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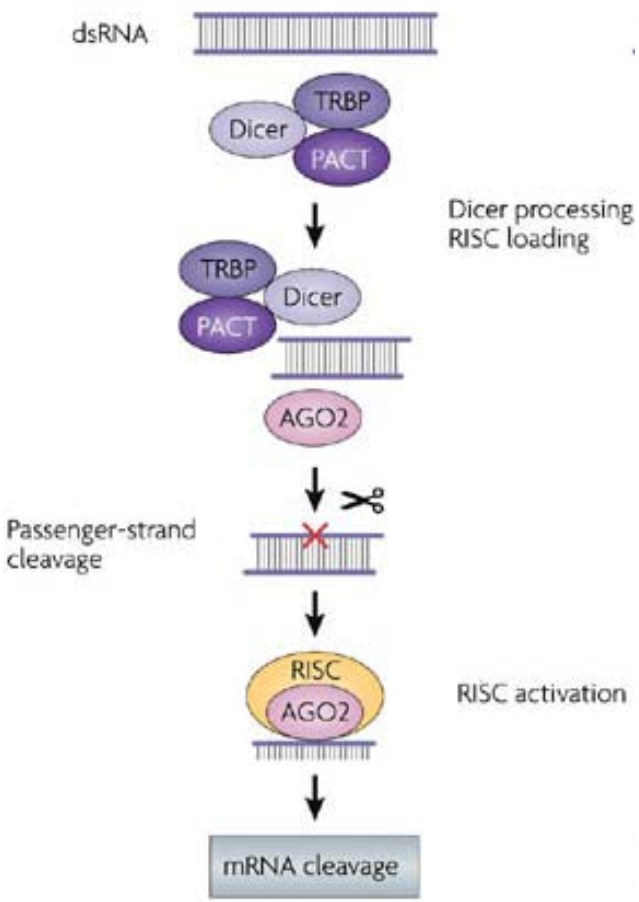
Discussion Points

- Mechanism of RNA interference
- Differences between synthetic DNA and RNA oligonucleotides
- Clinical experience with DNA and RNA oligonucleotides
- Issues of concern for synthetic RNA oligonucleotides mentioned by RAC*
  - Potential off-target effects
  - Long-term effects
- Conclusions

*As stated in December 1, 2009 public hearing on “Proposed Revisions to the NIH Guidelines: Responses to Public Comments”

Mechanism of RNA Interference (RNAi)

siRNA Pathway



- Synthetic small interfering RNAs (siRNAs) are 19-23 base pairs in length
- siRNAs harness this natural pathway
- Synthetic siRNAs enter pathway downstream of Dicer
- Results in specific mRNA cleavage
- Catalytic process mediated by RISC
- Numerous targets have been validated *in vitro* and *in vivo*

Kim and Rossi (2007) *Nature Reviews Genetics* 8; 173–184

Synthetic DNA and RNA Oligonucleotides

Characteristic	DNA Oligos	RNA Oligos
Typically are less than 40 base pairs in length	Yes	Yes
Mechanism of action is via down regulation of mRNA	Yes	Yes
Synthetic oligonucleotides have chemical modifications	Yes; some of these render them similar to RNA	Yes; some of these render them similar to DNA
Non-sequence specific off-target effects, including immunostimulatory effects are observed *in vitro*	Yes	Yes
Have characteristics common to recombinant DNA (integration, replication)?	No	No
Effects are reversible	Yes	Yes
Clinical experience	~175 trials involving ~15,000 subjects/patients*	~ 17 trials involving 1500 subjects/patients

*Includes antisense oligos, immunostimulatory oligos, and aptamers

RNAi Clinical Programs

Company	Product	Target Population	Clinical Experience
Active Programs			
Alnylam	ALN-RSV	Respiratory Syncytial Virus	• ~ 17 clinical trials involving approximately 1500 subjects/patients* have been conducted with small interfering RNAs • Programs include local and systemic delivery (including complex formulations) • Various organs targeted • Trials have ranged from Phase I to Phase III • Programs terminated have been due to lack of efficacy
Alnylam	ALN-VSP	Liver Cancer	
Calando	CALAA-01	Oncology – solid tumors	
Quark	PF-04523655	Diabetic Macular Edema; Wet AMD	
Quark	QPI 1002	Acute Kidney Injury; Delayed Graft Function	
Santaris	SPC3649	Hepatitis C (miR122)	
Silence Therapeutics	Atu027	Oncology – GI, Lung & Other	
Sylentis	SYL040012	Ocular Hypertension	
Tekmira	apoB SNALP	Hypercholesterolemia	
Transderm	TD101	Pachyonychia Congenita	
ZaBeCor	Excellair™	Asthma	
Terminated Programs			
Allergan	AGN211745	Wet AMD	
Opko	Bevasiranib	Diabetic Macular Edema; Wet AMD	

