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

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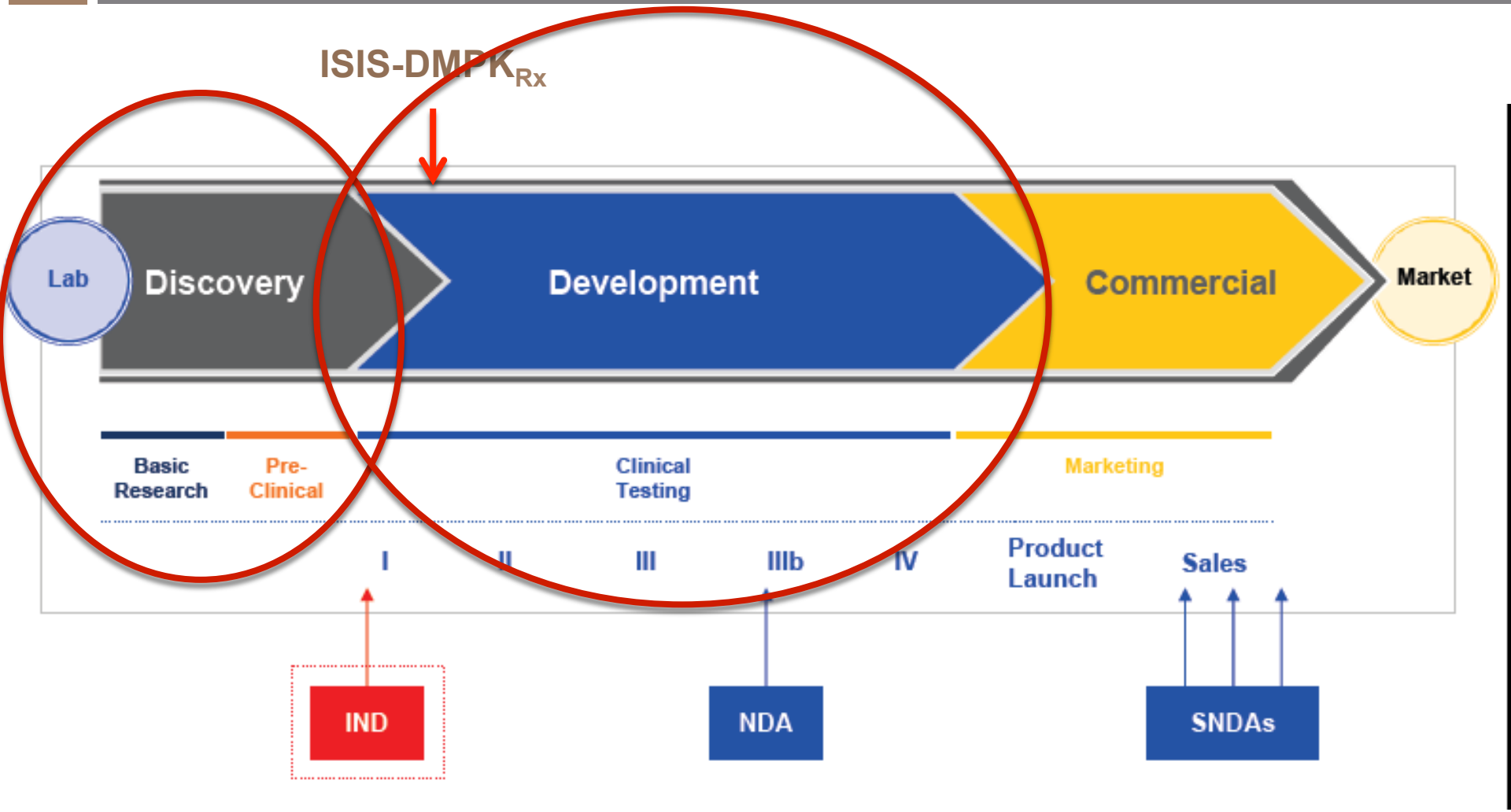
ISIS® PHARMACEUTICALS

Agenda

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

The Stages of Drug Development



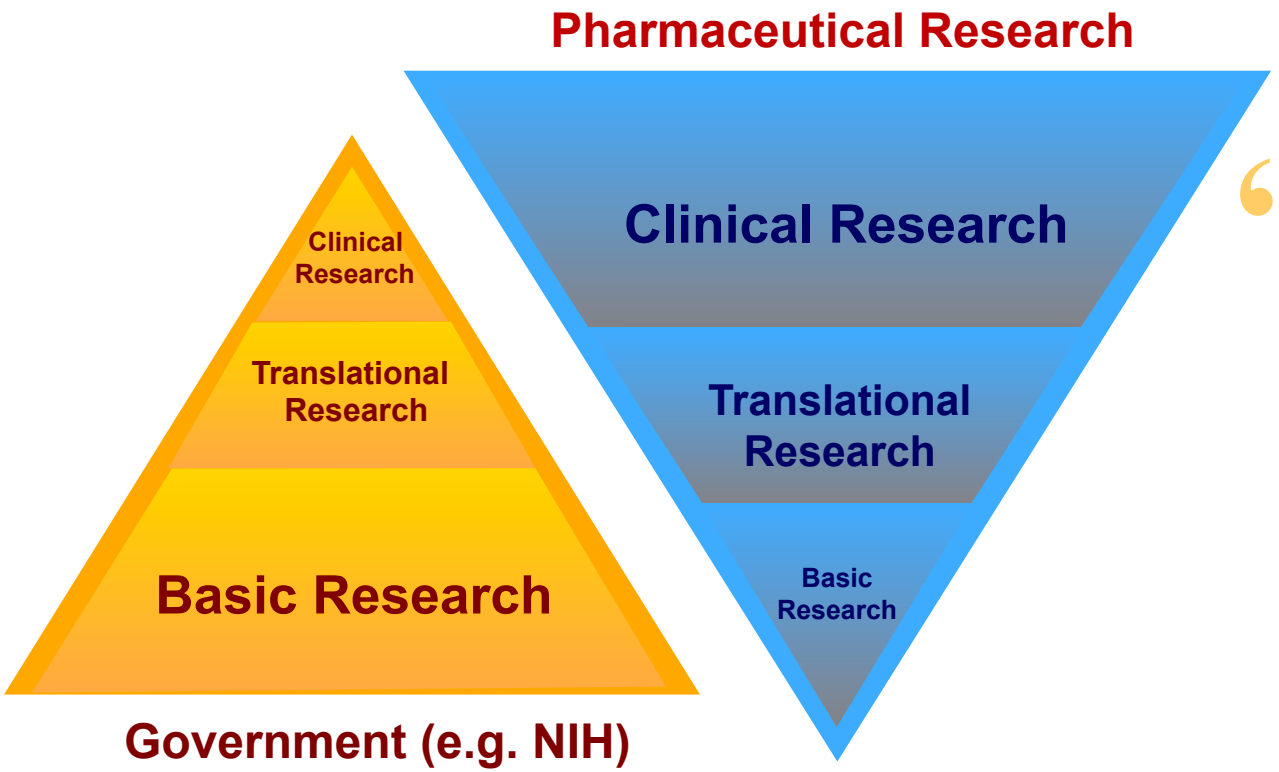
Drug Discovery Steps

- **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

Federal, Public & Company Roles in Research & 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

Cell, Vol. 68, 799–808, February 21, 1992.

