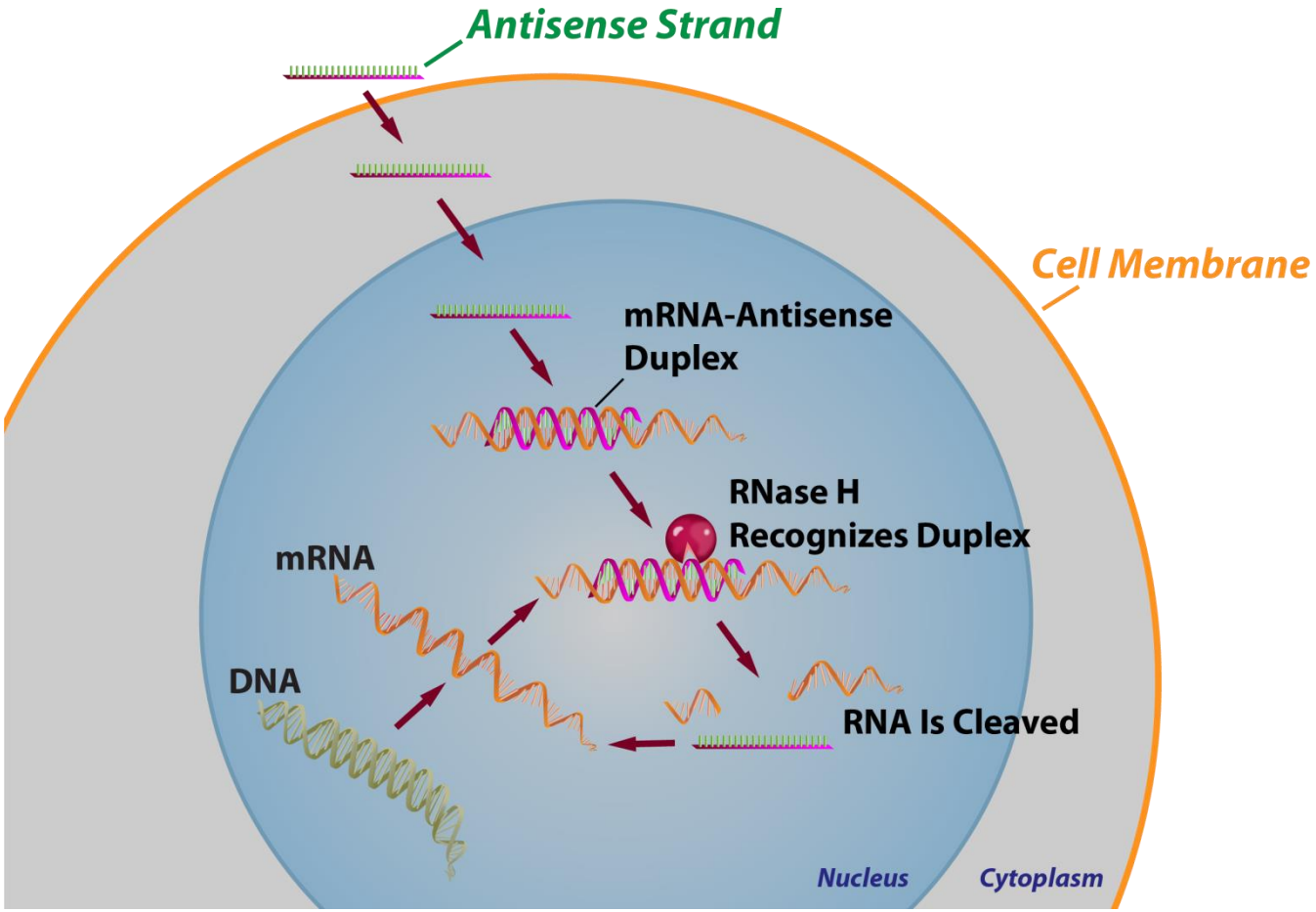
PK and PD Properties of Antisense Oligonucleotides: Bridging Nonclinical to Clinical

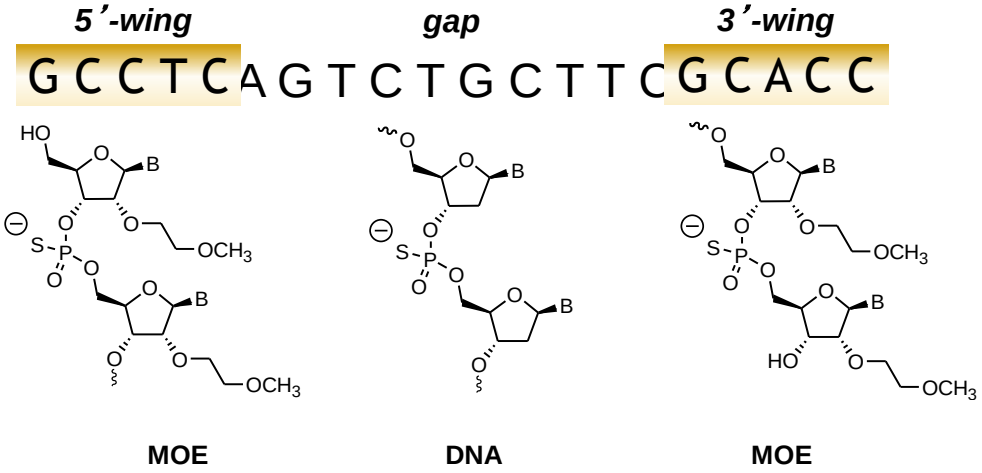
Rosie Z. Yu, Ph.D.
Pharmacokinetics & Clinical Pharmacology
Isis Pharmaceuticals, Inc.
Carlsbad, CA USA

Antisense Mechanism of Action
RNase H Oligonucleotides

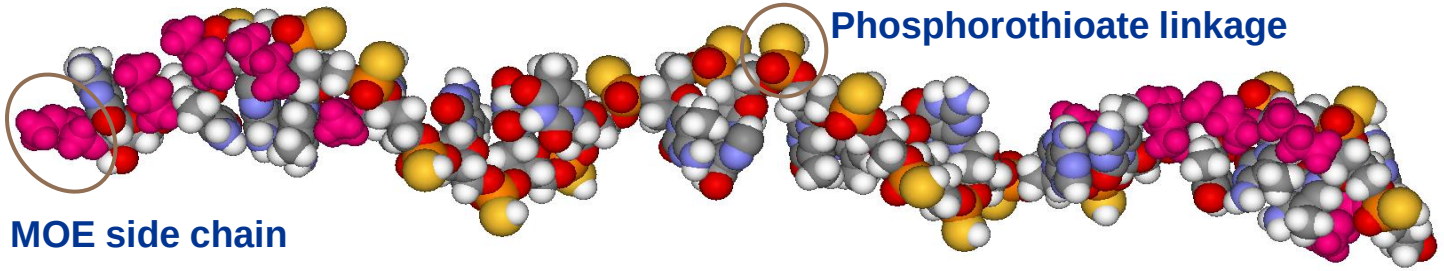


Structure of Representative 2nd Generation Antisense Oligonucleotide (ASO)

