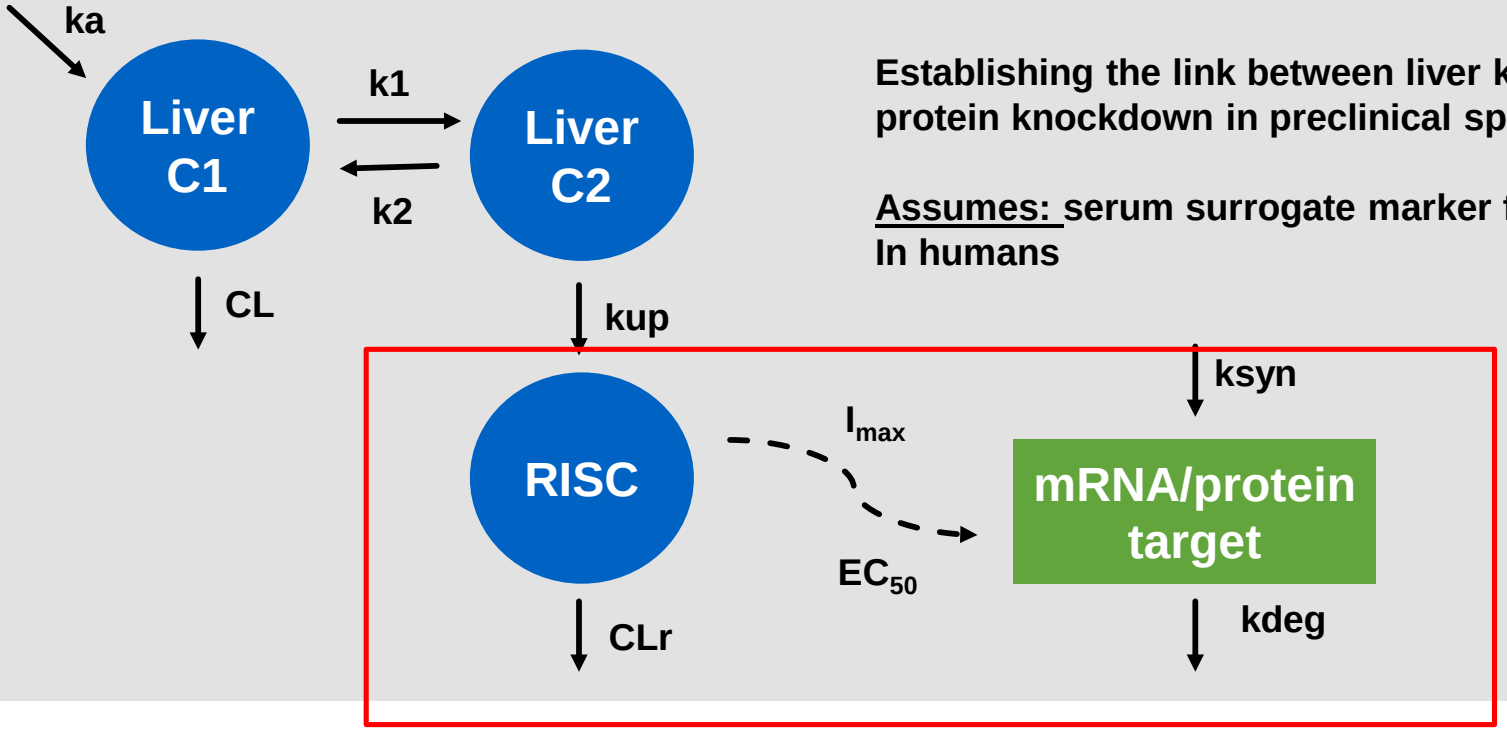
A CLOSER LOOK AT THE MECHANISM OF ACTION FOR LIVER-TARGETED SIRNA MOLECULES: SIMPLIFYING TO RATE CONSTANTS



Establishing the link between liver kinetics and protein knockdown in preclinical species