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

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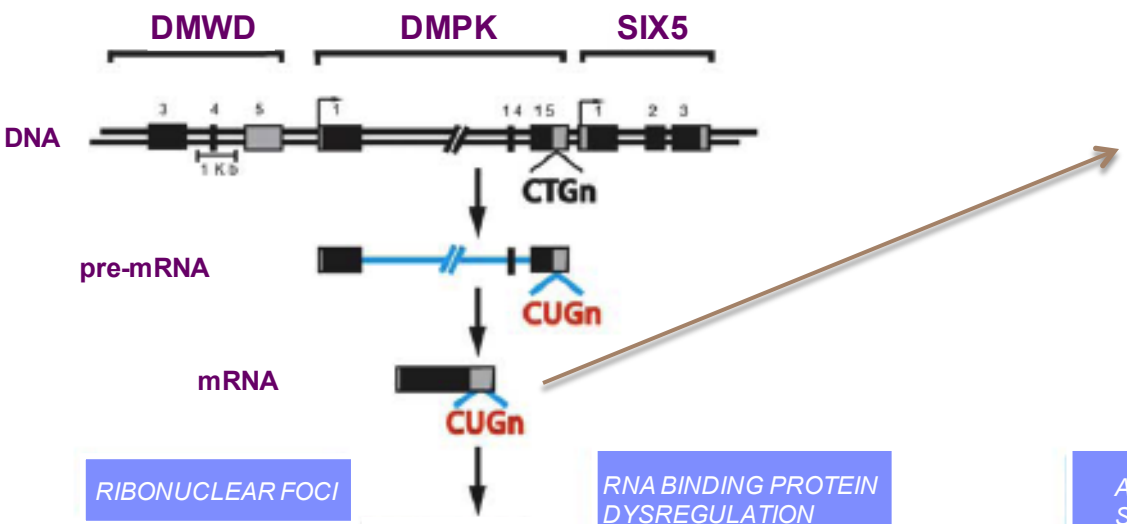
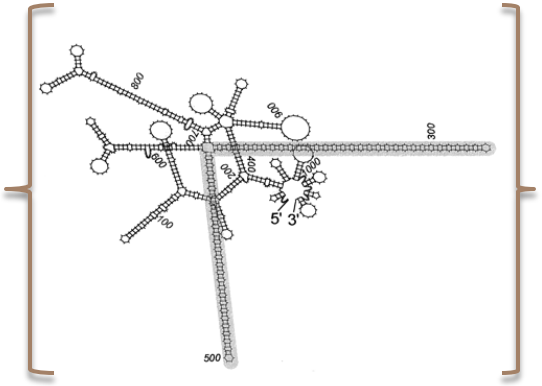
Summary

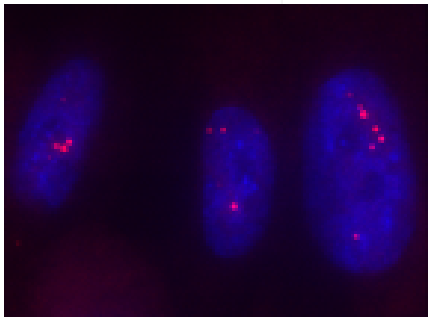
Using positional cloning strategies, we have identified a CTG triplet repeat that undergoes expansion in myotonic dystrophy patients. This sequence is highly variable in the normal population. PCR analysis of the interval containing this repeat indicates that unaffected individuals have between 5 and 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







RIBONUCLEAR FOCI

RNA BINDING PROTEIN DYSREGULATION

MBNL ↓

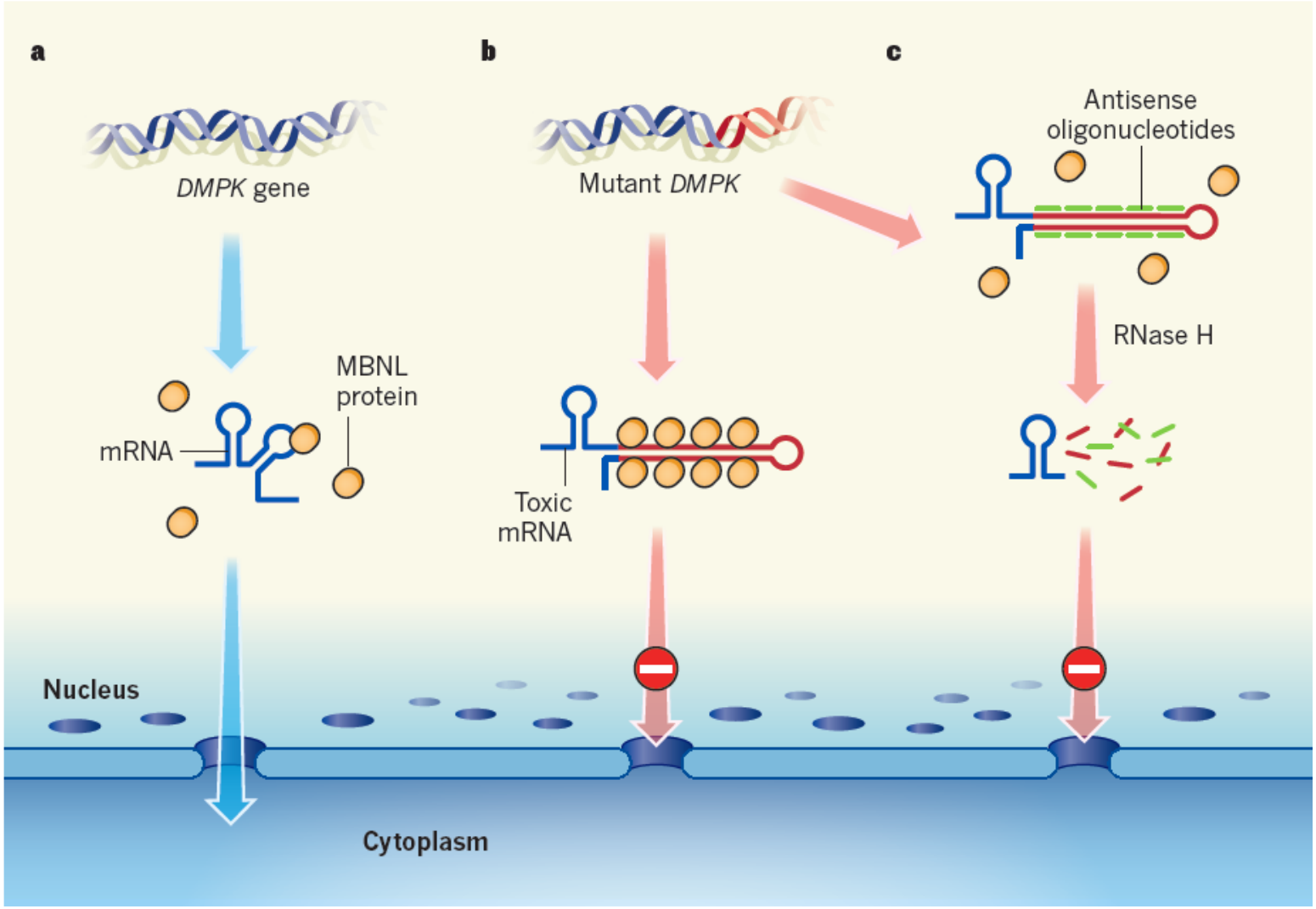
CUG-BP ↑

ABERRANT mRNA SPLICING

Chloride Channel	→	Myotonia
Insulin Receptor	→	Insulin Insensitivity
Cardiac Troponin T	→	?Cardiac Arrhythmia
Tau Protein	→	?CNS Effects
Myotubularin	→	?Myopathic Effects
?	→	Cataracts
?	→	Testicular Failure
?	→	Hypogammaglobulinemia

