DRAFT 8/28/2023

DART STRATEGIES FOR OLIGONUCLEOTIDES

October 17-18, 2023

In-Person: Open Gov Hub, 1100 13th Street, NW Ste 800 Washington, D.C. 20005
And Online: Zoom

Background: Oligonucleotides represent a novel pharmacotherapeutic drug class. While based in the specificity of Watson-Crick base pairing, construct (single and double stranded) and chemical modifications vary and result in distinct advantages but also specific challenges. Single stranded antisense oligonucleotides (ASO) were the first in this drug class. More recent are small interfering RNAs (siRNAs), with the first siRNA approval in 2018. Oligonucleotides have both small molecule and biotherapeutic attributes and there are no class-specific ICH or FDA guidance documents to provide a clear blueprint for nonclinical safety testing, including for developmental and reproductive toxicity (DART) evaluations. Currently, these compounds are regulated as small molecules, but certain compound characteristics aligning to that of biologics raises the need to evaluate if a traditional small molecule DART assessment is appropriate.

Meeting Purpose & Highlights: This workshop, organized by the Health and Environmental Sciences Institute (HESI) DART technical committee, will consider application of DART principles to fit attributes of ASOs, siRNAs and other oligonucleotide therapeutics. Gaps and challenges associated with determining the most appropriate strategies to account for potential risk to a varied patient population and spectrum of disease conditions will also be highlighted. Considerations unique to oligonucleotide therapeutics for DART evaluations that will be discussed include mechanism of action/target engagement, dosing schedule, toxicokinetics and biodistribution, and species or model selection and validation. Speakers will represent institutions actively working in oligonucleotide therapeutic development and will deliver case study examples to illustrate various DART considerations. It is anticipated that most of the session will be reserved for discussion rather than presentation. This workshop will be timely as RNA-based therapeutic development is expanding. Reviewing existing DART strategies and initiating discussion on how to address gaps and challenges will help advance the field toward a fit for purpose and robust characterization of DART risk for this class of compounds. The workshop will conclude with a forward-thinking scientific panel discussion based on emerging themes from the session. Panelists will include speakers and regulatory representatives who will provide their opinion (not necessarily that of their organizations) on key questions and challenges that have arisen in the meeting.

Anticipated Attendance: This meeting will engage representatives from the HESI DART committee and other invited experts and stakeholders. There will be a nominal registration fee, and registration will be open to virtual and in-person attendees.

Workshop Outputs: The goal will be to develop and submit a manuscript for peer review highlighting key workshop recommendations, gaps, and opportunities.

Updated information, including registration, will be posted online:
https://hesiglobal.org/developmental-and-reproductive-toxicology-dart/

Tuesday October 17
8:30 AM – 3:30 PM Eastern

7:30 – 8:30 **Registration, Breakfast**

8:30 AM – 10:25 AM Eastern
Opening Session

8:30 – 8:35 **Introduction to Workshop**
Bethany Hannas (Eli Lilly)

8:35 – 9:00 **Points to consider in design of science-based reproductive safety evaluations for oligonucleotides**
Tacey White (Tacey White Consulting)

9:00 – 9:20 **Regulatory Considerations- Japan**
Kazushige Maki (PMDA)

9:20 – 9:40 **Regulatory Considerations- EMA**
Kris Siezen (CBG-MEB)

9:40 – 10:00 **Regulatory Considerations- US**
Ronald Wange (US FDA/CDER)

10:00 – 10:15 **Break**

10:15 – 10:40 **Development and Reproductive Toxicity Safety Assessment Strategies for RNAi Therapeutics: Considerations in the Utilization of Standard Study Designs**
Camelia Saffarini (Alnylam)
Short interfering RNA (siRNA) is a novel class of human therapeutics that mimics the endogenous RNA interference mechanism mediating the translation of mRNA to protein. Through this mechanism, RNAi therapeutics can be designed to specifically target mRNA and silence the production of disease-associated proteins. This presentation will describe the unique characteristics of RNAi therapeutics and how this influenced the development and reproductive toxicity (DART) safety assessment of two approved siRNA therapeutics with different delivery systems, ONPATTRO® (patisiran) and GIVLAARI® (givosiran), for the treatment of polyneuropathy of hereditary transthyretin-mediated (hATTR) amyloidosis and acute hepatic porphyria, respectively, in adults. These examples will present an opportunity to discuss how the unique properties of siRNAs impacted DART study designs and how results from these studies must be placed into appropriate context when informing human risk assessment.

