Safety Assessment of Oligonucleotide Constructs

Scott P. Henry,
VP, Preclinical Development,
Isis Pharmaceuticals, Inc.,
USA

Drug Development of 2'MOE ASO Platform Technology

- Platform technologies offer the promise of increased efficiency throughout the development process
- Challenge is that ON drugs are becoming quite diverse
  - Chemistry, structure, mechanism, formulations, conjugates
- Safety profiles within a Chemical class are similar
  - But the magnitude of effects does vary widely with sequence
  - Differences between chemical classes can be profound
- Unique development challenges for ON drugs
  - Species-specific homology and pharmacologic activity
    - Animal-specific surrogates often to assess exaggerated pharmacology
  - Influence of sequence on magnitude of class effects

Distinct Chemical Classes of Oligonucleotide Therapeutics

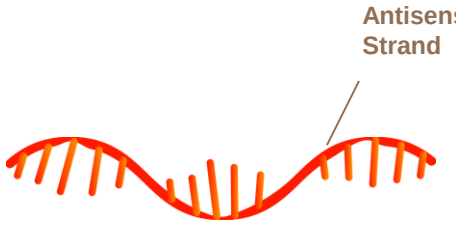
Antisense
Single-Strand

RNase H Mechanism

Saline vehicle

DNA
Phosphorothioate

2’-MOE, 2’-Me, cEt, LNA



siRNA
Double-strand

RISC Mechanism

Delivery vehicle for systemic use

RNA
Phosphodiester

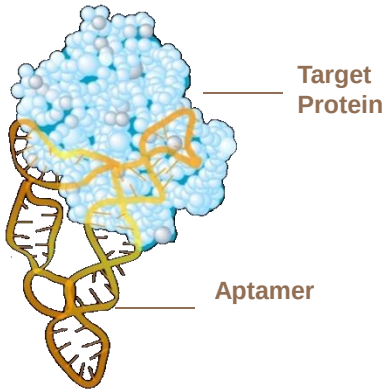
2’-Me, 2’-Fl

Alyphatic substituents

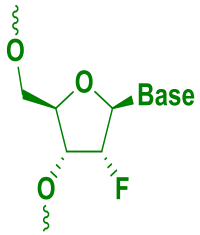
Aptamer
Structured oligo

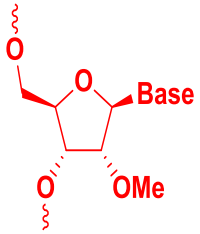
DNA or RNA
Mixed modifications

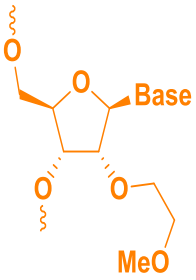
Pegylation