- **Gapmer' design (to activate RNase H)**
  - Phosphorothioate throughout
  - MOE modification at ends ("wings")
  - Unmodified in middle ("gap")



- **Molecular model of ASO**



Traits of Oligonucleotides in Comparison with Small Molecules and Biologics

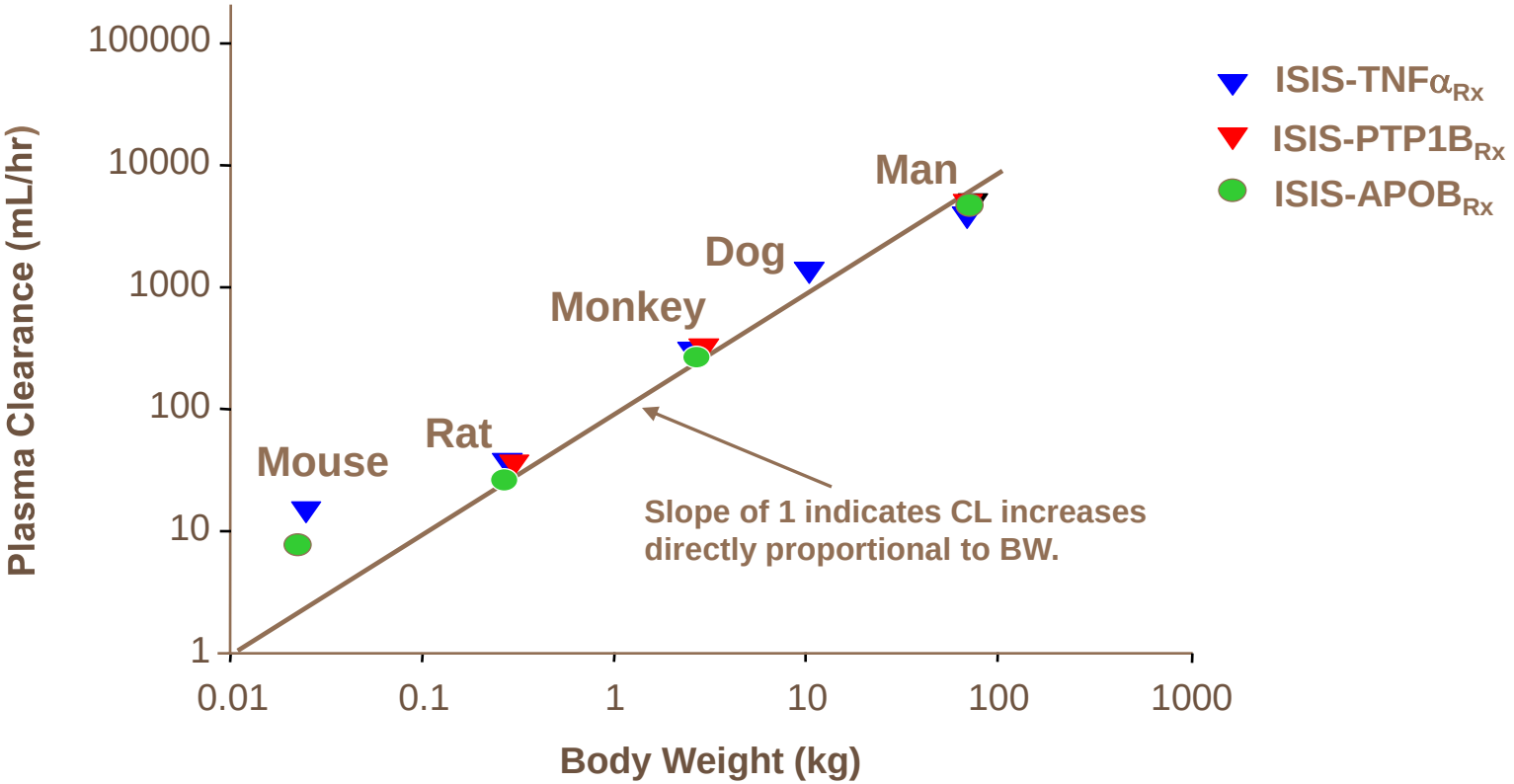
	Small molecules	Oligonucleotides	Biologics
Molecular weight	Low (<1 kDa)	6000–7000 Da	High
Manufacture	Chemical synthesis	Chemical synthesis	Biotechnology
Structure	Single entity, high purity	Single entity, high purity	Complex, heterogeneous
Target distribution	Intra- and extracellular	Intra- and extracellular	Largely extracellular
SAR	Database maybe available	No database but class effects	No database
PKDM	Species specific metabolites	Catabolized to nucleotides and other metabolites depending on modifications	Catabolized to amino acids
Immunogenicity	±	±	+++
Species specificity	±	+	+++
Off-target toxicity	+++	+	±

Originally presented to the American College of Toxicology, 9th November, 2008.
Adapted from Cavagnaro (*Nature Review: Drug Discovery* 1: 469, 2002) [1].

Ref: Lee S.. In Advanced Delivery and Therapeutic Applications of RNAi, First Edition. Edited by Kun Cheng and Ram I. Mahato. 2013.

Predictions of Human PK from Preclinical Species

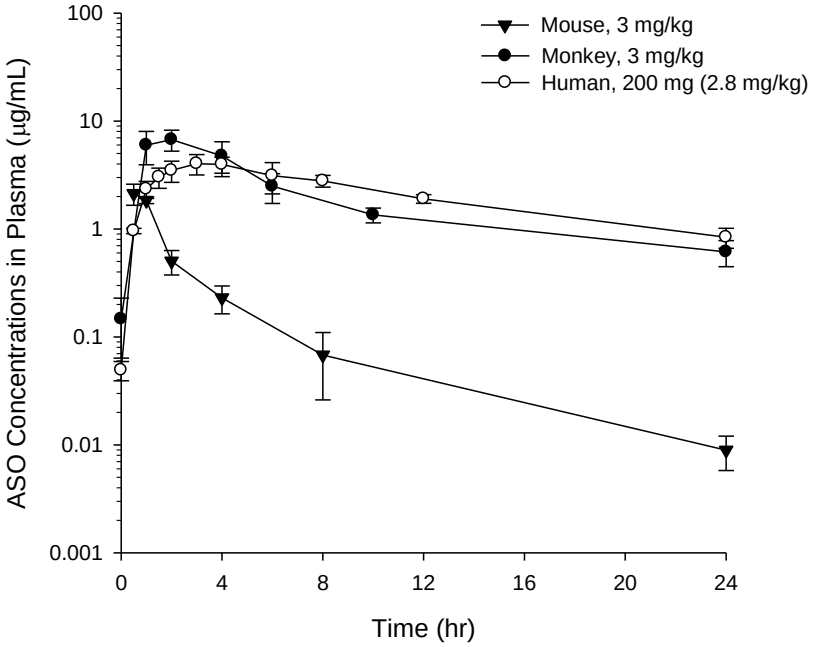
Allometric Scaling: Similar Plasma (Distribution Phase) PK Across Sequences and Between Species *(except mouse)*