10:40 – 11:00 **CRO Perspective: Common Questions, Strategies**
Michael Templin (Charles River Laboratories)

11:00 AM – 1:00 PM Eastern
Case Study Session 1: General Principles

Case studies in this session will cover various DART program designs. Speakers will describe some of the general principles applied to these DART programs, including justification for dose level selection, dosing schedule and overall study design. Specific factors considered to fit the design to the attributes of the oligonucleotide will be described.

Moderator: Tacey White (Tacey White Consulting)

11:00 – 11:15 **Comparison of Antisense Oligonucleotides (ASOs) with and without Ligand Conjugate Antisense (LICA) in Reproductive and Developmental Toxicology Studies in Mice**
Tae-Won Kim (Ionis)
In this presentation, reproductive and developmental tox study design including dosing schedule (differences between weekly dosing vs monthly dosing in clinic), justification of dose selection without conducting a separate DRF study, TK sampling, strategy of including surrogate molecule and safety margin calculation based on cumulative dose calculation, will be discussed with the same sequence ASO with and without LICA.

11:15 – 11:25 Discussion/Q&A

11:25 – 11:40 **Case S-antigen transport-inhibiting oligo polymer mouse and rabbit DART program**
Dinah Minser (Aligos)

11:40 – 11:50 Discussion/Q&A

11:50 – 12:05 **Case Study 3**
Nicola Powles-Glover (AstraZeneca)

12:05 – 12:15 Discussion/Q&A

12:15 – 12:45 **Discussion- identify key take aways/questions**

12:45 PM – 1:45 PM
LUNCH

1:45 PM – 3:30 PM Eastern
Case Study Session 2: Species/Model Selection

Case studies in this session will describe DART program considerations for scenarios when the clinical candidate oligonucleotide is not pharmacologically active in the routine DART species (rodent and rabbit). Aspects of designing programs using a routine DART species surrogate molecule will be described.

Moderator: Jen Sisler (Eli Lilly)

1:45 – 2:00 **Adaptation of a Combined Female Fertility/EFD Study Strategy using a Surrogate GalNAc siRNA Oligonucleotide**
Sara Blazejewski (GSK)
A surrogate molecule approach was used to conduct a combined female fertility/embryo-fetal development (EFD) study in rats. The surrogate is a N-acetylgalactosamine short-interfering RNA (GalNAc siRNA) oligonucleotide targeted to an endogenous protein. The subcutaneous route of exposure was selected because this is an intended route of human exposure. The surrogate molecule was shown to be pharmacologically active in the rat, resulting in 0.095X expression of the target protein as compared to controls at 14 days post-dose. Since the surrogate molecule will not be dosed in humans the focus was on having pharmacodynamics throughout estrous cycle evaluation, cohabitation, and organogenesis, as opposed to exposure to the surrogate molecule. Therefore, the dose at which complete knockdown was observed, time required to knockdown the target protein, and duration of target protein knockdown were key factors in determining dosing schedule and

levels, in addition to tolerability considerations. Once weekly dosing began 4 weeks prior to cohabitation to allow knockdown of the target protein to occur prior to estrous cycle evaluation, during cohabitation, and continuing through Gestation Day (GD) 20. Toxicokinetic evaluations occurred prior to mating on Day of Study 28 and on GD 21. Pharmacodynamic evaluations were performed to determine the concentration of target protein and mRNA in maternal and fetal livers, whole fetuses, and placenta on GD 21. These ongoing pharmacodynamic evaluations are expected to provide insight into the placental transfer of GalNAc siRNAs and whether GalNAc siRNAs localize to the fetal liver.