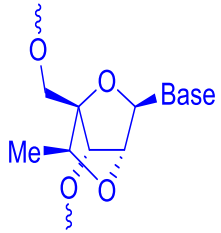


Physico-Chemical Properties of Common 2’-Modifications



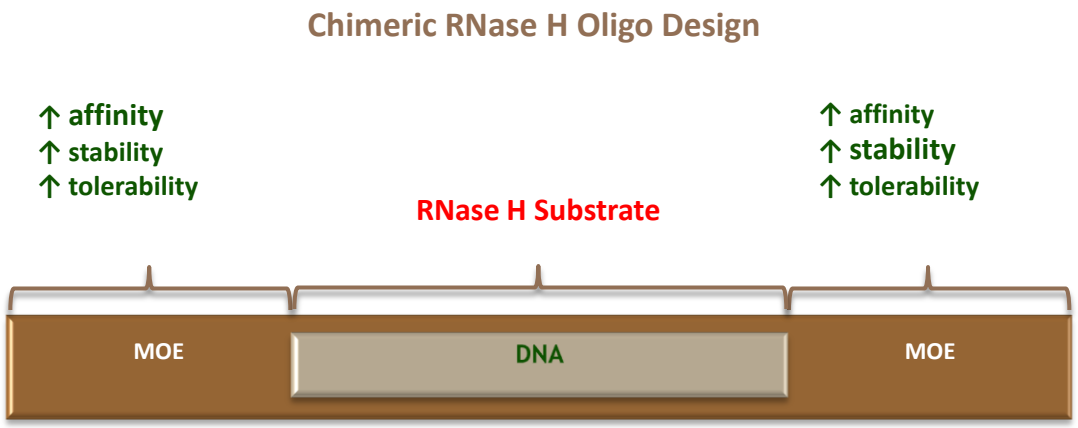


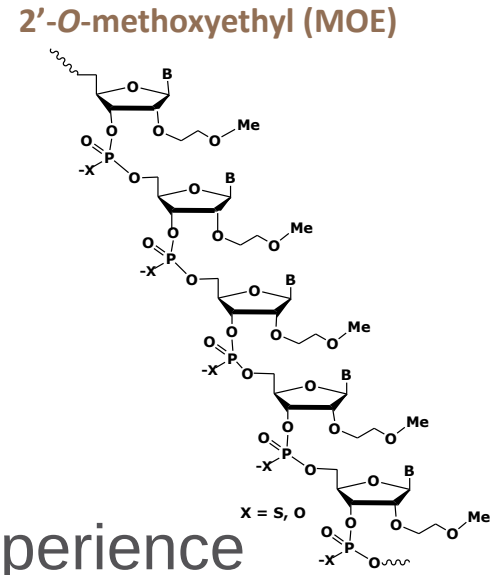




	2’-F RNA	2’-OMe RNA	2’-MOE RNA	cEt BNA
Affinity	+	+	+	+++
Stability	+	++	+++	++++
Hydrophobicity	++			+
Hydrophilicity		++	+++	

“2nd-Generation” 2’-O-Methoxyethyl Chimeric Antisense Drugs RNase H Mechanism





- Compared to first generation P=S ODNs, Chimeric MOE ASOs:
  - Increase potency
  - Increase duration of action
  - Decrease unwanted side effects

- Clinical Experience
  - > 3000 subjects dosed
  - > 90 clinical studies
  - Multiple therapeutic indications
  - > 140 patients dosed for > 1 year
  - > 70 patients dosed for > 2 years

Key Points in Designing Toxicology Evaluation for ASOs

- Hybridization – dependent
  - Exaggerated Pharmacology-
    - Human target sequence is often not conserved in animals
    - Simple sequence homolog for target ASO does not assure activity
    - Animal-Active surrogates are commonly used to assess pharmacology

- Hybridization - independent
  - Sequence-independent or class-related tox account for the majority of tox observed
  - Sequence-dependent – Examples of Toxicity arising from specific motifs that define a receptor interaction
    - e.g. TLR-9 interaction with CpG motifs
    - Avoided in development compound by diligent screening