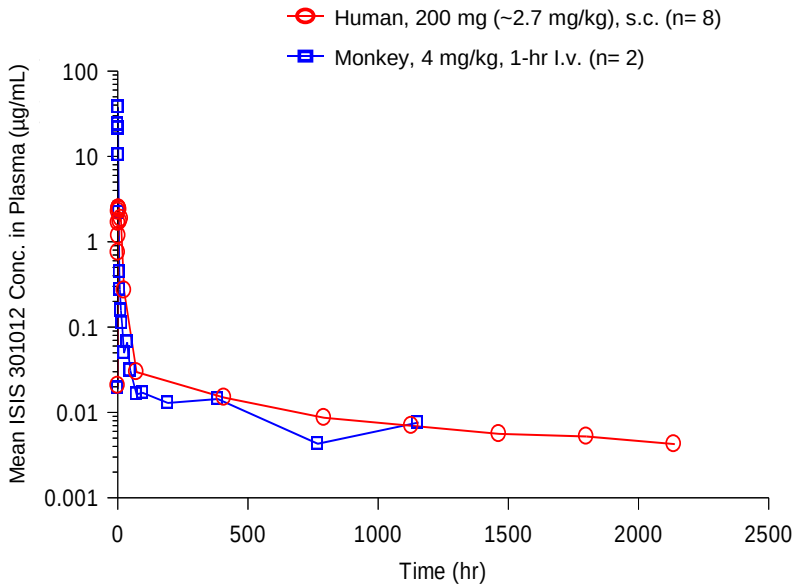
Refs: Yu et al. Drug Metab Dispos, 2007. 35(3): 460-468
Geary et al. Drug Metab Dispos, 2003. 31(11): 1419-1428

Comparison of Plasma Concentration-Time Profiles Across Species (mg/kg dosing)

Distribution Phase



Post-Distribution Phase



Ref: Yu et al. Drug Metab Dispos, 2007. 35(3): 460-468

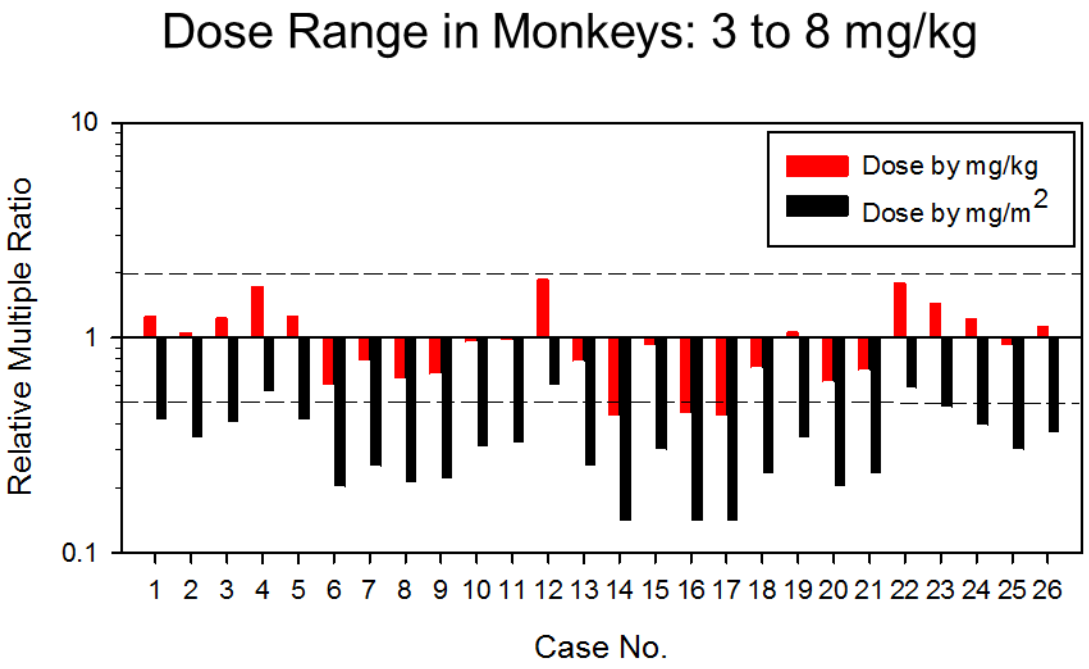
Plasma Distribution PK Scales Approx. by Body Weight from Monkeys to Humans

Compound	Dose* (mg/kg/week)	Route	Monkey		Human	
			C_{max} (µg/mL)	AUC (µg·h/mL)	C_{max} (µg/mL)	AUC (µg·h/mL)
ISIS-APOB$_{Rx}$	3	SC	3.2–5.5	18.5–26.7	2.7–6.7	32.1–79.9
ISIS-FXI$_{Rx}$	3	SC	9.2–9.8	54.3–68.1	6.1–7.2	62.3–68.8
ISIS-APOCIII$_{Rx}$	3	SC	4.0–9.8	43.0–60.4	4.0–4.1	49.1–50.7
ISIS-TTR$_{Rx}$	3	SC	5.6–8.7	37.1–39.8	7.0–12.4	62.9–76.7

*3 mg/kg/week = 200 mg/week dose in humans (~70 kg)
Some monkey data are dose-normalized.

Ref: Data on file (Isis Pharmaceuticals).

Monkey-to-Human Scaling
(TBW vs. BSA Dose Normalization Comparison)



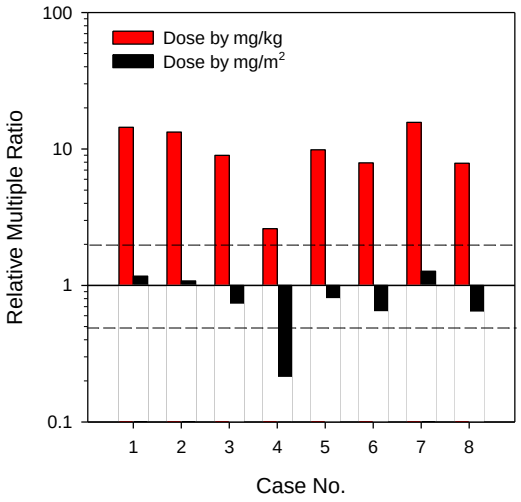
$$RMR = \frac{Dose\ Ratio}{AUC\ Ratio}$$

Dashed line shows acceptable relative exposure multiple ratio range of 0.5 to 2.0.