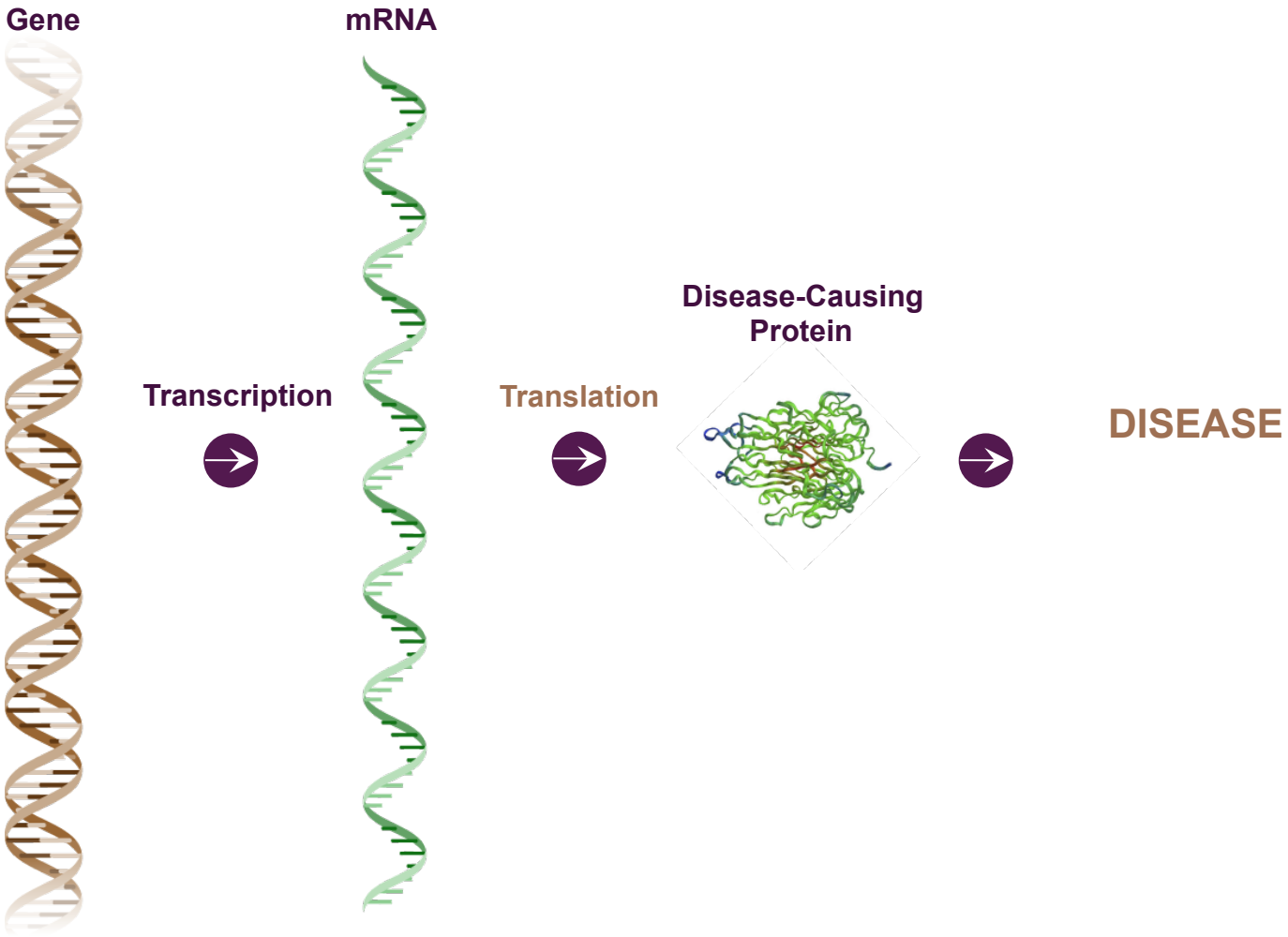
DM1 CLINICAL FEATURE

How to Target?
Antisense Drugs

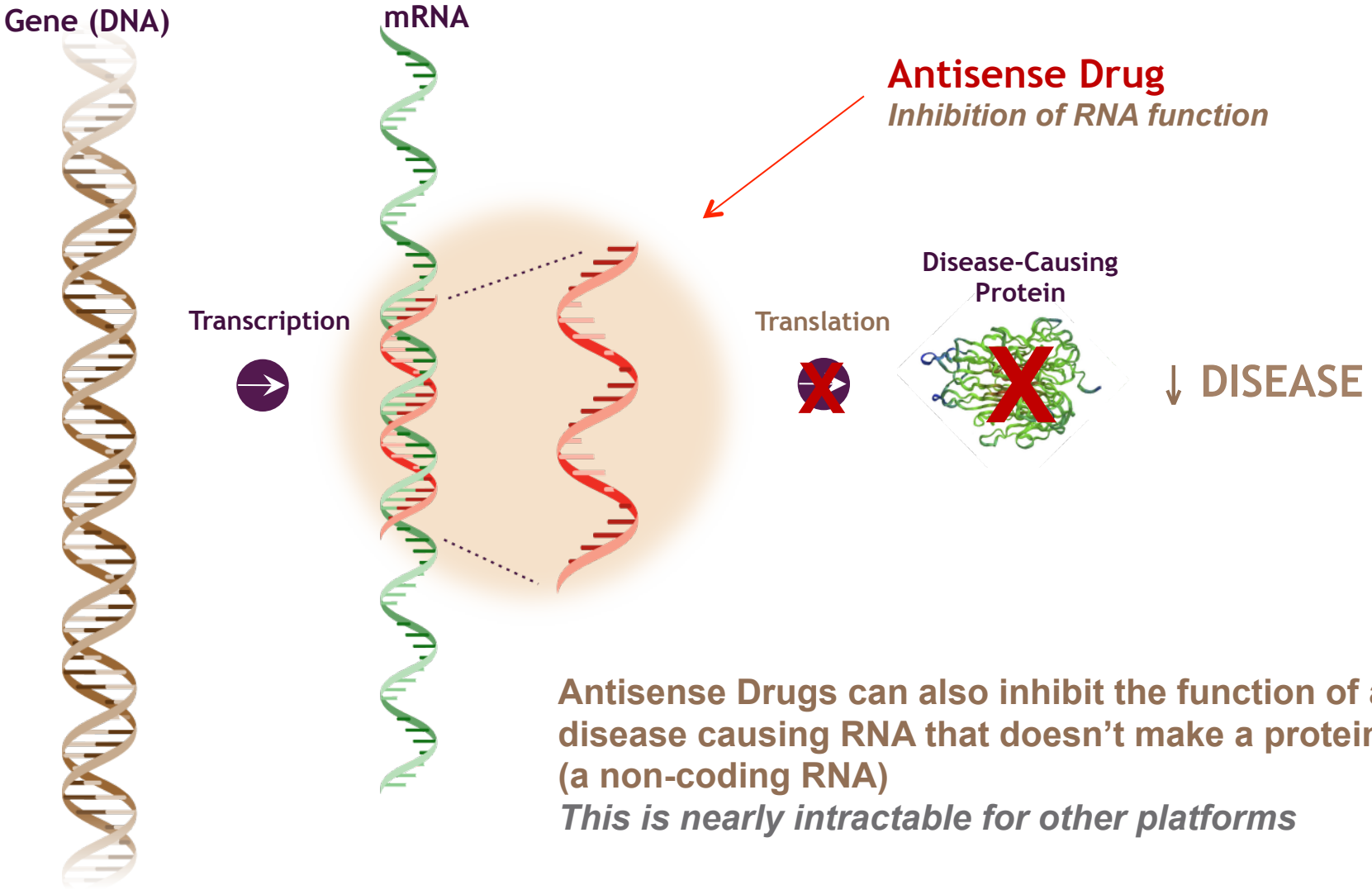


How Genetic Information Flows From in DNA to Protein

The “Central Dogma” of Molecular Biology



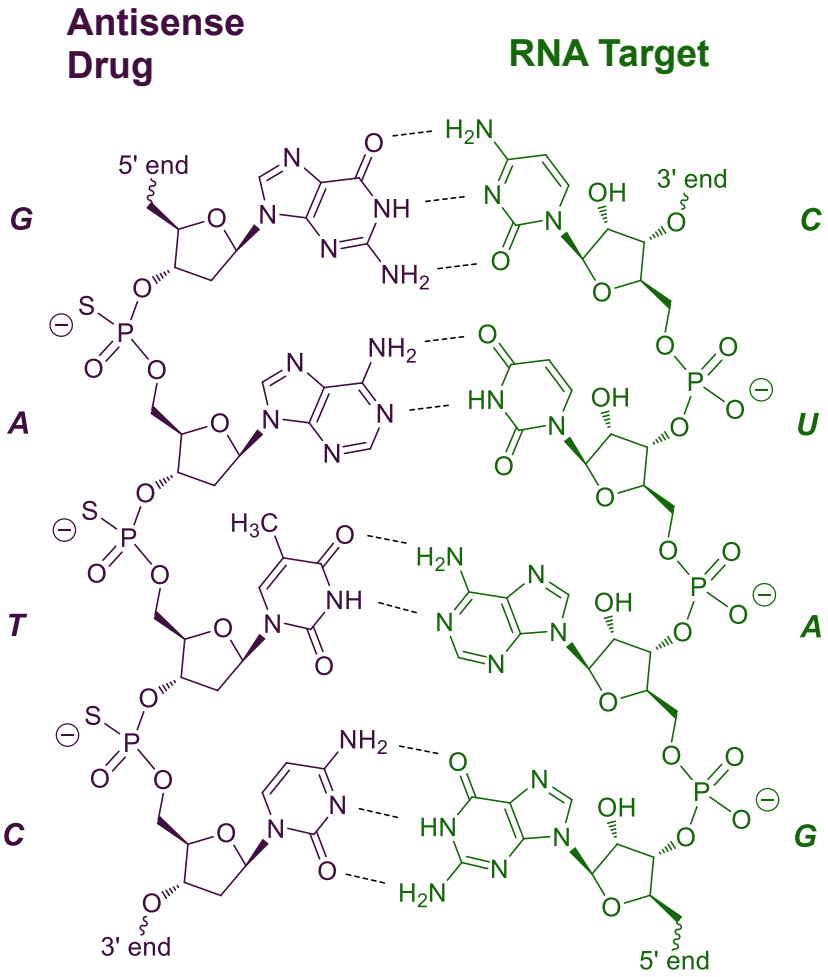
Antisense Drugs Target RNA, not Proteins

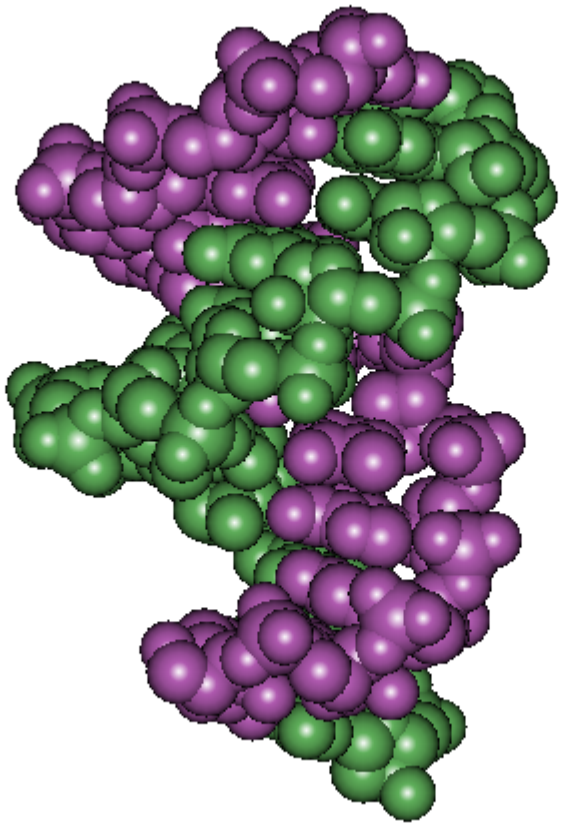


Antisense Drugs can also inhibit the function of a disease causing RNA that doesn't make a protein (a non-coding RNA)

This is nearly intractable for other platforms

The Antisense Drug-Receptor Interaction



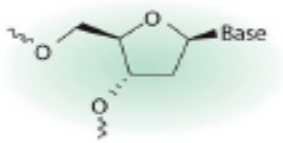


▲ ~16-20 base pairs required for specificity

