

Approaches to the assessment of oligonucleotide specificity

- Position of the Oligonucleotide Safety Working Group on Off-target effects (8+2 minutes)
- Santaris Pharma approach for single stranded antisense oligonucleotides (18+2 minutes)
- Merck approach for siRNA (18+2 minutes)

Assessing unintended hybridization induced biological effects of oligonucleotides

Based on a position paper by
Oligonucleotide Safety Working Group
Subcommittee on Off-target assessment

Draft position paper has been distributed with meeting materials

Presented by Morten Lindow, mol@santaris.com

The 'off-target subcommittee'

- **Arthur A. Levin**, Santaris Pharma A/S
- Donald Riley, DNA Consulting, LLC
- Douglas J. Kornbrust, Preclinsight
- Hans-Peter Vornlocher, Roche Kulmbach GmbH
- Husam Younis, Isis Pharmaceuticals
- James D. Thompson, Quark
- Joanne Kamens, RXi Pharmaceuticals
- Joel Parry, GlaxoSmithKline R&D Ltd.
- Morten Lindow, Santaris Pharma A/S
- Nicolay Ferrari, Topigen Pharmaceuticals Inc.
- Sara Nochur, Alnylam
- Scott P. Henry, Isis Pharmaceuticals
- Steven Bartz, Merck Inc

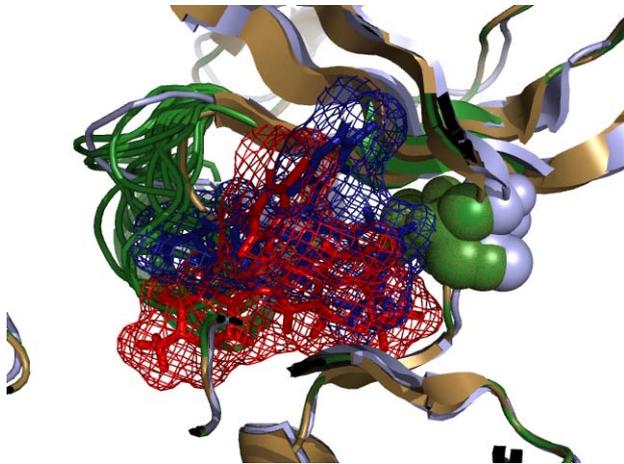
Mechanisms of toxicity for oligonucleotides

	Hybridization Dependent	Hybridization Independent
Preclinical	<p>On target toxicity: Exaggerated pharmacology</p> <p>Off target toxicity: Perfect match to nontarget gene Mismatch to nontarget gene MicroRNA (RNAi mechanism only)</p>	<p>Plasma protein interaction: Increase in APTT Complement activation</p> <p>Tissue/cell interaction: Proinflammatory effects Decrease in platelets Kidney: proximal tubule cell effects at high doses Liver: increase in liver enzymes</p>
Clinical	None described to date	<p>Plasma protein interaction: Increase in APTT</p> <p>Tissue/cell interaction: Injection site reactions Constitutional symptoms (fever, chills, arthralgia, headache, etc.) Decrease in platelets</p>

Scope of this session: off-target toxicity = toxicity caused by the Watson-Crick basepairing between an oligonucleotide and an unintended target

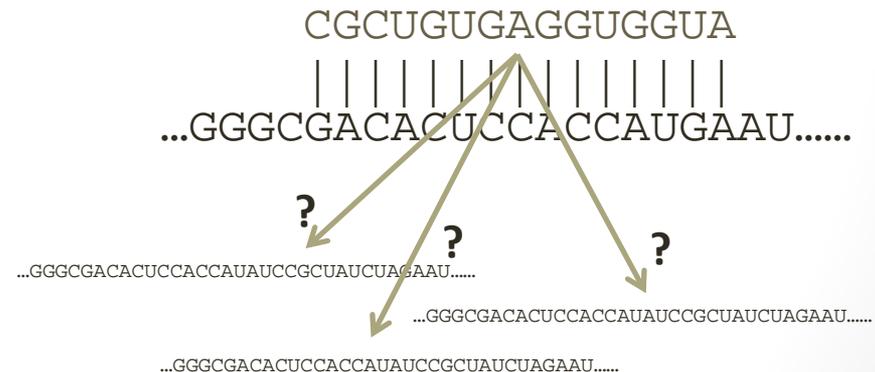
Predictability of putative off-targets is a strength