<u>Assumes:</u> serum surrogate marker for protein KD In humans

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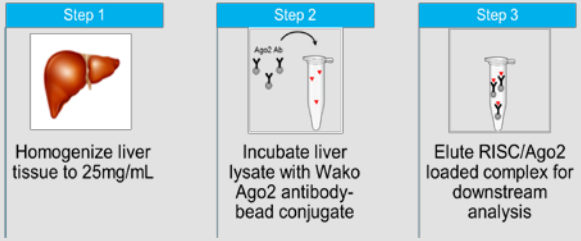
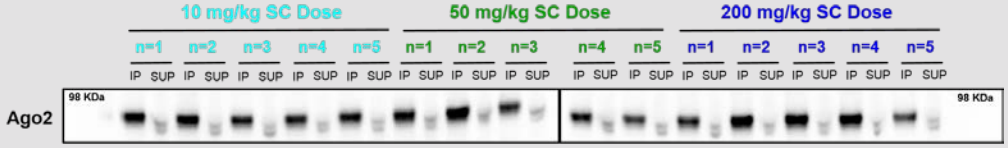
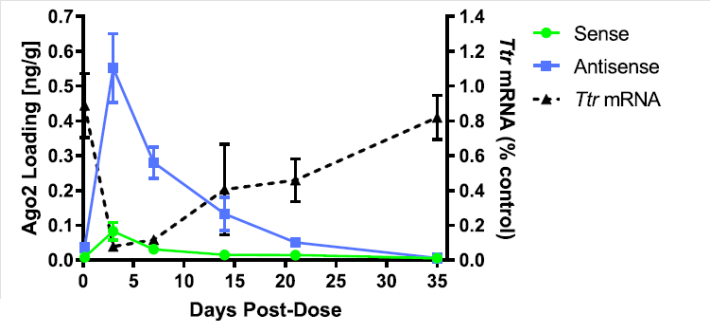
AGO2 PULLDOWN ASSAY TO MEASURE AMOUNT OF SIRNA BOUND TO RNA-INDUCED SILENCING COMPLEX (RISC)

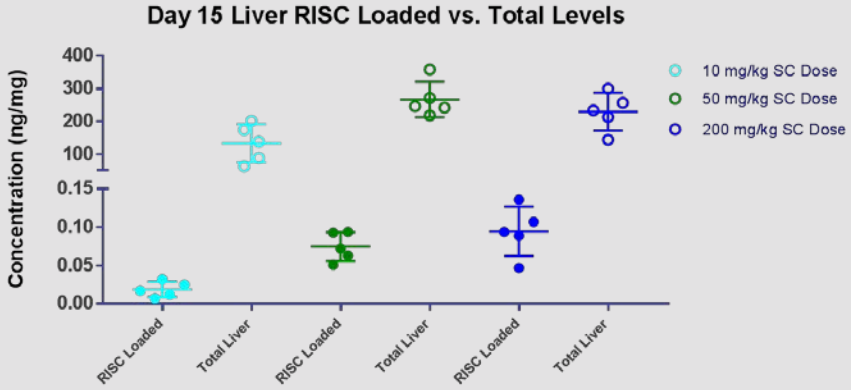
- **Commercially available kit from WAKO Chemical and previously published data* was utilized to pulldown AGO2 from liver lysate and in vivo from PK-PD studies**





Kdasljflas;kdjf;asljf;ljkasdf





*Methods modified Nucleic Acids Research 45:1469-1478 (2017)

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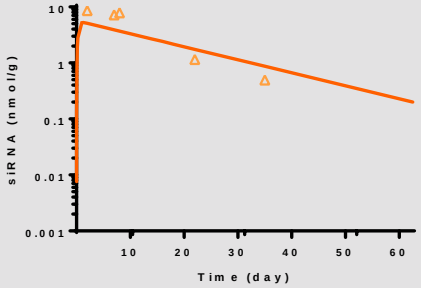
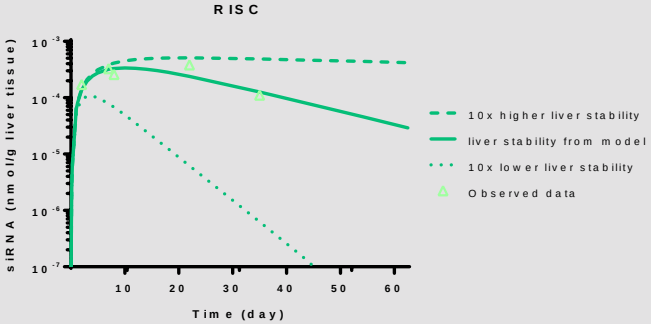
LINKING PRECLINICAL MEASUREMENTS TO SCALING HUMAN DOSE

Measurement of siRNA post 3 mg/kg dose (rat)





Human liver PK was predicted based on allometric scaling of PK parameters in rats with exponent of 1 for volume and 0.75 for clearance parameters

Predicted half-life of siRNA in the human liver determined to be 14 days at a dose of 70 mg