Scope of Toxicity Studies with ASOs

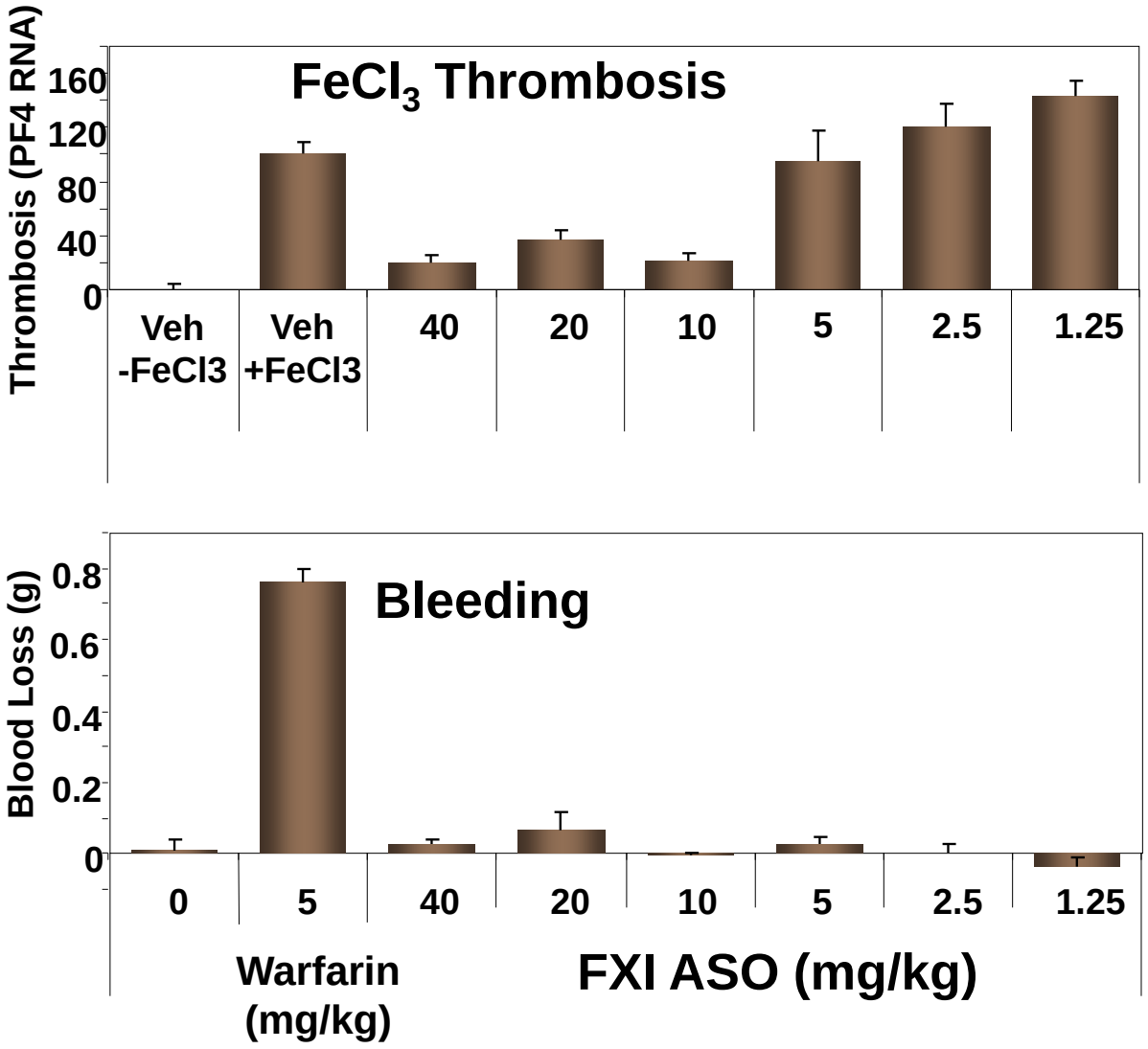
- IND Toxicology Studies for >28 MOE ASOs
  - Toxicity testing typically done in mice and monkeys
  - Doses up to 100 mg/kg/wk in mice and 80 mg/kg/wk in monkeys acutely tolerated
    - High dose in subchronic studies is typically 80 mg/kg/wk in mice and 35 mg/kg/wk in monkeys
  - Weekly dosing by IV infusion or SC injection
  - Duration of 6 and 9 months in mice and monkeys
  - Carcinogenicity study with 1st and 2nd generation drugs
  - Reprotox and Gentox batteries negative
  - Both human and species-active oligos used in Tox Studies when scientifically justified

Pharmacodynamic Assessment for Factor XI Project Was Performed in Mouse and Monkey

- Important to assess potential effect on bleeding risk compared to other drugs
  - Factor XI chosen based on intended lower risk of bleeding
- Included measurement of PD endpoints at multiple dose levels
  - Factor XI mRNA measurement in liver
  - Plasma Factor XI activity
  - APTT and other clotting endpoints
- Assessment of impact of Factor XI inhibition of clotting function vs. Heparin positive control
  - Skin laceration bleeding time
  - Oral mucosa laceration bleeding time
  - Tail amputation bleeding time