- TBW normalization: 23 of 26 cases (88%) acceptable RMR values.
  - $RMR_{(mg/m^2)} = 0.32 \pm 0.13$
  - $RMR_{(mg/kg)} = 0.98 \pm 0.41$

Mouse-to-Human Scaling (TBW vs. BSA Dose Normalization Comparison)

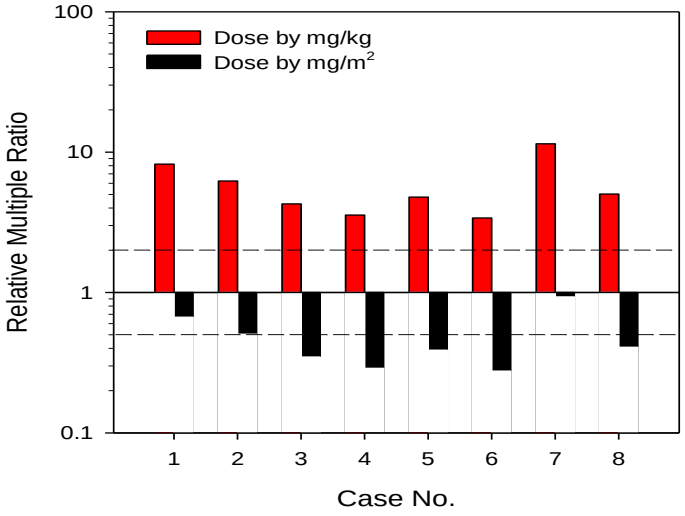
Single Dose



$$RMR = \frac{Dose\ Ratio}{AUC\ Ratio}$$

- Single dose BSA normalization has acceptable RMR values.
  - $RMR_{(mg/m^2)} = 0.82 \pm 0.35$
  - $RMR_{(mg/kg)} = 10.1 \pm 4.3$

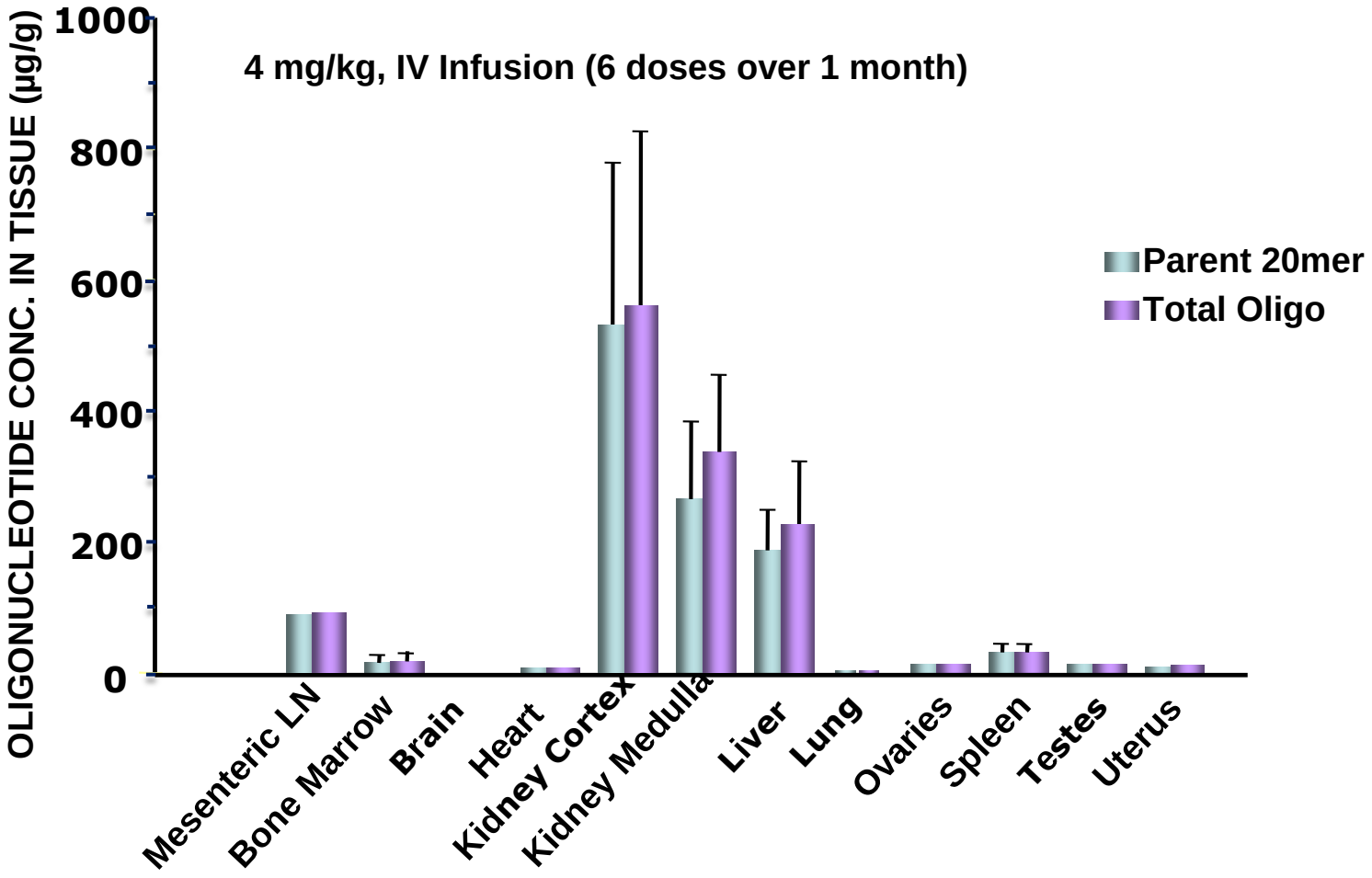
Multiple Dose



Dashed line shows acceptable relative exposure multiple ratio range of 0.5 to 2.0.