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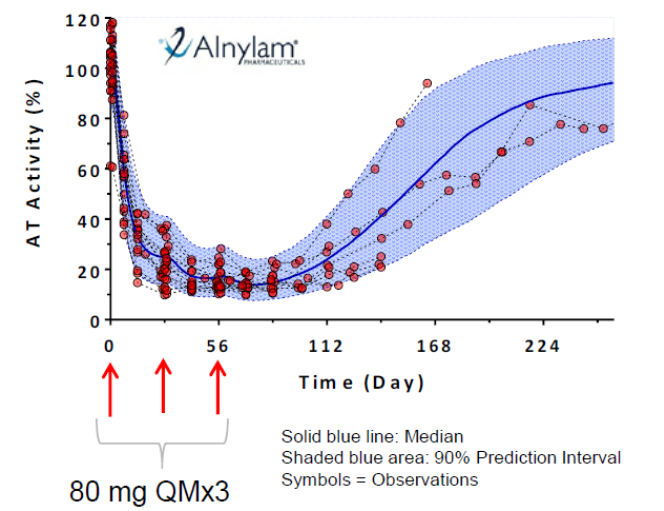
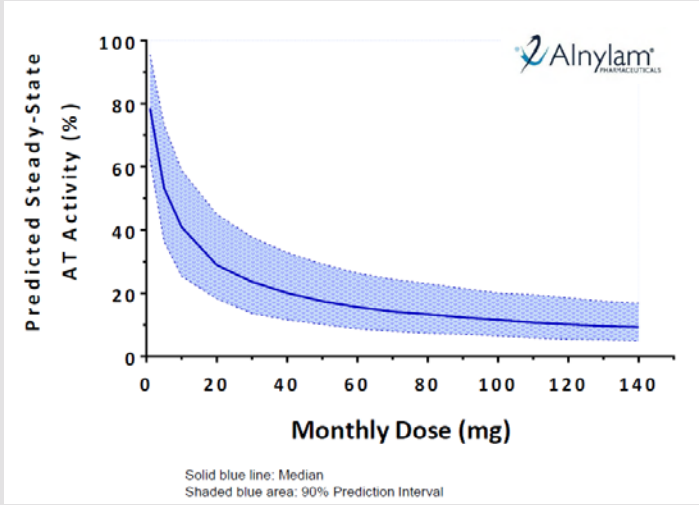
EXAMPLE OF PK-PD MODELING CONFIRMING HUMAN DOSE SELECTION AND PD OBSERVED IN THE CLINIC

The PK-PD model accurately described onset, SS and recovery phases, based on liver PK & RISC loading (rat)



Utilize the model to predict that doses greater than 80 mg would not increase efficacy



Husain Attarwala, Varun Goel, Kate Madigan, Akin Akinc, and Gabriel J. Robbie
Presentation July 10th 2017

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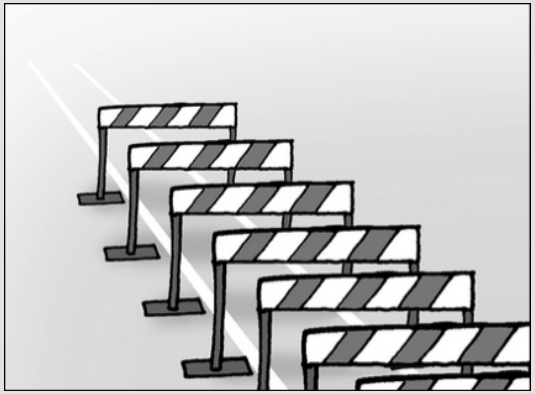
SUMMARY OF GALNAC CONJUGATES AND PK-PD PREDICTIONS

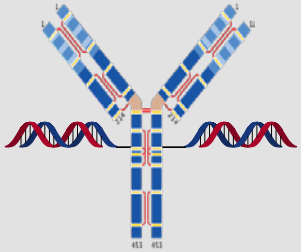
- **Mechanism of siRNA silencing via GalNac delivered constructs to the liver have substantially increased in the last five years**
- **Some parameters are still empirical; however bioanalytical techniques sensitive enough to measure drug levels throughout the duration of PD respond have improved modeling efforts**
- **Next step for siRNA modality is unlocking delivery to tissues beyond the liver**

QUESTIONS RELATED TO DISEASE INTERVENTION WITH SIRNA TARGETING THERAPEUTICS TO OTHER TISSUES

Many more hurdles targeting other tissues….



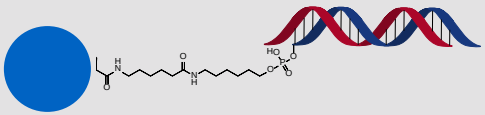
Antibody-siRNA



Feasibility for targeting extra-hepatic tissues

Can highly stabilized RNAs now allow for the pursuit of targets outside of liver targets

Alternative ligands
(peptides, small molecules)



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* Small activating RNA

TAKE HOME MESSAGES

- **Sensitive bioanalytical tools for siRNA are important in informing kinetics parameters for PK-PD modeling**
- **Major barriers restricting efficacious siRNA delivery are highly dependent on the delivery modality employed and tissue being targeted**
- **As the first siRNA modality is set for approval, the future landscape of siRNA is continually evolving**