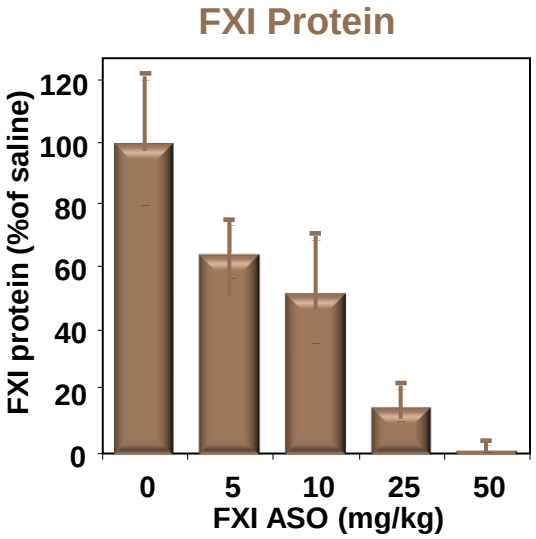
Antithrombotic Effects of FXI ASO Without Increased Bleeding

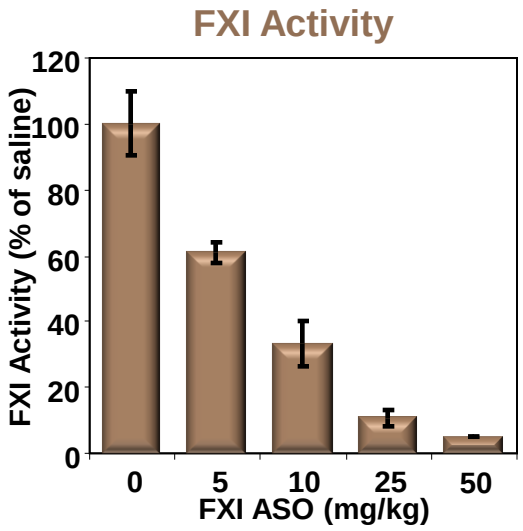
- Human FXI ASO is inactive in mouse
  - Homologous with monkey sequence but not optimal
- Mouse Pharmacology Models
  - Mouse FXI ASO produces up to 90% inhibition of RNA
  - >50% reduction in FXI Activity associated with reduced thrombosis

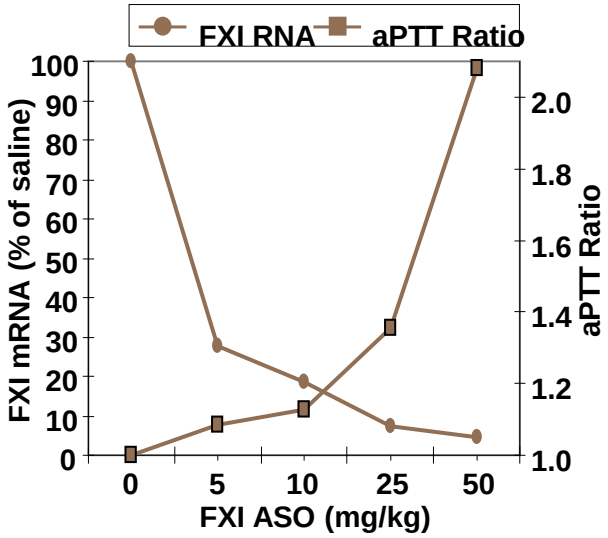


ASO Suppression of FXI in Mice Produces Specific, Dose-Dependent Anticoagulant Activity

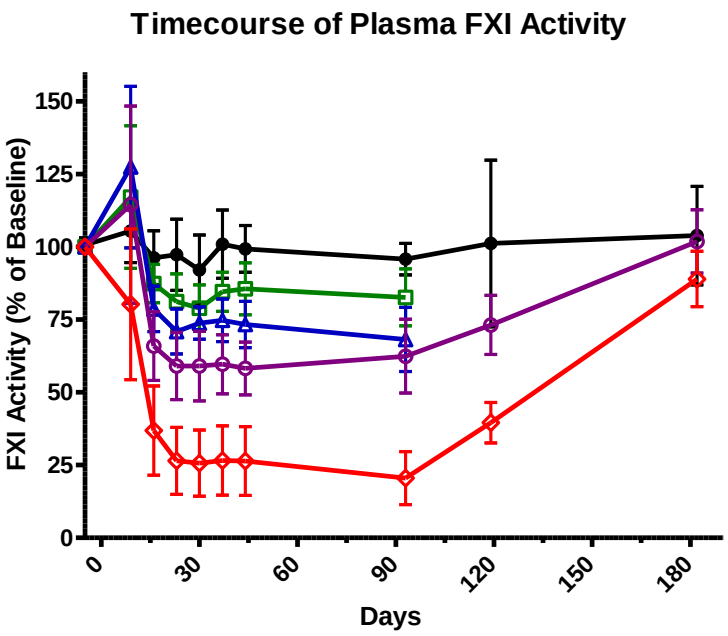
- Assessment of PD effect in mice
  - Correlation of RNA, protein, and APTT
    - No effect on PT
  - Exceeded intended clinical FXI reduction
    - 80 mg/kg/wk dose of mouse-active ASO in 13 week study
    - >90% FXI reduction