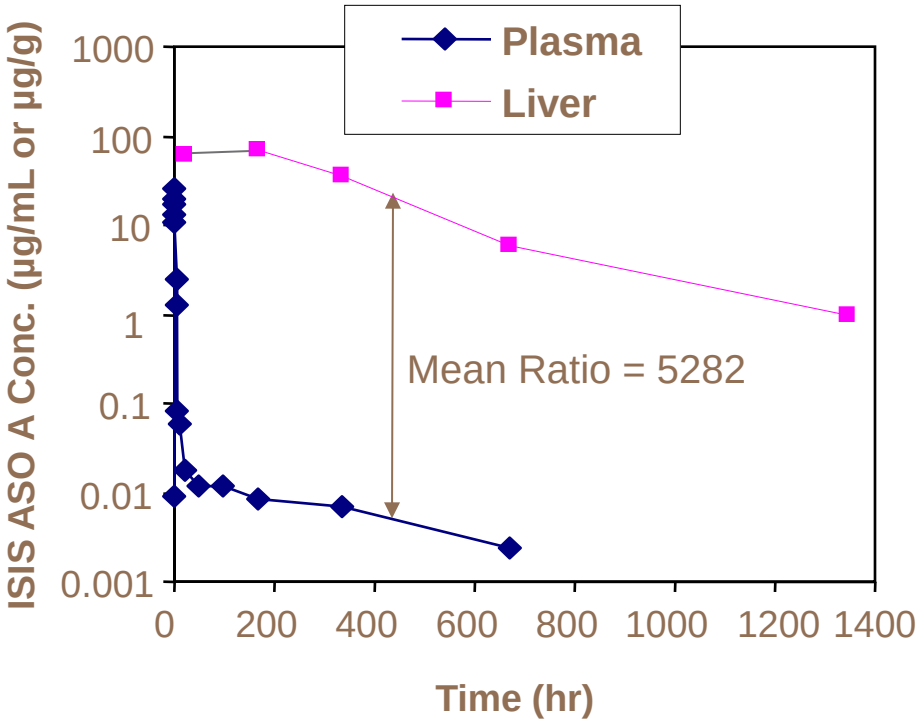
- Multiple dose neither BSA nor TBW normalization has acceptable RMR values.
  - $RMR_{(mg/m^2)} = 0.48 \pm 0.22$
  - $RMR_{(mg/kg)} = 5.87 \pm 2.75$

ASOs Rapidly/Extensively Distribute to Tissues after Dosing (similar between species; monkey shown)



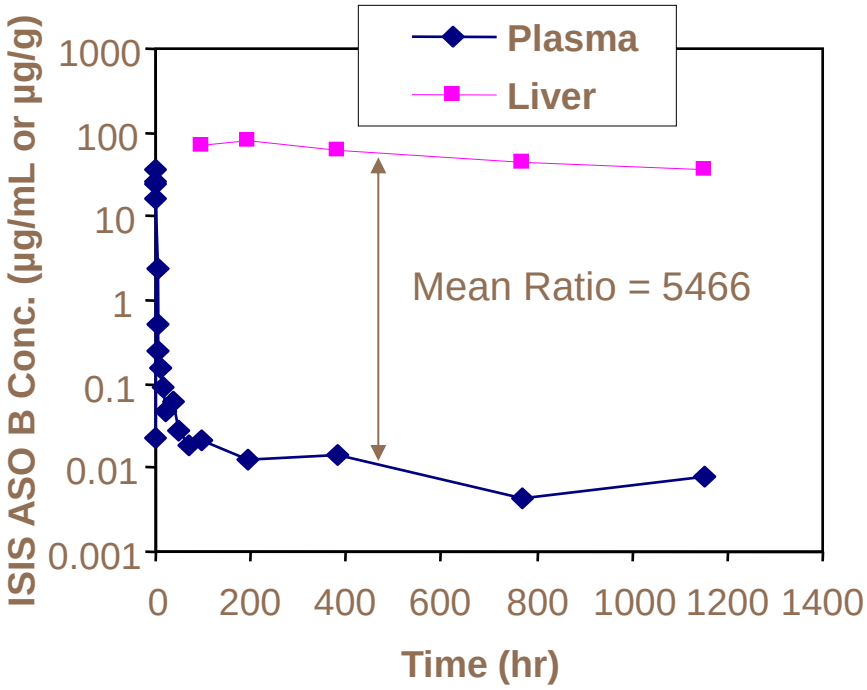
Post-Distribution Plasma Concentrations in Equilibrium with Tissue Concentrations in Monkeys

ASO A



Treatment: 3 mg/kg, 1 hr IV, q3d x 3

ASO B



Treatment: 4 mg/kg, 1 hr IV, q2d x 4

Consistent Liver to Plasma (Post-Distribution) Conc. Ratio Across Species and Between Different ASOs

Species	ASO	2'-MOE Motif	Liver : Plasma Conc. Ratio
Mouse	ISIS-APOB$_{Rx}$	5-10-5	5861
	ISIS-FAS	5-10-5	4500
	ISIS-CD49d$_{Rx}$	3-9-8	5000
Monkey	ISIS-APOB$_{Rx}$	5-10-5	5825
	ISIS-TNFα_{Rx}	5-10-5	5000
	ISIS-PTP1B$_{Rx}$	5-10-5	5300
	ISIS-FXI$_{Rx}$	5-10-5	4090
	ISIS-CRP$_{Rx}$	5-10-5	5434

Refs: Yu et al. Biochem Pharmacol 77 (2009) 910-919
Data on file (Isis Pharmaceuticals)

Plasma Post-Distribution PK Scales Approx. by Body Weight from Monkeys to Humans

Compound	Dose (mg/kg/week*)	Route	Plasma C_{trough} Range (ng/mL)	
			Monkey	Human
ISIS-PTP1B$_{Rx}$	3	IV (monkey) SC (human)	~10.0-15.0	8.6-16.7
ISIS-APOB$_{Rx}$	3	SC	11.7-53.3	11.1-42.2
ISIS-FXI$_{Rx}$	3	SC	48.0-52.1	23.6-30.3
ISIS-APOCIII$_{Rx}$	3	SC	30.7-56.9	49.2-95.6
ISIS-TTR$_{Rx}$	3	SC	10.9-16.4	9.99-11.2

*3 mg/kg/week = 200 mg/week dose in humans (~70 kg)

Ref: Data on file (Isis Pharmaceuticals). Some monkey data are dose-normalized.

