Biodistribution – local vs. systemic delivery

- Site and route of administration impact biodistribution
  - Some organs easier than others
    - Some target cells within organs easier than others
      - Brain e.g., go into CSF may provide broader distribution than directly into brain parenchyma
        - Focal parenchyma injection more approachable than delivery to whole brain or entire brain region (e.g., hindbrain).
      - Delivery via blood provides access to liver, spleen, etc, particularly organs with a fenestrated vasculature
      - Eye – anterior chamber vs. neural retina
      - Delivery to airway – product likely to stay in lung, but airway epithelia designed to keep stuff out
        - Entry may require inducing a chink in the armor

Biodistribution – local vs. systemic delivery

- Site and route of administration impact biodistribution
  - Some organs easier than others
    - Several target cell types within organs easier than others
      - Muscle, multiple injections or mechanism to make vasculature leaky
      - Solid tumors – does the vasculature help or hinder?
      - Other?

Persistence of Oligos and Physiological Effects – Points for Consideration

- Persistence is often a desired effect for an oligonucleotide
  - Decrease frequency of administration
  - Minimize the potential for escape (virus, tumor growth, etc.)
- Balance must be established between the benefits of persistence and the potential consequences
- Exaggerated Pharmacology
  - Represents an unwanted or adverse effect resulting from the intended pharmacology of an oligonucleotide drug candidate
- Toxicology
  - Represents an unwanted or adverse effect resulting from the oligonucleotide drug candidate, not directly associated with pharmacology

Persistence of Oligos and Physiological Effects – Toxicology

- In general the profile of target organ for toxicity follows the biodistribution pattern for the oligo in question
- Threshold levels have been noted for organ toxicity
  - Acute effects are dose-dependent
  - Subacute/chronic effects are dose-, schedule, duration-dependent
- Elimination of the oligo from the tissues results in resolution of the toxicity/observations of granules
  - Kinetics can be highly variable among oligonucleotide constructs and chemistries, but likely to be consistent for a specific construct or chemistry
- Non-clinical program is the appropriate means to identify the toxicities and kinetics

Persistence of Oligos and Physilogical Effects – Exaggerated Pharmacology

- Unlikely to be a major factor for persistence and (adverse) physiological effects?
  - Pharmacology (should be) dose-dependent for magnitude
  - Dose-dependence often observed for duration

- The organ distribution and biology of the intended target is a primary factor for persistence and (adverse) physiological effect
  - Located in a tissue other than intended
  - Cell has a low turnover rate
  - Extensive duration/slow recovery (mRNA or protein turnover)

What has been learned about immune and inflammatory responses and what models are best for predicting the Response?

- A clear distinction should be made between oligonucleotides intended to stimulate the immune system (immunostimulatory) and non-specific immune stimulation

- For immune stimulatory oligos the questions and models are the same as those for any indication
  - Specificity/profile of the stimulation
  - Magnitude of effect
  - Duration of effect
  - Recovery from effect
  - Model selection is based on indication

Immune and inflammatory responses

- For non-immunostimulatory oligos, this effect has often been addressed within toxicology studies
- The magnitude of immune and inflammatory responses varies among sequences and constructs
  - Motifs have been identified but absolute predictive value has not been achieved
  - Chemical/Physical characteristics can play a significant role
    - Length of the oligonucleotide
    - Conjugation (PEG, proteins, Abs)
- Each oligonucleotide should first be considered/evaluated for its own unique effects
  - “class effects” are important in the design of studies and in the evaluation of effects

What models are best for predicting the Response?

- Models are intended to mimic a specific disease or physiological condition ….
- In the evaluation of a novel agent it is important to evaluate the broad picture…..
- The best model for predicting an immune response for an oligonucleotide is likely to be a well designed toxicology study
  - Two species (rodent and non-rodent, when appropriate)
  - Relevant dose levels, route, and frequency
  - Kinetics of oligo in blood and tissues (include cytokines)
  - Serum chemistry and hematology at key time points
  - Histopathology of multiple tissues

What models are best for predicting the Response? (cont.)

- Case-by-case and results driven decision point for further evaluation
  - Based on observations, a determination can be made
    - Innate response vs. humoral response
    - Tissue/organ specific
  - If additional information is needed or specific studies are appropriate the *in vivo* model can be selected that best represents the situation

- *In vitro* (cell) models can be useful for identification or selection of oligonucleotides early in development
  - Whole blood vs. cell-specific assays
  - Good for eliminating highly stimulatory oligos

What models are best for predicting the Response? (cont.)