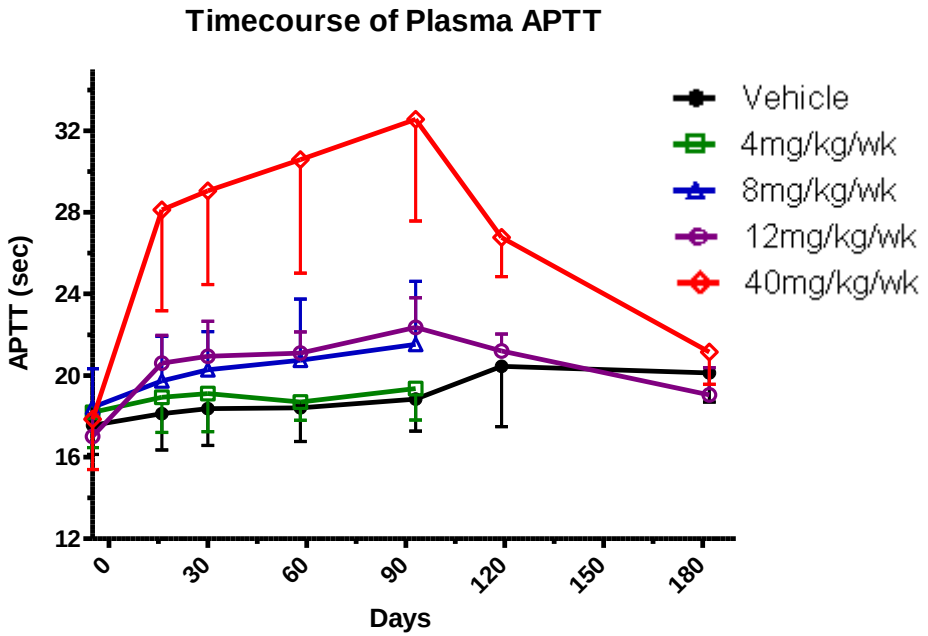






Time and Dose-Dependent Correlation Between Factor XI mRNA, Plasma Protein, and APTT





No evidence of spontaneous bleeding in animals with up to 75% inhibition of Factor XI for 13 weeks.

Summary of Pharmacodynamic Results

- Dose dependent reduction in FXI mRNA levels in mouse and monkey
  - Comparable reductions in FXI plasma protein and elevations in APTT
  - Maximum inhibition in monkey was 70%
- FXI ASO did not produce bleeding where FXI mRNA levels were reduced by 90% in mice
  - No Spontaneous bleeding
  - No evidence of excess bleeding was noted under a surgical setting of partial tail amputation or skin or gum laceration