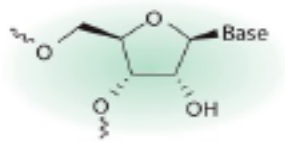
Antisense Drugs

Chemical Modifications Produce Desired Effects in the Body

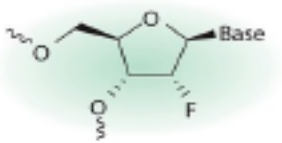
Commonly Used Chemical Modifications for Antisense Drugs



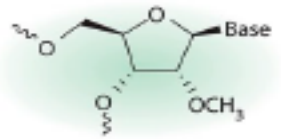
Deoxy
(2'-deoxyribofuransyl)



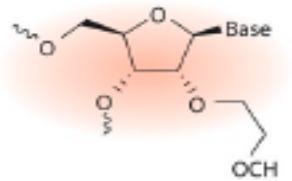
Ribo
(ribofuransyl)



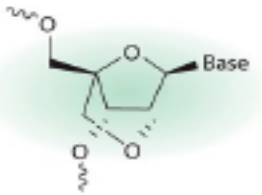
2'-Fluoro



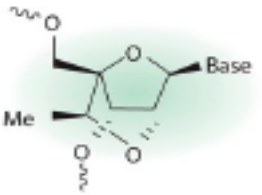
OMe
(2'-O-methyl)



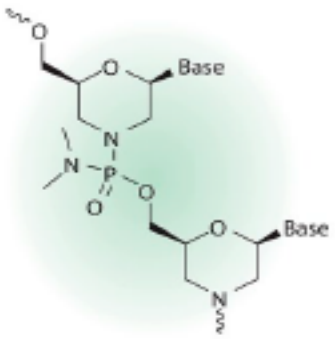
MOE
(2'-O-methoxyethyl)



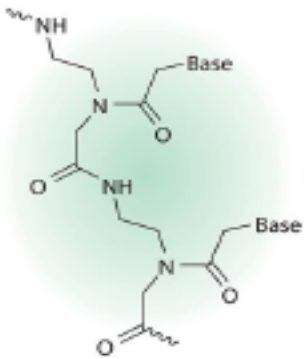
LNA
(locked nucleic acid)



S-cEt
(2',4'-constrained-
2'-O-ethyl BNA)



