Development of a Factor XI Antisense Inhibitor: From Concept to Phase 1
Antisense Mechanisms of Action

To date there are more than 12 different antisense mechanisms that have been characterized.
RNase H Antisense Mechanism
The Most Advanced Antisense Mechanism

Chimeric RNase H Oligo Design

- Increased affinity
- Increased stability
- Increased tolerability

RNase H Substrate

2’-O-methoxyethyl (MOE)

Compared to first generation P=S ODNs, Chimeric MOE ASOs:
- Increase potency 10-15 fold
- Increase duration of action 10-20 fold (50 to 100 fold less drug)
- Decrease unwanted side effects

Clinical Experience
- > 2200 subjects dosed
- > 60 clinical studies
- Multiple therapeutic indications
- > 100 patients dosed for > 1 year
- Doses as high as 1200 mg tolerated
Why Target Coagulation Factors with Antisense Drugs for the Prevention of Thrombosis?

✦ The medical need for safer antithrombotic agents is high.

✦ Small molecule specificity & drugability against coagulation factors is highly challenging.

✦ Liver is highly sensitive to antisense drug action.

✦ Attractive pharmacokinetic profile for 2nd-Generation antisense drugs.
  - Once/week injectable
  - Steady & predictable PK profile
  - Drug interactions very rare

✦ Coagulation factors have been linked to many diseases that go beyond thromboembolic disease.
  - Inflammatory disease
  - Cancer
Antisense Drugs Selectively Reduce Levels of Individual Clotting Factors Produced in the Liver

*Analysis in murine liver samples
Thrombus Formation

Intrinsic Pathway

Extrinsic Pathway

Collagen / Tissue Factor

Endothelium

Platelet

Activated Platelet

Vascular Injury
(TF/Collagen Exposure)
Factor XI
An Attractive Target for Drug Development

✧ Human deficiencies in Factor XI not associated with major bleeding

✧ Deficiencies in Factor XI are associated with reduced risk for thromboembolic diseases
  - In animal models and in patients

✧ Humans with increased levels of Factor XI are at increased risk for venous thrombosis, myocardial infarction and potentially stroke
ASO Suppression of FXI in Mice is Specific, Dose-Dependent, & Produces Anticoagulant Activity

A

FXI ASO (mg/kg)

0 5 10 25 50

TPO
FVIII
FXI
FVII
FIXII
FXII
FX
FII
FV
TAFI

FXI ASO (mg/kg)

0 5 10 25 50

FXI protein (% of saline)

0 20 40 60 80 100

B

FXI ASO (mg/kg)

0 5 10 25 50

FXI Activity (% of saline)

0 20 40 60 80 100

C

FXI ASO (mg/kg)

0 5 10 25 50

FXI mRNA (% of saline)

0 10 20 30 40 50 60 70 80 90 100

D

FXI ASO (mg/kg)

0 5 10 25 50

FXI mRNA (% of saline)

0 10 20 30 40 50 60 70 80 90 100

aPTT Ratio

0 1 2 3 4 7 14 28 56

E

FXI RNA, % of saline

0 20 40 60 80 100

PT/aPTT INR

8
Antithrombotic Effects of FXI ASO in IVC FeCl3 Injury Model

IVC Thrombosis

Bleeding

Warfarin (mg/kg)

F11ASO (mg/kg)
Transition from Discovery to Development

- Oligonucleotides have always been reviewed under CDER guidelines
  - Based on chemical synthesis methods of manufacturing
  - Presents some challenges in manufacturing and toxicology for a novel mid-size biomolecule
- Manufacturing and Quality Standards
  - GMP manufacturing at 0.5-ton capacity is available
  - Linear nature of solid phase oligonucleotide synthesis makes it practically impossible to control unspecified impurities to the small molecule identification and qualification thresholds
- Toxicology and PK Characterization
  - Follows traditional thorough rodent/non-rodent evaluation
    - Toxicology often requires the use of species-active analog to address exaggerated pharmacology
  - PK assessment has required the development of sensitive/specific bioanalytical techniques
Manufacturing of Drug Product for Development Work

- Initial batch of material made is GMP quality batch sufficient to do toxicology and Phase 1 clinical trial
  - Manufacturing method is similar from project-to-project
  - Assures all process-related impurities are characterized in toxicology studies

- Quality testing of API by LC-MS is the same method and level of detail for non-clinical and clinical studies
  - While chemically synthesized, the linear synthesis method for oligos can not meet the purity standards of small molecules
  - Purity determined by LC-MS is typically around 90%

- Sterile Drug Product is made to support each dose solution concentration needed for pivotal toxicology studies
  - Avoids mistakes and potential for contamination by repeated preparation of small batches at the Toxicology CRO
Typical Non-Clinical Package for MOE ASO to Support Phase 1

- FHD Enabling General Toxicology Studies -
  - 4/13-Week Subcutaneous Toxicity Study in CD1 Mice
  - 4/13-Week Subcutaneous Toxicity Study in Asian Cynomolgus Monkeys
  - GeneTox
    - Mouse Micronucleus assay only
- Safety Pharmacology
  - In vivo cardiovascular safety pharmacology in monkey
  - In vivo CNS safety pharmacology in mice
  - In vivo pulmonary safety pharmacology in mice
  - hERG in vitro
Unique Considerations in Development Package for MOE ASO

- Consistency of biophysical and pharmacokinetic properties of ASO results in relatively consistent toxicology profile
  - Allows for efficient design of studies to support subchronic dosing
    - Pro: Move directly from discovery to 13-week tox
    - Con: Results in relatively small tox package
      - Need to design pretty complex studies (Tox, TK, PK, PD)
      - Increases early development cost and can slow time to Phase 1