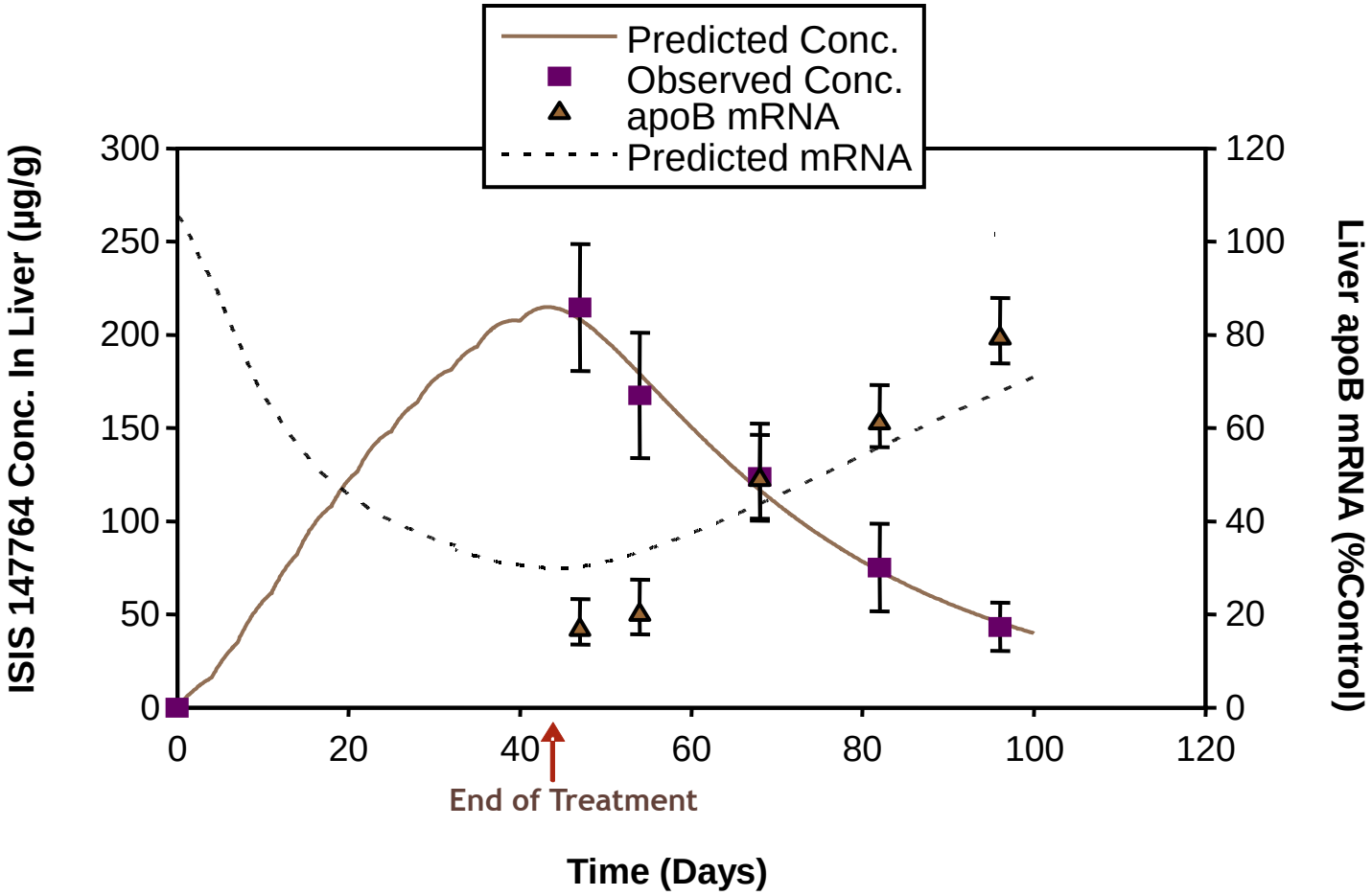
Tissue Elimination $t_{1/2}$ in Monkeys is Consistent with Plasma Elimination $t_{1/2}$ in Humans

ASO	$t_{1/2}$ (days)		
	Monkey Liver	Monkey Kidney	Human Plasma
ISIS-TNFα_{Rx}	13	17	13
ISIS-PTP1B$_{Rx}$	8	16	16
ISIS-APOB$_{Rx}$	34	33	31
ISIS-FXI$_{Rx}$	19	13	14-23
ISIS-APOCIII$_{Rx}$	19	13	12

Refs: Yu et al. DMD 35(3) 2007: 460-468; Data on file (Isis Pharmaceuticals)

Predictions of Human PD from Preclinical Species

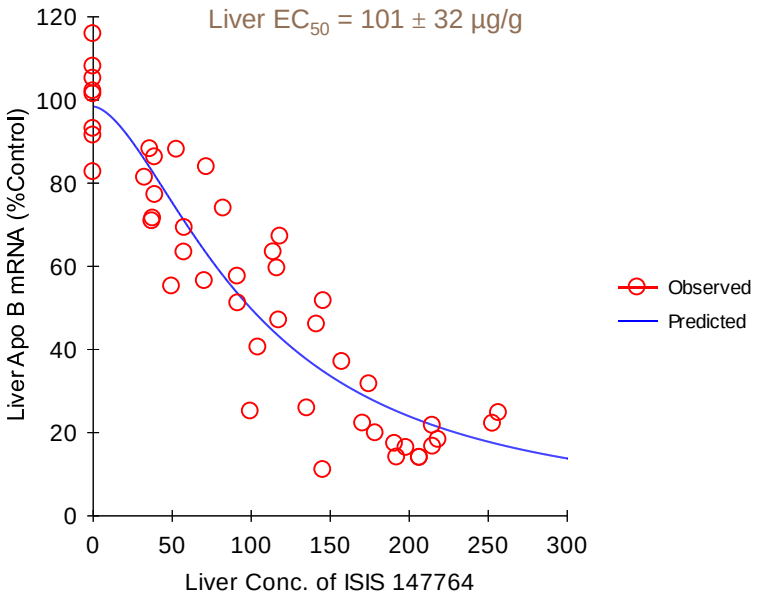
In Vivo (Murine) apoB Inhibition by an ASO: 6 Week Dosing Study



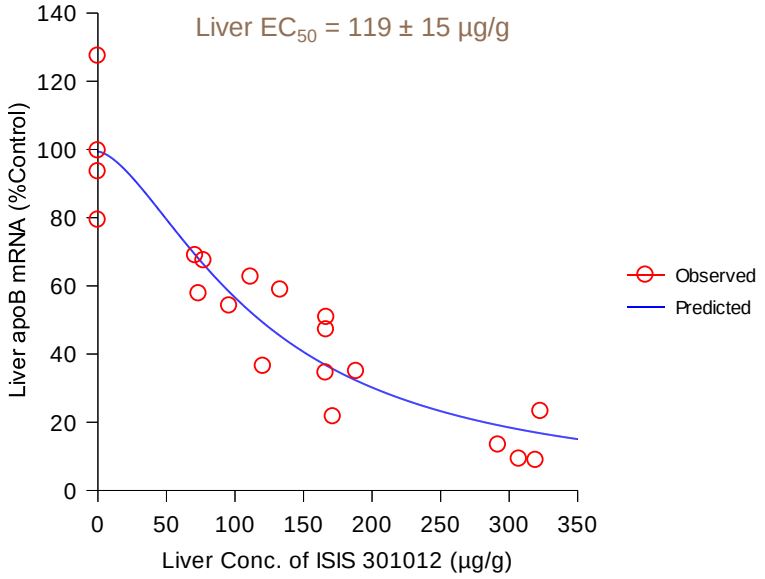
Ref: Yu et al. Biochemical Pharmacology 77 (2009): 910-919