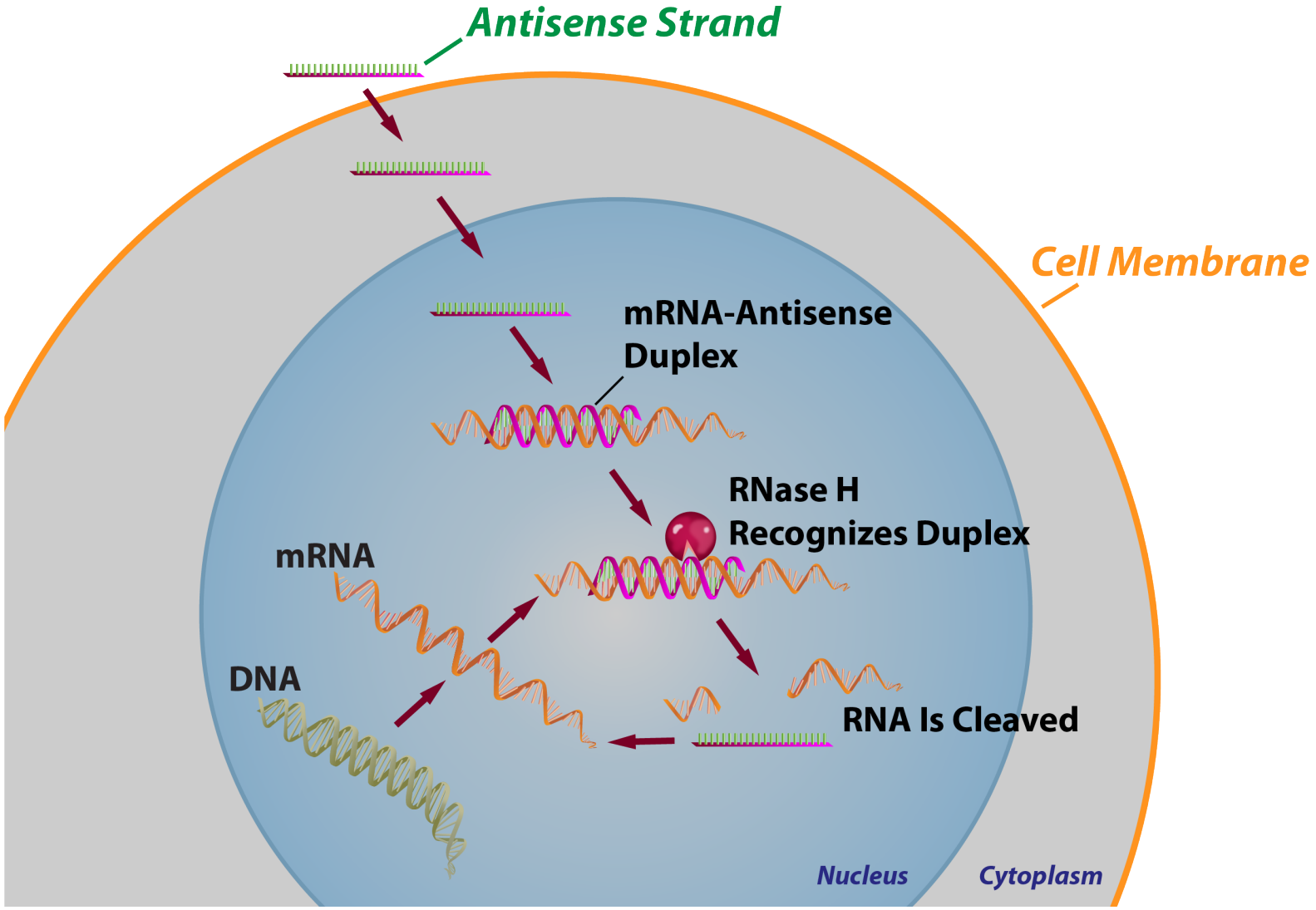
Morpholino



PNA
(Peptide nucleic acid)

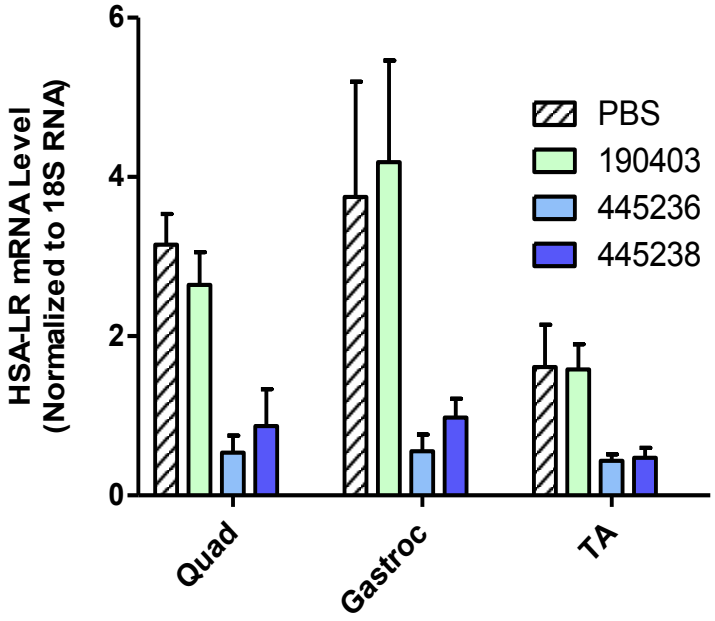
ISIS-DMPK$_{Rx}$: Promotes Degradation of the Toxic DMPK RNA

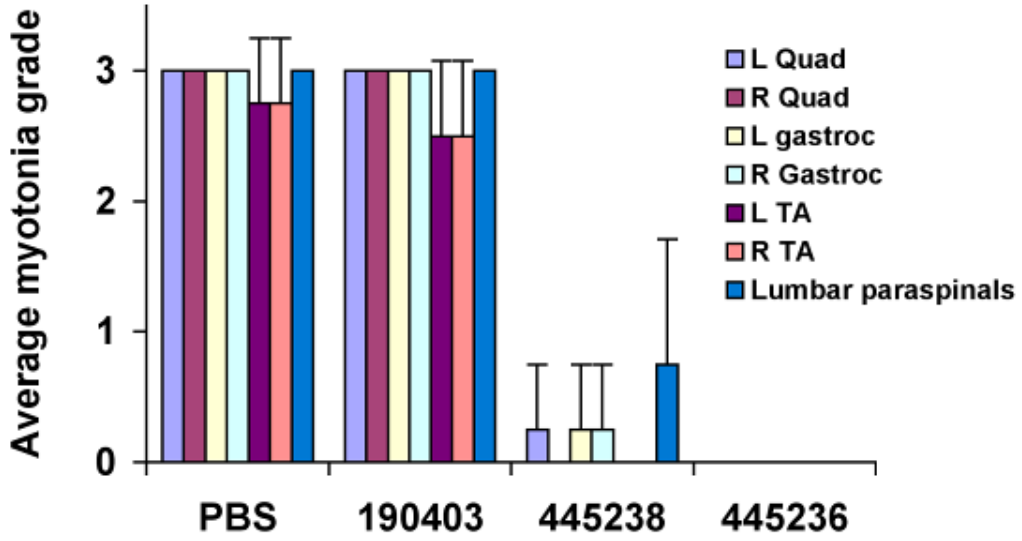
An RNase H Mechanism of Action



Proof of Concept Studies:

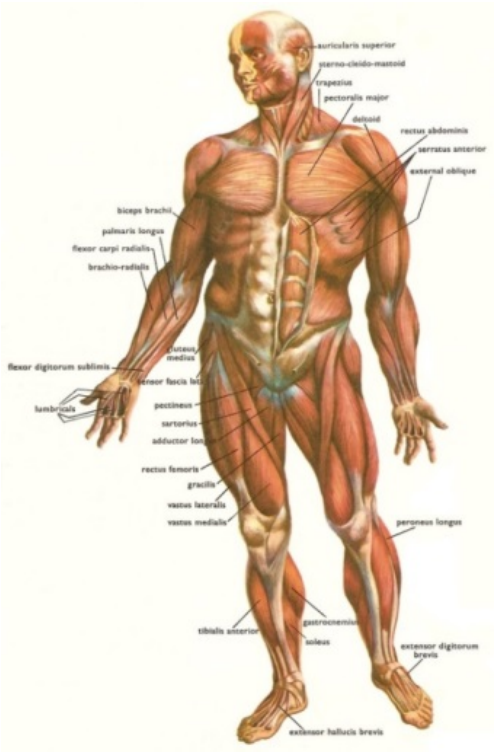
Systemic Delivery of Antisense Drug Reverses Myotonia and Spliceopathy in a Mouse Model of DM1 (HSA-LR)