- Assessment of effects related to inhibition of intended target can require use of a surrogate animal-specific inhibitor
  - Needed to address potential for exaggerated pharmacology
  - Presence of sequence specific toxicities can affect interpretation
  - Desirable to have a compound that is active in at least 1 tox species
Toxicology and Pharmacodynamic Assessment of a Factor XI Inhibitor

▲ Animals: Male and female Cynomolgus monkeys (Chinese origin)

▲ Safety Evaluation Study: 4, 8, 12, 40 mg/kg/wk or PBS vehicle control; subcutaneous injection for up to 13 weeks
  - Endpoints (collected at several time points throughout the studies)
    - Clinical pathology
    - Complement activation (Bb)
    - Coagulation parameters
    - Factor XI plasma and hepatic mRNA levels
    - Histopathology - full tissue list

▲ Surgical Model of Bleeding Study: 20 mg/kg/wk, enoxaparin (2 mg/kg) or PBS vehicle control; subcutaneous injection for up to 6 weeks
  - Endpoints (collected at several time points throughout the studies)
    - Coagulation parameters
    - Factor XI plasma and hepatic mRNA expression
    - Skin laceration bleeding time assessment
    - Bleeding time assessment following tail amputation or laceration of oral mucosa
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
    - 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
  - Transient ↑ in APTT at high doses during the first 4hr
  - Minimal proximal tubular epithelial cell degeneration at ≥10 mg/kg/wk and Kupffer cell hypertrophy
**Most Factor XI MOE ASO Effects in Monkey Were Typical of the Chemical Class**

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<th>Dose (mg/kg)</th>
<th>Noteworthy Findings</th>
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| 4            | **Liver**: basophilic granules in Kupffer cells (minimal to slight)  
**Lymph nodes**: histiocyte hypertrophy (minimal to slight) |
| 8            | **Liver and lymph nodes**: same as above, slight to moderate in severity |
| 12           | **Clinical pathology**: ↑ Bb (2-fold, sc)  
**Kidney**: basophilic granules in tubular epithelium (slight), tubular dilation and degeneration/regeneration (minimal)  
**Liver, lymph nodes**: same as above (slight to moderate)  
**Spleen**: ↑1.7X weight female only |
| 40           | **Clinical pathology**: ↑ Bb (3-fold, sc)  
**Kidney**: basophilic granules in tubular epithelium (minimal to moderate), granular casts (minimal to slight), tubular dilation (slight), tubular degeneration/regeneration (minimal), tubular vacuolation (moderate)  
**Liver and lymph nodes**: same as above, ↑1.3X liver weight  
**Spleen**: ↑1.7X weight female only |
Pharmacodynamic Assessment for Factor XI Project Was Performed in Monkey

- Human Factor XI ASO was homologous and active in monkey
- Included measurement of PD endpoints at all 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
Time and Dose-Dependent Correlation Between Factor XI mRNA, Plasma Protein, and APTT

- mRNA Expression
- Timecourse of Plasma FXI Activity
- Timecourse of Plasma APTT

**Liver FXI mRNA (\% of Vehicle)**
- Interim Sac (Day 44)
- Terminal Sac (Day 93)
- Recovery (Day 182)

**FXI Activity (\% of Baseline)**
- Days: 0, 30, 60, 90, 120, 150, 180

**APTT (sec)**
- Days: 0, 30, 60, 90, 120, 150, 180

- **Vehicle**
- **4mg/kg/wk**
- **8mg/kg/wk**
- **12mg/kg/wk**
- **40mg/kg/wk**
Dose-Dependant Relationship Between FXI Inhibition and Elevation in APTT

Dose Response (Day 93)

- **Protein**
- **mRNA**
- **APTT**

Response (% of Baseline)

Log Dose (mg/kg/wk)
Assessment of Impact of Factor XI Inhibition in 3 Bleeding Models

- No apparent increase in associated with Factor XI inhibition
  - Mucosal tissue has fibrinolytic activity
  - Tail Amputation similar to Surgical model

- Enoxaparin (low MW Heparin) produced increased bleeding time in mucosal and amputation models
Summary of Results

▲ FXI ASO was well tolerated in mice and cynomolgus monkey for up to 13 weeks of treatment

▲ FXI ASO produced dose dependent reduction in FXI mRNA levels in monkey and subsequent reductions in FXI plasma protein and elevations in APTT

▲ FXI ASO did not produce bleeding at doses up to 40 mg/kg where FXI mRNA levels were reduced by 90%
  ◆ No evidence of excess bleeding was noted under a surgical setting of partial tail amputation or skin or gum laceration

▲ Findings were limited to dose-dependent uptake of FXI ASO into Kupffer cells of the liver, proximal tubular epithelial cells of the kidney and histiocytes of lymphoid tissue
  ◆ These effects were not associated with organ dysfunction and not considered adverse
FXI ASO Therapy For The Treatment of Thromboembolic Diseases

Summary

- Specific suppression of FXI levels and activity demonstrated across multiple animal models including NH primate

- FXI ASO treatment was well tolerated despite suppression of FXI levels ≥ 90%.
  - No bleeding (including NH primate)
  - No toxicity

- Antithrombotic activity comparable to standard of care agents demonstrated in multiple animal models including NH primate

- Superior anticoagulation profile when combined with antiplatelet agents and other standard of care agents relative to FXa small molecules

- **FXI ASO** is currently under evaluation in Phase I studies in healthy volunteers