ISIS-DMPK$_{Rx}$: An Antisense Drug for The Treatment of Myotonic Dystrophy Type 1

C. Frank Bennett Ph.D.
Senior Vice President of Research
Agenda

- Stages of Drug Development
- Drug Development Strategies for Myotonic Dystrophy
- Introduction to Antisense Technology
- \( \text{ISIS-DMPK}_{\text{Rx}} \):
  - Why we are excited about the drug
  - Where we are in the development process
The Stages of Drug Development

ISIS-DMPK$_{Rx}$

Lab

Discovery

Development

Commercial

Market

Basic Research

Pre-Clinical

Clinical Testing

Marketing

Product Launch

Sales

IND

NDA

SNDAs
Drug Discovery Steps

- Identify the therapeutic target → The Gene

- Determine the therapeutic strategy
  - What to target?
  - How to target?
  - When to target?

- Screen to identify the drug candidate
  - Activity
  - Safety

Drug Development
Government, Foundation Supported, Pharmaceutical Industry Research are Complementary

There is an ecosystem of science and biotechnology. Public organizations, patient organizations, universities, Congress, FDA, all of this is an ecosystem that is envied in the rest of the world.

– E. Zerhouni, Director of NIH
**1st Clue → What to Target?**

DM is Caused by a 3 Nucleotide Expansion in the DMPK Gene

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**Molecular Basis of Myotonic Dystrophy: Expansion of a Trinucleotide (CTG) Repeat at the 3’ End of a Transcript Encoding a Protein Kinase Family Member**

J. David Brook,*† Mila E. McCurrach,*
Helen G. Harley,† Alan J. Buckler,* Deanna Church,*
Hiroyuki Aburatani,* Kent Hunter,*
Vincent P. Stanton,* Jean-Paul Thirion,*
Thomas Hudson,* Robert Sohn,* Boris Zemelman,*
Russell G. Snell,† Shelley A. Rundle,† Steve Crow,†
June Davies,‡ Peggy Shelbourne,‡ Jessica Buxton,‡
Clare Jones,‡ Vesa Juuvonen,‡ Keith Johnson,‡
Peter S. Harper,† Duncan J. Shaw,†
and David E. Housman*

*Center for Cancer Research
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
†Institute of Medical Genetics
University of Wales College of Medicine
Heath Park
Cardiff CF4 4XN
Wales
‡Department of Anatomy
Charing Cross and Westminster Medical School
London W6 8RF
England

**Summary**

Using positional cloning strategies, we have identified a CTG triplet repeat that undergoes expansion in myotonic dystrophy patients. This sequence is highly variable in the normal population. PCR analysis of the interval containing this repeat indicates that unaffected individuals have between 5 and 27 copies. Myotonic dystrophy patients who are minimally affected have at least 50 repeats, while more severely affected patients have expansion of the repeat containing segment up to several kilobase pairs. The CTG repeat is transcribed and is located in the 3’ untranslated region of an mRNA that is expressed in tissues affected by myotonic dystrophy. This mRNA encodes a polypeptide that is a member of the protein kinase family.
2nd Clue → Nucleotide Expansion Sequesters Proteins
A Gain of Function Toxic RNA

DNA → pre-mRNA → mRNA

- RIBONUCLEAR FOCI
- RNA BINDING PROTEIN DYSREGULATION
- ABERRANT mRNA SPLICING
- DM1 CLINICAL FEATURE

- Chloride Channel
- Insulin Receptor
- Cardiac Troponin T
- Tau Protein
- Myotubularin
- ?
- ?
- ?

- Myotonia
- Insulin Insensitivity
- ?Cardiac Arrhythmia
- ?CNS Effects
- ?Myopathic Effects
- Cataracts
- Testicular Failure
- Hypogammaglobulinemia
How to Target?
Antisense Drugs

a. DMPK gene
   - mRNA
   - MBNL protein

b. Mutant DMPK
   - Toxic mRNA

(c). Antisense oligonucleotides
   - RNase H

Nucleus

Cytoplasm
How Genetic Information Flows From in DNA to Protein
The “Central Dogma” of Molecular Biology
Antisense Drugs Target RNA, not Proteins

Antisense Drugs can also inhibit the function of a disease causing RNA that doesn’t make a protein (a non-coding RNA). This is nearly intractable for other platforms.
The Antisense Drug-Receptor Interaction

~16-20 base pairs required for specificity
Antisense Drugs
Chemical Modifications Produce Desired Effects in the Body