Protein ← small molecule



In silico drug design and specificity assessment is HARD

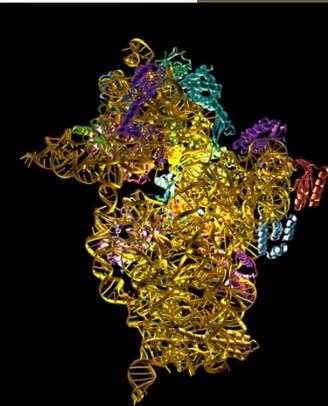
RNA ← oligonucleotide



In silico drug design and specificity assessment is 'EASY'

Recommendation #1: “Candidate drug sequences (ASO and siRNAs) should be selected to minimize potential binding to and inhibitory activity on unintended RNAs“

- Perhaps not so “EASY”
- Specificity is a quantitative trait. The objective is to:
 - Maximize effect on intended target knock down, while minimizing effect on off-target knockdown
- Molecular factors governing activity of an oligonucleotide on an RNA (off)-target
 - Affinity to (off)-target site
 - Tolerance of RISC and RNaseH
 - Position of mismatches and bulges, possible context requirements
 - Target site accessibility (RNA folding, protein binding)



In silico screening of candidates

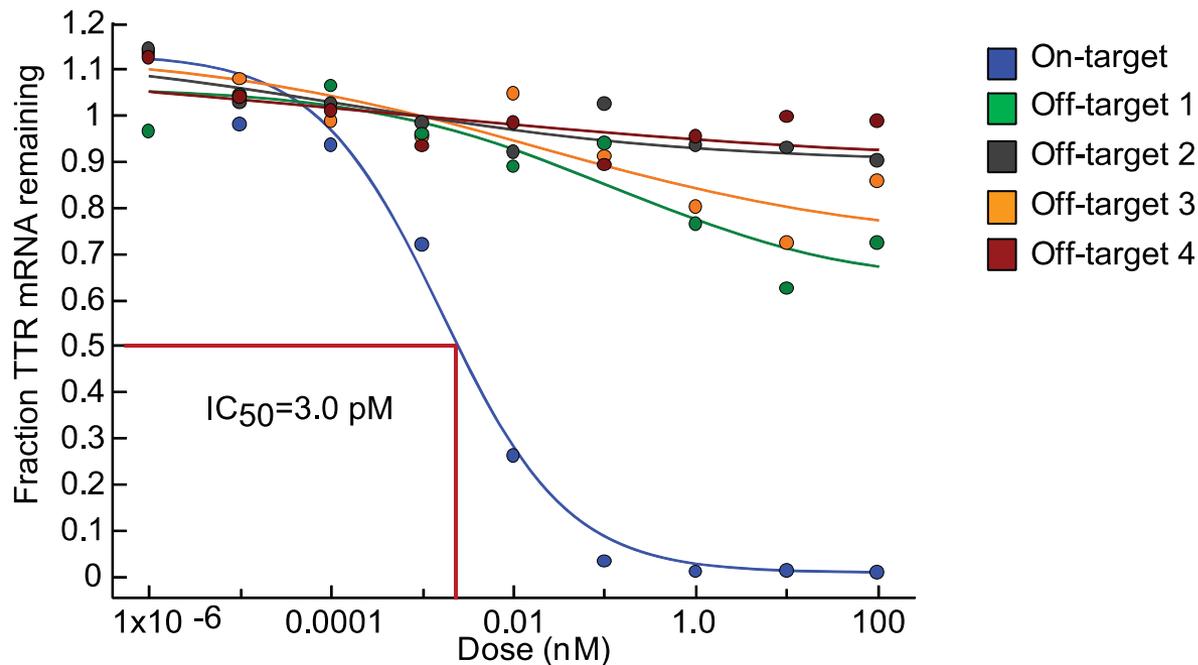
- In silico algorithms will never be perfect
- Algorithms will depend on
 - mechanism (RNAseH, RISC, steric block)
 - chemistry of the oligonucleotide
- Algorithms are under continuous development to align with increasing experimental data
 - → Merck talk
 - → Santaris talk

Recommendation #2: Interpretation of in silico predicted off-targets

- *In vivo* expression pattern of the off-target RNA
 - Overlap with tissues of drug accumulation
- Function of off-target RNA
 - knock-out / knock-down phenotype
 - OMIM etc
- Is the off-target site also present in species used in toxicity studies?

Experimental follow-up on critical off-target hits

- Validation. Relative potency between on- and off-target
 - E.g. qPCR in human cell lines.



Recommendation #3: in vivo preclinical tox studies

- Oligonucleotides are not essentially different from other drug classes
- Penultimate test of relevance of off-target findings are in vivo studies in animal models
- Understanding that the only way to truly test for human responses is in carefully controlled and monitored clinical trials

Summary

- Off-target screening is only one among many other factors used to select compounds