Wheeler et al., Nature 488: 111, 2012

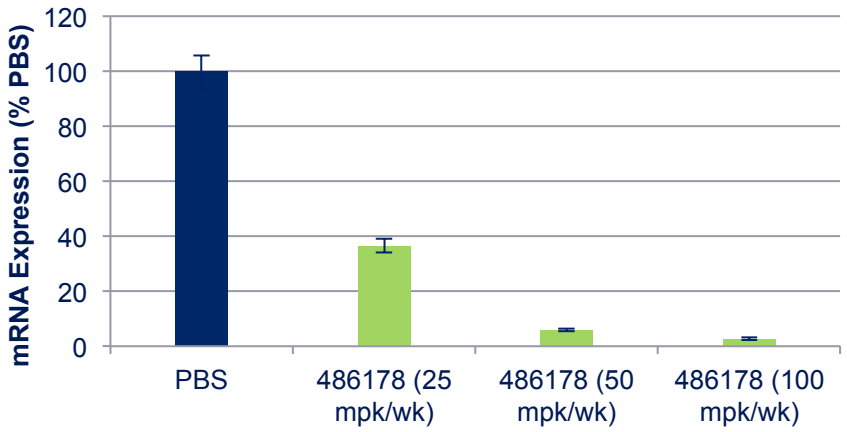
Next Step:
Demonstrate Reduction of DMPK RNA in Muscle of Different Species



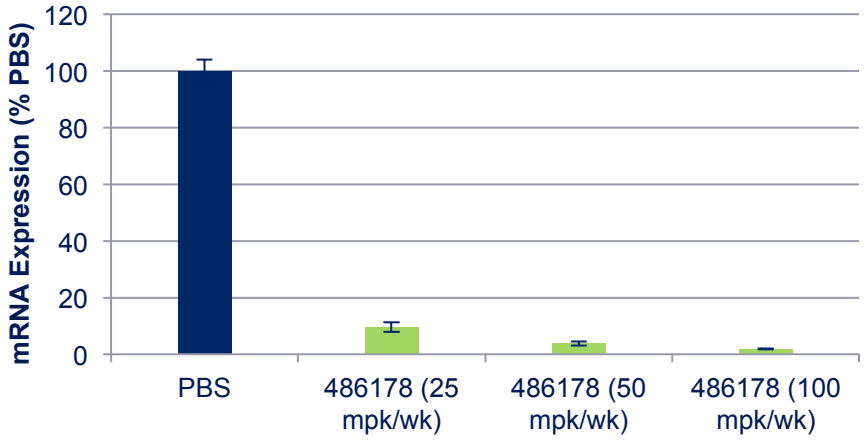
Proof of Concept:

Marked Reduction of Endogenous mDMPK RNA Levels in Mice by an RNase H Antisense Drug