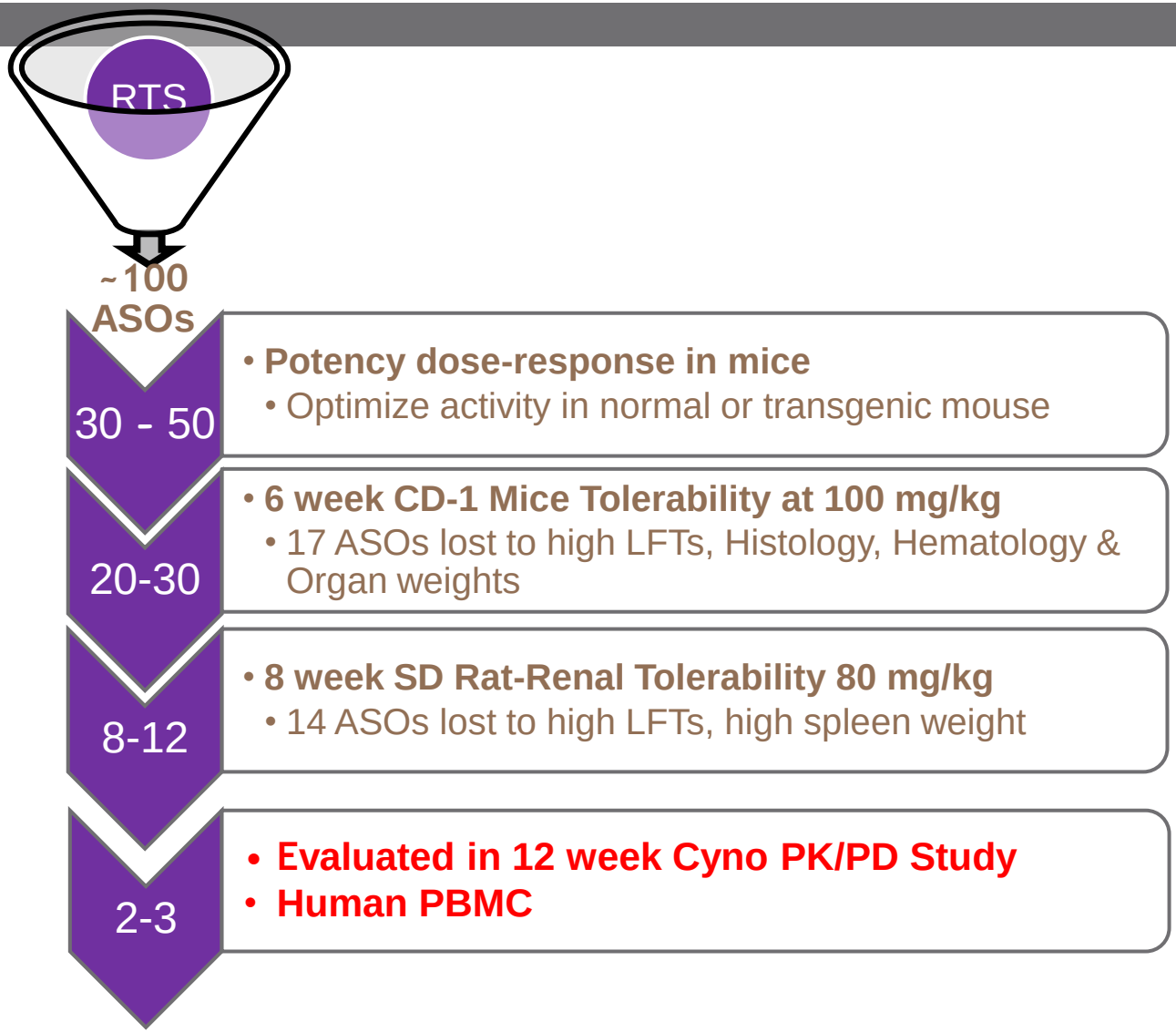
Class-Related Effects for 5-10-5 MOE ASO at Toxicologically Relevant Doses

- Rodents (Mice and Rats)
  - Pro-inflammatory effects
    - Lymphohistiocytic infiltrates in various tissues at ≥10 to 20 mg/kg
      - Splenomegaly and Lymphoid hyperplasia
    - Slight increase in AST and ALT at ≥25 mg/kg
  - Endosomes accumulate oligo in basophilic granules in Kupffer and proximal convoluted tubules at ≥10 mg/kg
  - Rats are more sensitive to renal effects, especially male rats
    - Increased proteinuria at ≥10 mg/kg
- Monkey
  - Proinflammatory effects not prominent
    - Minimal SC injection site reaction
  - Complement activation at doses ≥10 mg/kg
  - Minimal proximal tubular epithelial cell degeneration at ≥10 mg/kg/wk

ASO Class Effects in Non-Clinical Models -Translation to Man-

Effect	Mechanism	Effects in Man
Transient aPTT Increases	Inhibition of tenase complex	Transient, C_{max}-related prolongation, i.v.
Complement activation (in monkeys)	Inhibition of Factor H	No effects in man
Pro-inflammatory effects (especially in rodents)	Release of cytokines or chemokines via toll-like or other receptors	Occasional constitutional symptoms, local injection site reactions.
Renal tubular cell degeneration at high tox doses (monkey)	Concentration in kidneys; mechanisms not known	No renal effects in man
Platelet count reductions	? Transient sequestration	not Gen-2 class effect in man