- Case-by-case and results driven decision point for further evaluation
  - Based on observations, a determination can be made
    - Innate response vs. humoral response
    - Tissue/organ specific
  - If additional information is needed or specific studies are appropriate the *in vivo* model can be selected that best represents the situation

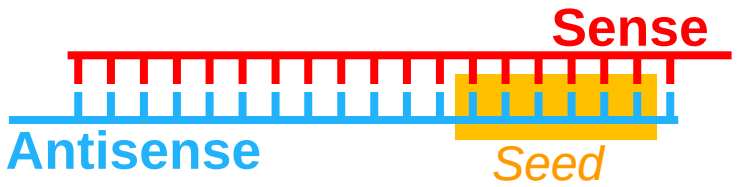
- *In vitro* (cell) models can be useful for identification or selection of oligonucleotides early in development
  - Whole blood vs. cell-specific assays
  - Good for eliminating highly stimulatory oligos

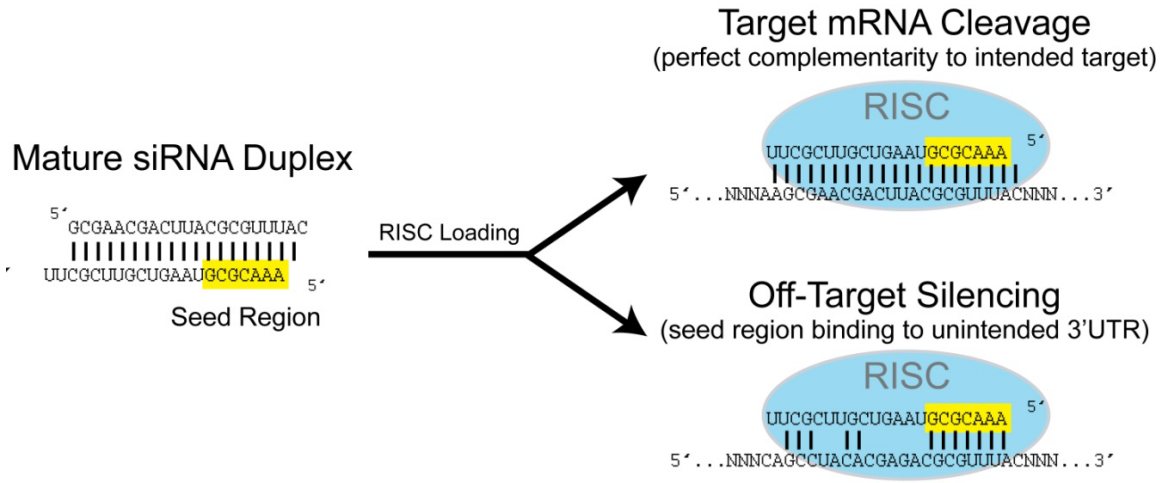
How has the potential for off-target activity been addressed?

- Off target activity
  - Cellular responses due to formulation, toxicity, etc.
  - Off-sequence silencing

Off- sequence silencing

- Optimize loading of the appropriate strand
- Consideration of perfect complements – BLAST, Bowtie (antisense)
- Consideration of seed region





How has the potential for off-target activity been addressed?

- Evaluate toxicity
  - For siRNAs, high frequency seeds often toxic after 24-72 hrs in vitro, and within several days *in vivo (dose dependent) (*Anderson et al, 2008*)*
  - Antisense and morpholinos – toxicity sequence dependent?
    - Same sequence toxic in multiple species?
- What about chronically infused oligos?
  - Small reduction in non-target genes could cause unintended toxicity (e.g., splicing regulators, transcriptional repressors)
    - Evaluate off-targeting by microarray?
      - Predict direct effects bioinformatically?
      - Best readout is a clinical readout – does the tissue tolerate the product
  - Animal models best for predicting toxicity?
    - For miRNA-based off targeting, sequences different between models and man

Assays and animal models to address these questions?

- Cell based assays – human cells modeling disease state
  - Standard cell viability and cell plating assays

- Rodent and non-rodent testing (NHPs)
  - Both may give an idea of the general toxicity of the oligo but may not be predictive of clinical toxicity in patients because of sequence differences between these species and man
  - Nonetheless, toxicity (of assumed off-target silencing) in rodents/NHPs a no-go for acute testing in humans
    - Balance oligos for reduced miRNA-like off-targeting in multiple species with safety and efficacy
    - Antisense and morpholinos – toxicity sequence dependent?
      - Is the same sequence toxic in multiple species

What Are the Key Challenges that need to be addressed to move the field Forward?

<u>Non-clinical Development</u>

1. Key areas that involve oligonucleotides overall have been identified and progress is being made to define and address
   1. Off-target effects
   2. Exaggerated pharmacology
   3. Genetic toxicology, reproductive toxicology, carcinogenicity
   4. Immune-based effects

- The oligonucleotide Safety Working Group (OSWG) has taken-up these points for discussion and drafting of “white-papers”

What Are the Key Challenges that need to be addressed to move the field Forward?

Non-clinical Research

1. Similar to all areas of research, publication and presentation in “peer-reviewed” forums provides the underlying data to support the entire field
2. Individual scientists have the responsibility to conduct scientifically sound studies
   1. Use of proper controls
   2. Address alternative mechanisms
   3. Report data set and not selected data
   4. Additional

What Are the Key Challenges that need to be addressed to move the field Forward?

Clinical Development

1. Demonstration of activity in humans
2. Demonstration of therapeutic benefit in humans
3. Safety in humans
4. Additional?

What Are the Key Challenges that need to be addressed to move the field Forward?

<u>Activity</u>

- Robust demonstrations of “proof of concept”
  - Mechanism of Action
    - Reduction in target mRNA or miRNA
    - Reduction in target protein
    - Biological effect (vs. therapeutic effect)

<u>Therapeutic Effect</u>

- Consideration of diseases that provide a means of “efficient” evaluation
  - Measureable effect in blood, biological fluid, or readily obtained sample
  - Primary endpoint can be achieved in a reasonable (< 6 months?) time frame