Commonly Used Chemical Modifications for Antisense Drugs
ISIS-DMPK$_{Rx}$: Promotes Degradation of the Toxic DMPK RNA
An RNase H Mechanism of Action
Proof of Concept Studies:
Systemic Delivery of Antisense Drug Reverses Myotonia and Spliceopathy in a Mouse Model of DM1 (HSA-LR)

HSA-LR mRNA Level (Normalized to 18S RNA)

Serca-1

Average myotonia grade

Next Step:
Demonstrate Reduction of DMPK RNA in Muscle of Different Species
Proof of Concept:
Marked Reduction of Endogenous mDMPK RNA Levels in Mice by an RNase H Antisense Drug
**Proof of Concept:**
Reduction of DMPK RNA Levels in Non-human Primates (Cynomolgus Monkey) by an Antisense Drug

**mRNA expression from several monkey tissues**

Dose and duration: 40 mg/kg BW; day 1,3,5,7 and then once weekly for the next 12 weeks (total of 16 doses)
Proof of Concept:
Prolong Duration of Effect with a DMPK Targeting Antisense Drug in Monkey Muscle

![Graph showing mRNA expression (% PBS) over time with DMPK Drug at 7, 13, 19, and 26 weeks post-dosing, compared to PBS after dosing stopped and 3 months later.](image-url)
The Screening Process
The Discovery of ISIS-DMPK$_{Rx}$

Design and Synthesis of Antisense Drugs
Screen in Cell Culture

Screen in Rodents (Safety and Tolerability)

Screen in Rodents (Pharmacology)
Target reduction
Benefit in a disease model

Screen in Non-human Primate
Target reduction
Safety and tolerability

$\sim$3000 Antisense Drugs

$\sim$300 Antisense Drugs

$\sim$100 Antisense Drugs

$\sim$10 Antisense Drugs

ISIS-DMPK$_{Rx}$
- Generation 2.5 antisense drug

Constrained ethyl nucleotide  
2’-MOE (methoxyethyl)

- Promotes degradation of the mutant DMPK transcript by the RNase H mechanism of action

- Delivered by subcutaneous injection ~once per week
Preclinical Phase
Before Testing in Humans

- **Characterize potential adverse drug effects**
  - Define potential organ or tissue damage induced by the drug
  - Define reversibility of toxicity

- **Characterize pharmacokinetics**
  - Drug levels in blood

- **Characterize beneficial pharmacodynamic effects**
  - Does the drug do anything to the target in the body

- **Guide safe use in human clinical studies**
  - Determine safe and reasonable starting does
  - Provide monitoring guidelines for the clinical study

- **Provide sufficient data to conclude that patients are not exposed to unreasonable risks**
**ISIS-DMPK$_{Rx}$**
IND Enabling Toxicology Summary

- **Mouse**
  - 13 Week repeat-dose study with 13-week recovery

- **Monkey**
  - 13-week repeat dose study and a 13-week recovery

- **Standard genetic toxicology and safety pharmacology studies**

- **Results from toxicology study support continued development of ISIS-DMPK$_{Rx}$**
**Study Objective:** to assess the safety and tolerability of \( \text{ISIS-DMPK}_{\text{Rx}} \) in healthy volunteers

- Blinded, randomized, placebo-controlled single ascending dose
- 4 single dose cohorts (randomized 3 active: 1 placebo);
- Doses: 50 mg, 100 mg, 200 mg, 400 mg
- Single subcutaneous injection on Day 1, and subjects are followed for 28 days

**Primary Endpoints:**
- Safety and Tolerability
- Pharmacokinetetic measures (plasma drug levels)
Phase 1 SAD in Healthy Volunteers → completed
- No safety or tolerability concerns identified
- Up to 400 mg single dose tested
ISIS-DMPK$_{Rx}$ Phase 2
Multiple Dose Study in DM1 Patients

- Planned to start late this year in the United States
- Drug will be given as a subcutaneous injection
- Primary Goals:
  - Safety & Tolerability, and PK
- In addition, markers of biological activity and clinical biomarkers will be collected
ISIS-DMPK$_{Rx}$

Summary

- Antisense drugs demonstrate selectivity for nuclear retained RNAs

- Systemic delivery of Gen 2.5 ASOs profoundly inhibited mouse DMPK RNA levels in normal mice and monkeys: treatment was well tolerated

- Generation 2.5 antisense drugs have long duration of action in skeletal muscle

- ISIS-DMPK$_{Rx}$ Phase 1 single dose study in normal volunteers has been completed

- ISIS-DMPK$_{Rx}$ Phase 2 multiple dose study in DM1 patients to start late this year
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