Selection Strategy for 2'-MOE ASO Development Compound (MOE 20-mer)



ASO Producing Poor Tolerability are Eliminated in Screening Studies

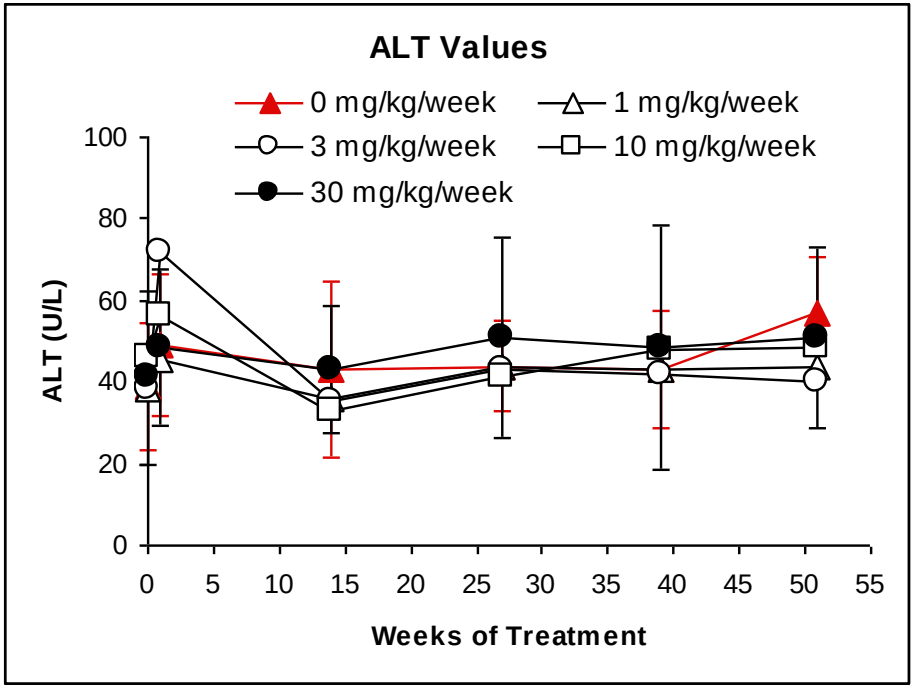
- CD-1 Mice treated with 100 mg/kg/wk for 4 weeks
- 404161 Eliminated for elevation in ALT
  - 2- to 4-fold increase in ALT expected at this dose level
  - 409998 and 409975 have border line acceptable ALT/AST levels

			Fold over Saline	
ISIS	Chemistry	Walk Position	ALT	AST
409988	2-13-5	-5	5.1	2.1
409821	5-10-5	-1	2.7	1.5
404176	5-10-5	0	2.5	1.2
409975	2-13-5	+3	5.0	1.9
409976	2-13-5	+4	1.5	0.8
404161	2-13-5	0	9.6	2.6
404169	5-10-5	0	1.3	0.8
409815	5-10-5	+1	1.2	0.9
409826	5-10-5	-3	3.4	1.6
410003	2-13-5	+1	1.5	1.1
373125	5-10-5	0	1.2	0.9

Hepatic Tolerability of MOE ASO in Mice and Monkey

- Kupffer cell hypertrophy in monkey and mice –
  - Reflects dose dependent uptake of oligo
- Increased mononuclear cell infiltrate in mice.
  - Increased AST/ALT and single hepatocyte necrosis in mice - mild to moderate at ≥50 mg/kg
- No alteration of hepatic function in monkey
  - Consistent with the lack of cellular infiltrates in monkey liver
  - Includes chronic administration for at least 6 ASO