*Based on public disclosures

Potential Off-target Effects
Non-Sequence Specific Immune-stimulation by siRNAs

- Immune-stimulation by siRNAs likely occurs through cellular receptors in the endosome and cytoplasm[1,2]
- Such effects are minimized by several strategies[3-6]
  - Chemical modifications on sugar residues
  - Cell-based assays to screen for immune-stimulatory potential
- Immune-stimulation is not unique to siRNAs and has been observed for DNA oligonucleotides[7,8], small molecules and biologics[9]
- Therapeutic immune-stimulatory oligonucleotides are currently in clinical testing[10,11]
- Potential clinical candidates undergo toxicology testing in rodents and non-human primates to eliminate oligonucleotides with this effect

[1]Armstrong et al., (2008) *J Immunol* 180:7125; [2]Marques et al., (2005) *Nature Biotech* 23:1399
[3]Sioud et al.,(2006*) Eur J Immunol* 36:1222; [4]Judge et al.,(2006) *Mol Ther* 13:494;
[5]Cekaite et al.,(2007) *JMB* 365:90; [6]Robbins et al.,(2007) *Mol Ther* 15:1663; 135;
[7]Henry, S.P. et al., (2006) Antisense Drug Technology, 2nd ed., 327-363; [8]Senn, J.J. et al., (2005) *J Pharm and Exp Ther* 314:972-979;
[9]Suntharalingam, G. et al., (2006) *NEJM*, 355:1018-1028; [10]Halperin, S. A. et al., (2003) *Vaccine 21,* 2461–2467;
[11]Klinman (2004) *Nature Reviews Immunol* 4:1.

Specificity of Drugs

- All drugs have potential for non-specific effects
  - May be due to
    - Non-specific binding
    - Downstream effects of metabolites
    - Sequence or non-sequence-mediated effects (in the case of oligos)
- Thus, off-target effects observed *in vitro* with oligos, including siRNAs are not unique
  - Knowing the sequence for the specific intended target enables minimization of potential off-targets
  - Bar should not be higher for synthetic oligonucleotides because of theoretical reasons

Potential Off-target Effects
siRNAs Do Not Impact miRNA Pathway

- Sequence selection is used to eliminate siRNAs with homology to known miRNAs thereby minimizing the effect on miRNA downstream targets
- No significant saturation of endogenous miRNA machinery evident at pharmacologically relevant doses[1]

[1]John et al., *Nature* 449:745

Potential Off-target Effects
Sequence-specific Off-targeting by siRNAs

- Off-target down modulation occurs through unintended base pairing to RNA targets with incomplete homology to delivered siRNA[1]
- Such interactions are minimized by several strategies[2,3]
  - Bioinformatic selection of specific siRNA sequences
  - Chemical modifications
  - Cell-based screening to select for specificity
- Off-targeting is not unique to siRNAs and has been observed for DNA oligonculeotides as well[4-6]
- Effect typically small and *in vivo* relevance not demonstrated
  - At protein level modulation shown to be quite modest[7]

[1]Jackson et al., (2003) *Nature Biotech*, 21:635; [2]Jackson et al., (2006) *RNA* 12:1197;
[3]Bramsen et al., *NAR* (May 2010) ; [4]Bilanges and Stoke, (2005) *Biochem J* 388:573;
[5]Cho-Chung and Becker(2003) *Nature* 21:492; [6]Fisher et al., (2002) *JBC* 277:22980;
[7]Selbach et al., (2008) *Nature* 455:58

Epigenetic Effects?
Modulation of Transcription by siRNAs

- Small number of published reports in mammalian cells[1-5]
  - » Requires high siRNA concentrations
  - » Has not been shown to occur in animal studies
- Proposed mechanism involves changes in chromatin (histone) methylation patterns similar to those induced by small molecule modulators of transcription
  - » Histone de-acetylase inhibitors, nuclear receptor ligands (e.g. retinoids), etc.
- Reported changes are transient

[1]Ting et al., (2005) *Nat Genetics* 37:906;[2]Kim et al (2006) *Nat Struct Mol Bio*, 13:793;
[3]Janowski et al (2007) *Nat Chem Biol* 3:166; [4]Han et al (2007) *PNAS* 104:12422; [5]Schwartz et al (2008) *Nat Struct Mol Bio*, 15:842

In Conclusion

- Short, synthetic DNA and RNA oligonucleotides that do not use viral vectors for delivery do <u>not</u> pose concerns similar to those of recombinant DNA constructs
- DNA and RNA oligos should be reviewed by the same processes and agencies
- Off-target effects (whether sequence-mediated or not) are <u>not</u> unique to oligos and are observed with all classes of drugs
- Safety of these synthetic oligos are well supported by toxicological studies (as is currently done) prior to studies in humans
- Clinical data to date support similar review of RNA and DNA oligonucleotide therapeutics

Synthetic RNA oligonucleotides should also be exempt in Section III-F-1 of the NIH Guidelines along with synthetic DNA oligos