2:00 – 2:10 **Discussion/Q&A**

2:10 – 2:25 **Qualifying a murine surrogate siRNA for use in the DART package**
Bethany Hannas (Eli Lilly)

2:25 – 2:35 Discussion/Q&A

2:35 – 2:50 **The minipig as a juvenile animal model for oligos and its use as a DART model**
Isabelle LeConte (Charles River Laboratories)

2:50 – 3:10 **Case Study 4**

3:10 – 3:20 Discussion/Q&A

3:20 – 3:50 **Discussion- identify key take aways/questions**

3:50 – 4:00 **Day 2 Preview/Adjourn**

Wednesday October 18
8:30 AM – 1:30 PM Eastern

8:00 – 8:30 **Breakfast**

8:30 AM – 8:40 AM
Day 1 Recap/Day 2 Overview
Michael Templin (Charles River Laboratories)

8:40 AM – 10:40 AM Eastern
Case Study Session 3: Newer Oligos/Novel Approaches
Case studies in this session will cover scenarios where a unique aspect of the oligonucleotide calls for a novel or alternative approach for DART strategy planning. Speakers will describe approaches taken or challenges faced in fitting the DART program to the attributes of the unique oligonucleotide.

Moderator: Alan Hoberman (Charles River Laboratories)

8:40 – 8:55 **Ab-conjugated siRNA DART program**
Eileen Blasi (Avidity Biosciences)
In this presentation, an initial strategy to assess embryofetal development risk of an antibody oligonucleotide conjugate (AOC) will be discussed. The unique attributes of an AOC with dual pharmacology and limited species cross reactivity allows for a potential alternative approach to avoid unnecessary DART studies in nonhuman primates. To this end, a strategy using a using a

combination of rodent surrogate molecule plus weight of evidence in an integrated analysis will be described.

8:55 – 9:05 **Discussion/Q&A**

9:05 – 9:20 **DART approach for IT clinical route and waiver considerations**
Lutz Mueller (Roche)
This case study will cover weighing the evidence from patient population, surrogate feasibility and tolerability information from the intrathecal treatment route.

9:20 – 9:30 Discussion/Q&A

9:30 – 9:45 **Case Study 3**
TBD

9:45 – 9:55 Discussion/Q&A

9:55 – 10:25 **Discussion- identify key take aways/questions**

10:25 – 10:45 **BREAK**

10:45 AM – 12:30 PM Eastern

Case Study Session 4: Exposure Considerations/ADME relevant to DART considerations

Case studies in this session will describe exposure and ADME considerations that influence DART study design for oligonucleotides. Some points that will be described include placental transfer potential, most appropriate TK or exposure parameter to consider in determining dosing schedule, and TK/exposure sample collection design for DART studies.

Moderator: Rudiger Cordts (Boehringer Ingelheim)

10:45 – 11:00 **Case Study 1: Placental Considerations**
TBD (AstraZeneca)

11:00 – 11:10 Discussion/Q&A

11:10 – 11:25 **Case Study 2:**

11:25 – 11:35 Discussion/Q&A

11:35 – 11:50 **Case Study 3**

11:50 – 12:00 Discussion/Q&A

12:00 – 12:30 **Discussion- identify key take aways/questions**

Scientific Round Table Discussion

12:30 – 1:30 PM Eastern

12:30 – 1:30 **Revisit key take away topics from case study sessions. Highlight main recommendations & points to consider**

Moderator:
Joy Cavagnaro (Access BIO)

Panelists:
Jeff Foy (Pepgen)
Kazushige Maki (PMDA)
Kris Siezen CBG-MEB
Ron Wange (US FDA)

1:30 **Wrap-up/Adjourn**