No increase in ALT values in 1-yr monkey study



Attributes of Safety Assessment for MOE ASO Platform Chemistry

- Tremendous efficiencies in design, data collection and interpretation for selected MOE ASO
- High success rate for supporting clinical trials
  - Only 3 cases where compound did not pass 13 wk tox
- Increase in confidence of interpretation of safety data
  - Data from each tox study is interpreted in the context of the chemical class
    - Partially solves of the issue with low animal numbers
  - Relative tolerability of any given ASO-drug in animals is a good predictor of clinical tolerability
  - Helps interpret significance of animal findings

Attributes of 2’-MOE ASO Platform Technology in Safety Assessment

- Data on Class Toxicity profiles enhances our overall Safety/Risk assessment
  - Any new MOE ASO can be compared/benchmarked relative to broader experience
    - Multiple disease states, compounds, doses and greater patient number
  - Phase 1 have the benefit of dozens or hundreds of subjects treated with similar compounds
    - Can compare nonclinical behavior in rodent and monkey

Preclinical Monkey Toxicology Database Established for ISIS MOE ASOs

- GLP Toxicology Studies for Development ASOs
  - Dose response 1 to 30/40 mg/kg
  - Discovery Pharmacology Screening Toleration Studies (non-GLP)
    - 13-week high dose 30/40 mg/kg
  - Investigative Toxicology Studies (non-GLP)
- Preclinical and clinical data in similar format to support integrated queries

Composition of Monkey DB

Characteristic	Number
Studies	35 (16 GLP)
Compounds (multiple chemistry)	>100 (85, 2'-MOE 20mers)
Animals (multiple sources)	Placebo (~400); ASO (~1500)
CROs	4
Endpoints (Clin path, BW, Tissue Wt, Cytokines, Complement, TK, PD)	>150

Clinical Safety Database Established for Isis MOE ASO

- Clinical studies conducted or initiated for 8 PS ODN oligonucleotides and 28 MOE ASOs

	Number of Subjects Treated (est.)		
	1st Gen	2nd Gen	Total
IV & SC	2,570	3,840	6,410
Local / Oral	1,210 / 20	420 / 100	1,630 / 120
Total	3,800	4,340	8,150

- ISIS MOE ASOs
  - 16 treatment populations; 100 studies
  - IV/SC doses up to 1200 mg / week (17 mg/kg/wk)
  - > 650 patients treated ≥ 12 weeks, > 330 treated ≥ 6 months, > 140 treated ≥ 1 year

Utilize Database to Understand Translational Effects of Standard Target Organ Effects - Kidney

- No trend for a treatment affect on Renal Function in Monkey or Humans

Human Parameter	Placebo (N=)	ASO Treated (N=)
Creatinine > 30% Increase vs. Baseline	6.2% (534)	6.6% (1775)
BUN > 2X Baseline	1.7% (472)	1.6% (1953)
GFR > 30% decrease vs. Baseline	3.3% (509)	3.7% (1797)
Urine Protein (qualitative)	In Progress	
Monkey Parameter	**Placebo (N=306)**	**ASO Treated (N=1142)**
Creatinine > 30% decrease vs. Baseline	7.8%	10.2%
BUN > 30% Increase vs. Baseline	17.3%	20.0%
Urine P/C Ratio > 50% Increase vs. Baseline	9.2%	10.6%

Confirms adequate safety margins for common class effects

Despite the Consistency – Exceptions Will Occur

- Exceptions could be related to pharmacologic effect or patient population
  - Increased ALT present for Kynamro
    - Pharmacologic effect of lowering apoB and related LDL changes
- Exceptions need to be interpreted in the context of their screening process and specific chemical class
  - Renal effect of PCSK9 - LNA ON gapmer
  - DMD splicing ASO – Full 2’-Me

Conclusions on Consistency and Translatability of MOE ASO Safety Profile

- Assessment of Exaggerated Pharmacology must consider sequence homology across species
  - Animal-Active surrogates are effect tools in the safety assessment
- Generalization of ASO Class Effects can be made, but best done on defined set of criteria
  - Consistent chemical class
  - Defined set of performance criteria
  - Exceptions to the rule will be found
- Overall Safety Assessment is facilitated by consistency
  - Focuses attention on key class effects
  - Databases facilitate translational assessment of animal toxicology
  - Greater confidence in safety margins for class effects
  - Facilitates the identification of compounds with suboptimal tolerability