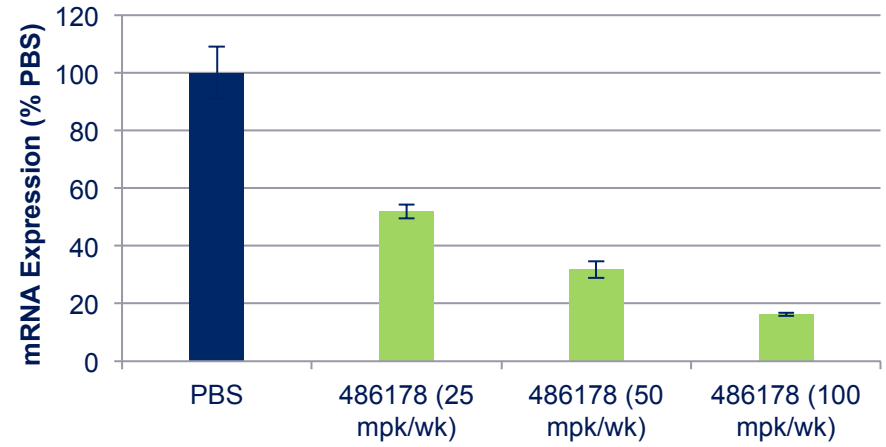
Tibialis Anterior



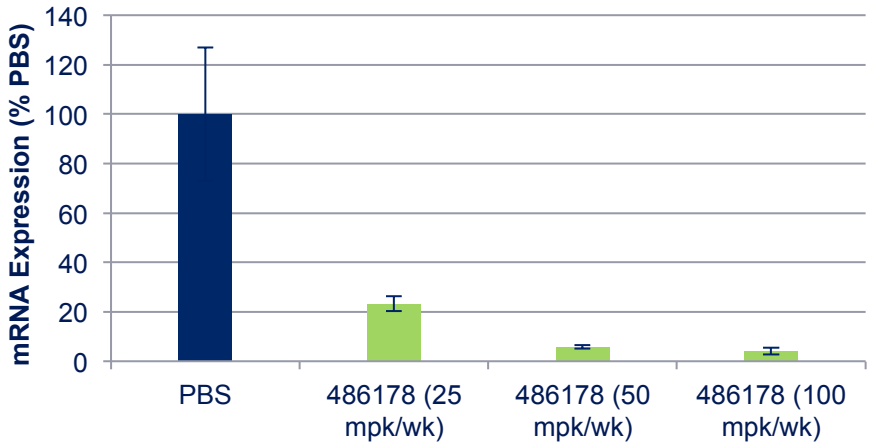
Diaphragm



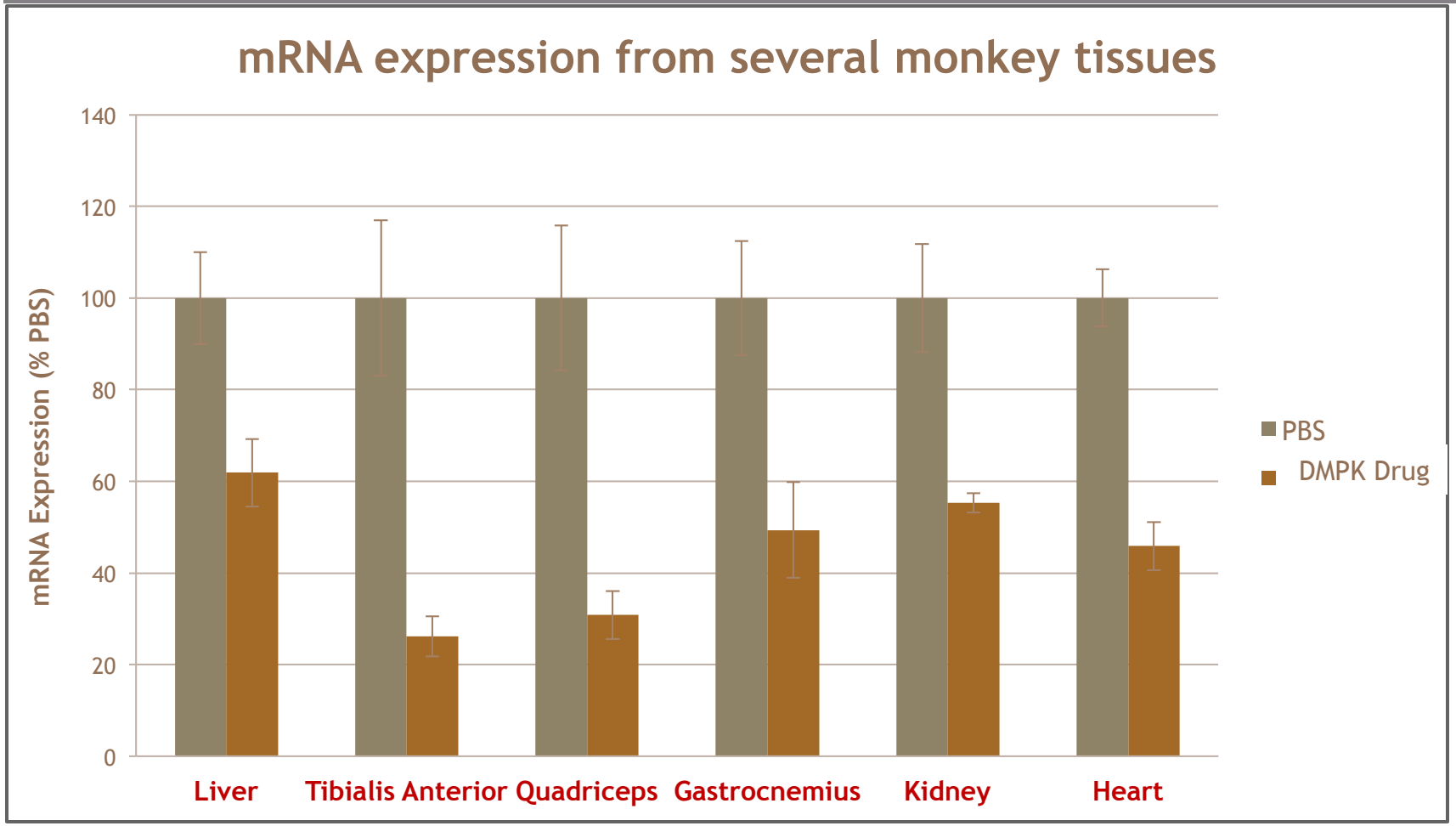
Heart



Gastrocnemius



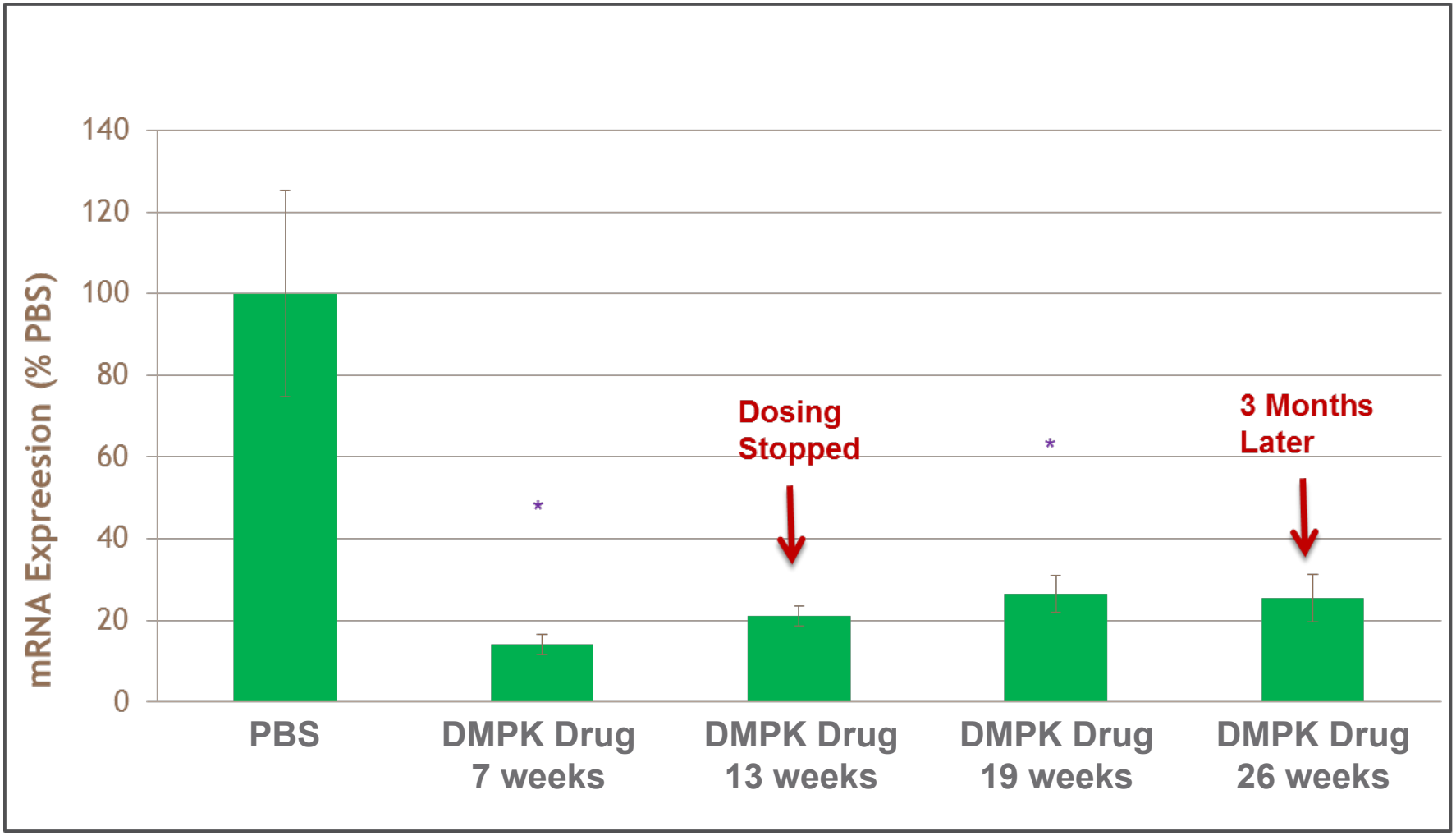
Proof of Concept:
Reduction of DMPK RNA Levels in Non-human Primates (Cynomolgus Monkey) by an Antisense Drug



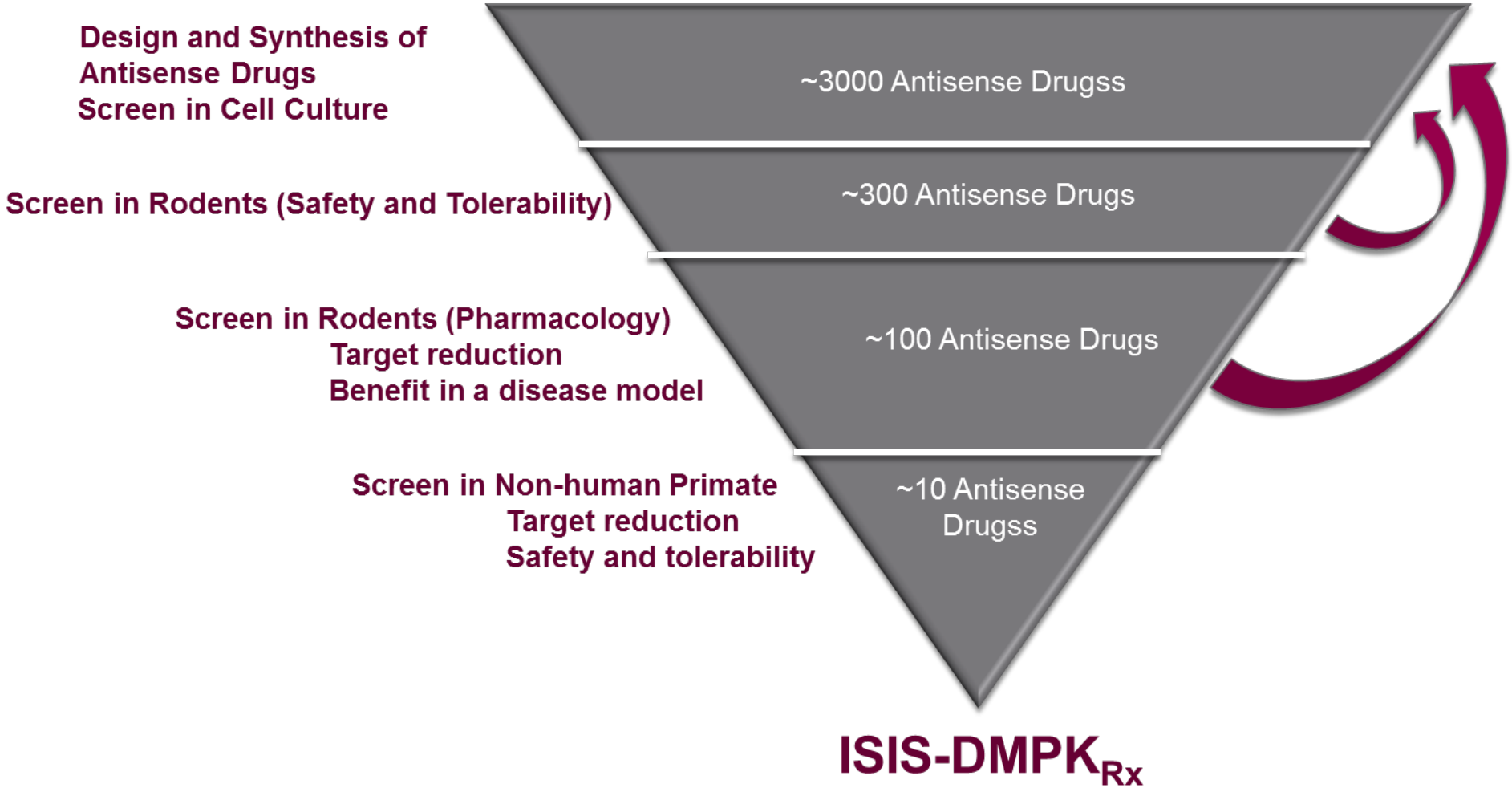
Dose and duration: 40 mg/kg BW; day 1,3,5,7 and then once weekly for the next 12 weeks (total of 16 doses)