Target (Liver) Exposure-Response Relationship in Mouse Models

HF-Fed C57BL Mice



Human ApoB Tg Mice

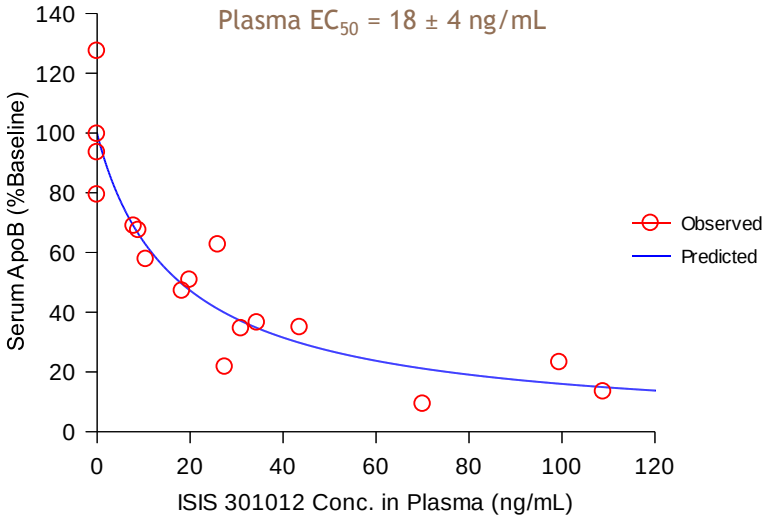


*Best Fit Inhibitory Effect E_{max} Model of liver ApoB mRNA Levels vs. liver ASO conc.

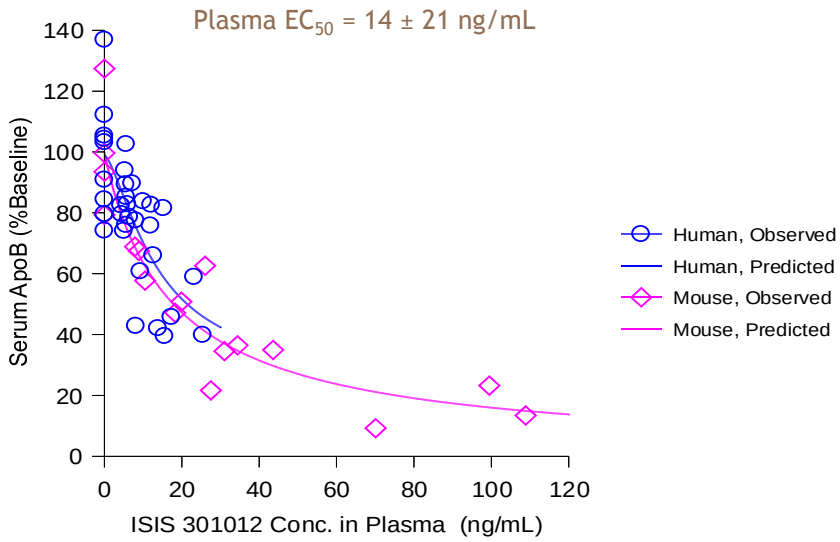
Ref: Yu et al. Biochemical Pharmacology 77 (2009): 910-919

Similar Plasma Exposure-Response Relationship in Human ApoB Tg Mouse and Human

Tg Mice



Tg Mice Vs. Human

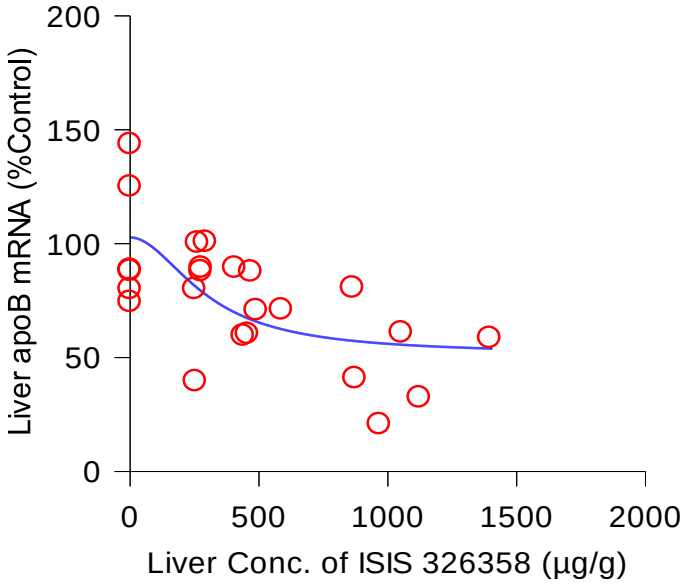


*Best Fit Inhibitory Effect E_{max} Model of liver ApoB mRNA Levels vs. liver ASO conc.

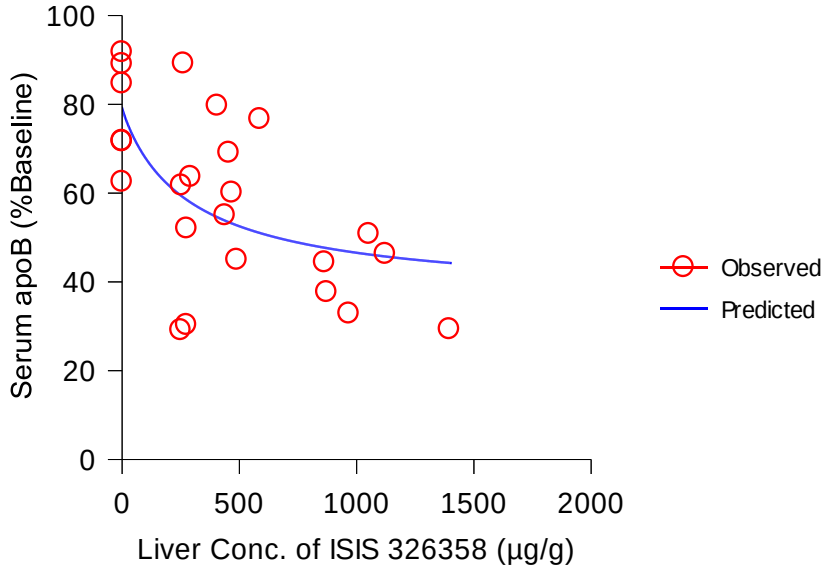
Ref: Yu et al. Biochemical Pharmacology 77 (2009): 910-919

Less Sensitive Exposure-Response Relationship in Monkeys

Liver apoB mRNA



Serum apoB



Ref: Yu et al. Biochem Pharmacol 77 (2009) 910-919.

ED_{50} (EC_{50}) in Target Tissue across Species

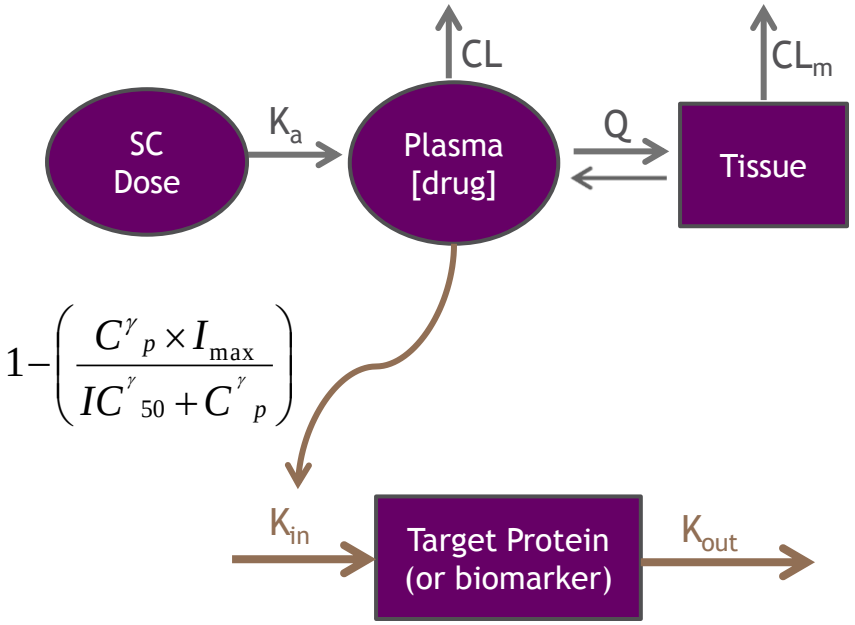
ASO Target	Mouse	Human
ApoB in Liver (Mipomersen)	25 mg/kg/wk (85 µg/g)	3 mg/kg/wk (110 µg/g*)
ApoC-III in Liver (Isis)	12.5 mg/kg/wk (55 µg/g)	2 mg/kg/wk (150 mg/wk) (65 µg/g*)
TTR in Liver (GSK/Isis)	12.5 mg/kg/wk (50 µg/g)	2 mg/kg/wk (150 mg/wk) (65 µg/g*)
Survivin in Lung/Colon Tumor (Lilly/Isis)	50 mg/kg/wk (20 µg/g ED50)	7 mg/kg (500 mg/wk) (est. based on PK modeling 20 µg/g)[a]
Clusterin in Prostate Tumor (Oncogenix)	62.5 mg/kg/wk (5 µg/g)	6 mg/kg (320 mg/wk) (3 µg/g measured median)[b]
STAT3rx (Gen 2.5) in Tumor (AstraZeneca/Isis)	15 mg/kg/wk (5 µg/g)	2 mg/kg (140 mg/wk) (est. based on PK modeling 1-3 µg/g)

*Predicted based on measured trough plasma levels of drug in man

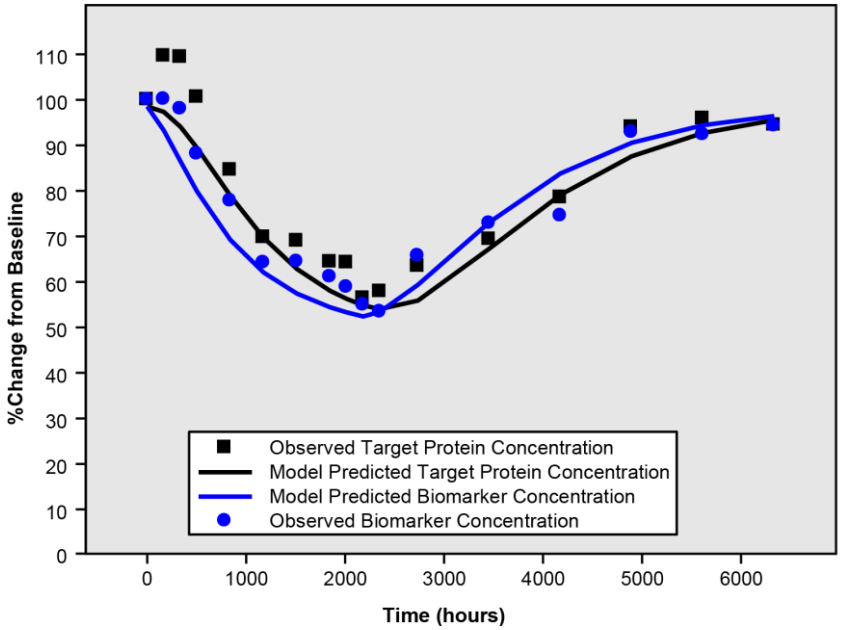
[a]*Talbot, Ranson and Davies et al., Clin Cancer Res, November 2010*
[b]*Chi, Eisenhauer and Gleave et al., JNCI, Vol 97, 2005*

Cross-Species PK/PD Model Predictions vs. Actual Clinical Observations for an ASO

PK-PD Model Diagram



Predicted vs. Observed Clinical Response



Treatment: 200 mg/week for 13 weeks.

Conclusions

- **Plasma PK in monkeys well extrapolates to humans**
  - Monkey plasma AUC directly extrapolates to human by mg/kg dosing
  - At post-distribution phase, plasma levels are in equilibrium with tissue and thus provide surrogate measure of tissue concentrations
  - Plasma elimination $t_{1/2}$ is similar to tissue $t_{1/2}$ and similar across species (typically 2–4 weeks)
- **PD relationship from mice well extrapolates to humans**
  - Pharmacologic effects of ASOs directly are related to drug concentrations in target organ.
  - Similar EC_{50} (based on plasma trough levels, or expected tissue levels)
- **Challenges:**
  - Altered plasma post-distribution to target tissue concentration relationship due to immunogenicity with chronic ASO administration
  - ASO may be less well distributed to certain target tissue/cell type
  - Distribution to productive vs. non-productive pathways

Acknowledgements

- John Grundy
- Richard Geary
- Scott Henry
- Yanfeng Wang
- Jennifer Burkey
- Dan Norris