Proof of Concept:

Prolong Duration of Effect with a DMPK Targeting Antisense Drug in Monkey Muscle



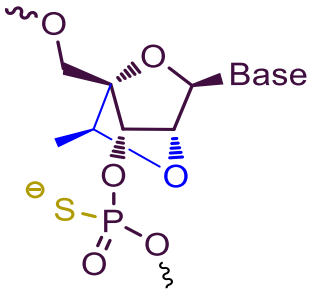
The Screening Process
The Discovery of ISIS-DMPK$_{Rx}$



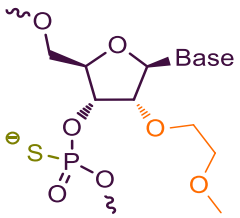
ISIS-DMPK$_{Rx}$

Partnered with biogen idec

- Generation 2.5 antisense drug



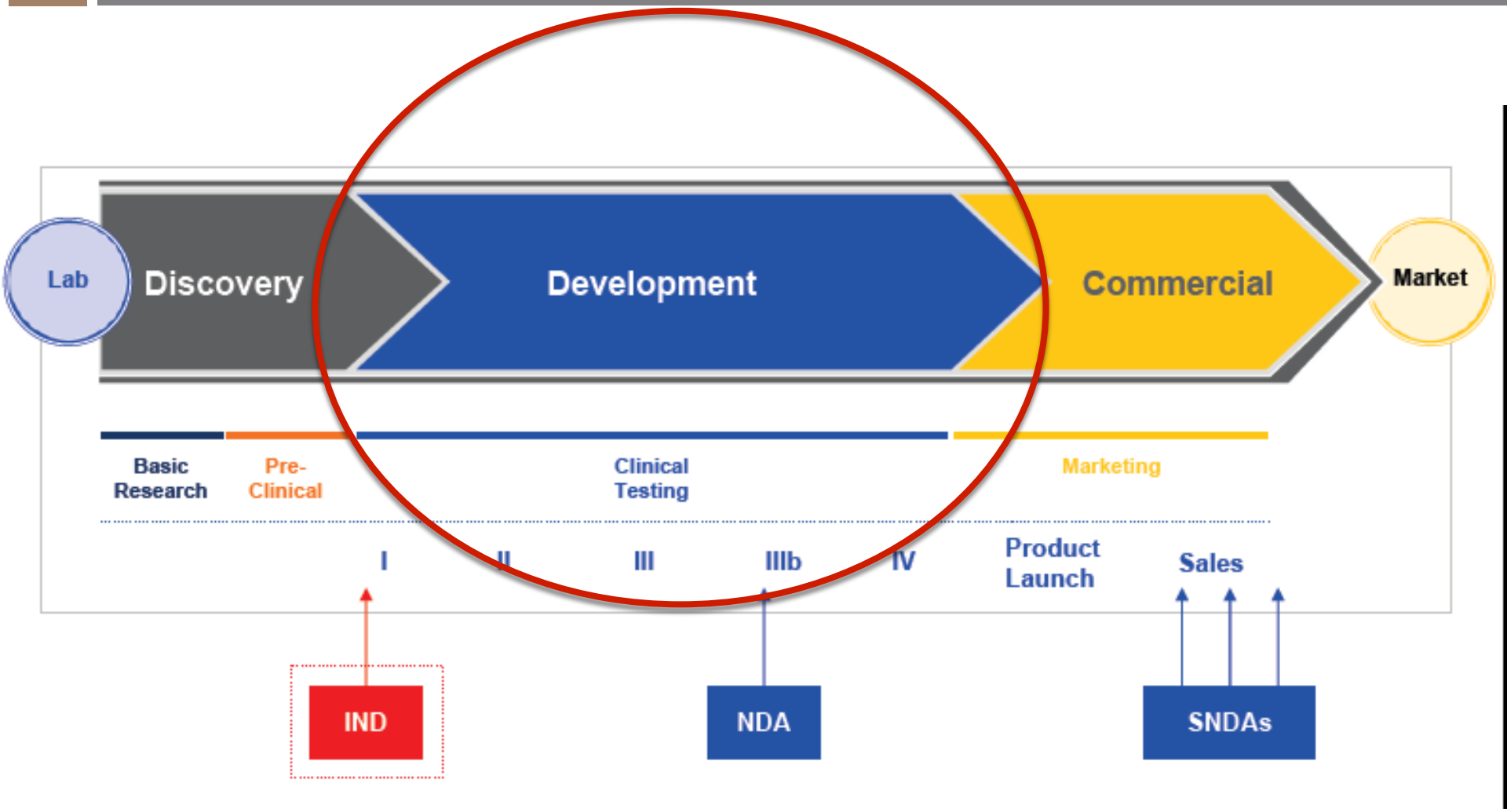
Constrained ethyl nucleotide



2'-MOE (methoxyethyl)

- Promotes degradation of the mutant DMPK transcript by the RNase H mechanism of action
- Delivered by subcutaneous injection ~once per week

The Stages of Drug Development



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$_{Rx}$**

ISIS-DMPK$_{Rx}$ Phase 1

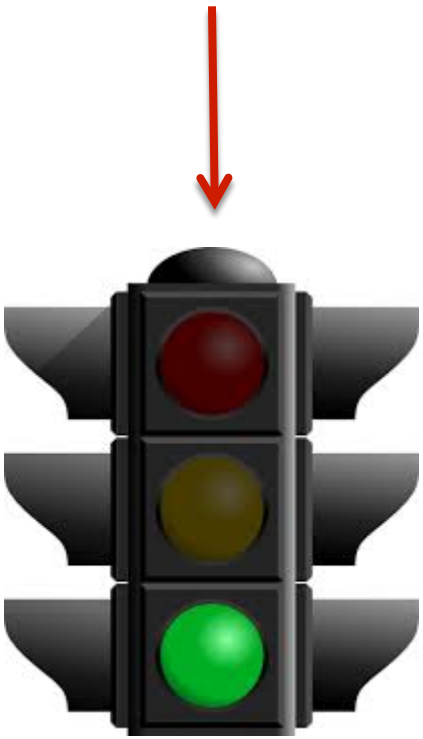
CS1 Experimental Design - Single Dose

Study Objective: to assess the safety and tolerability of ISIS-DMPK$_{Rx}$ 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
  - Pharmacokinetic measures (plasma drug levels)

ISIS-DMPK$_{Rx}$ Phase 1
CS1 Single Ascending Dose Results

- **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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