

# Toxicological Considerations For Oligonucleotide Therapeutics

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Northern CA SOT, May 2010



# Oligonucleotide therapeutics

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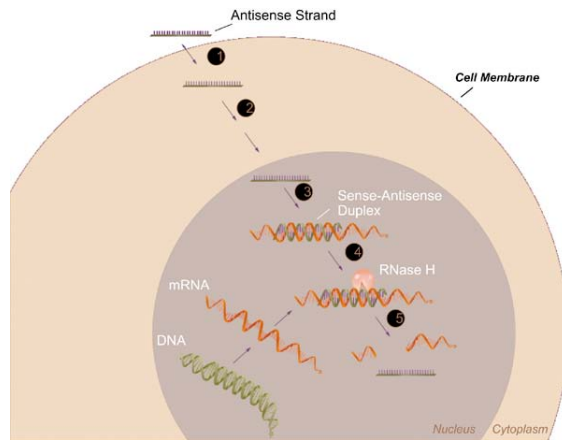
- What do I include?
  - Short strands of chemically linked, native or modified nucleotides, single or double stranded
  
- What is not included?
  - Nucleosides/nucleoside analogs
  - Long sequences of coding genetic material (e.g. gene therapy)
  - Non-nucleic acid transcriptional regulators (e.g. nuclear hormones, small molecule transcriptional regulators)

# The Major Classes of Oligonucleotide Therapeutics

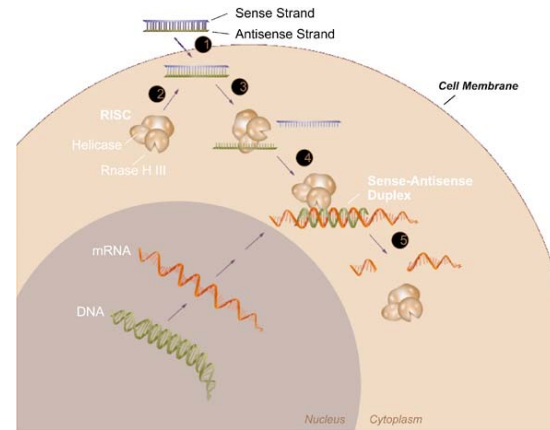
Class	Structure	Length	General MOA	Specific MOA
Antisense	ssDNA	13-25	Inhibit protein expression via complementary hybridization to mRNA	Cleave the target mRNA or inhibit translation by steric hindrance
Ribozyme / deoxyribozyme	ssRNA or ssDNA	>20		Catalytically active; cause RNA cleavage
siRNA	Duplex RNA or ssRNA	19-21		Induce mRNA degradation via RNAi pathway
miRNA	ssRNA	20-25		Inhibit translation via binding to 3'UTR and/or promote mRNA degradation
Decoy	dsDNA	>20	Inhibit protein function through high affinity binding	Binds to DNA binding site of transcription factor, inhibiting gene expression
Aptamer	ss or dsRNA, DNA	30-40		Binds to target protein
Immunostimulatory (CpGs)	dsDNA	>20	Trigger immune response by activating Toll-like receptors (TLRs)	

# Mechanisms of the Four Major Classes of Oligonucleotide Therapeutics

## Antisense

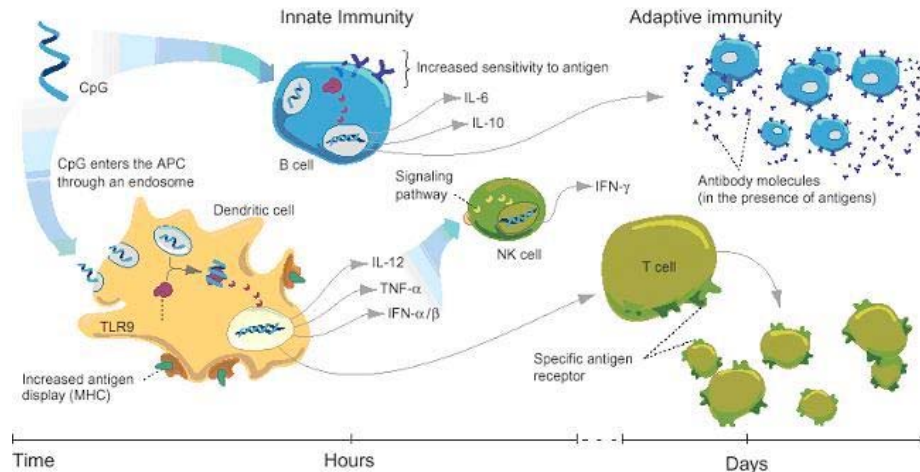


## siRNA



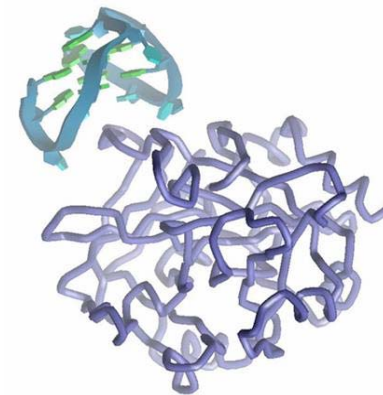
From: Antisense oligonucleotide-based therapeutics for cancer  
*Oncogene* (2003) 22, 9087–9096. doi:10.1038/sj.onc.1207231

## Immunostimulatory



From: [adjuenet.net/pubadjuvantdatabase/id\\_cpg.htm](http://adjuenet.net/pubadjuvantdatabase/id_cpg.htm)

## Aptamer



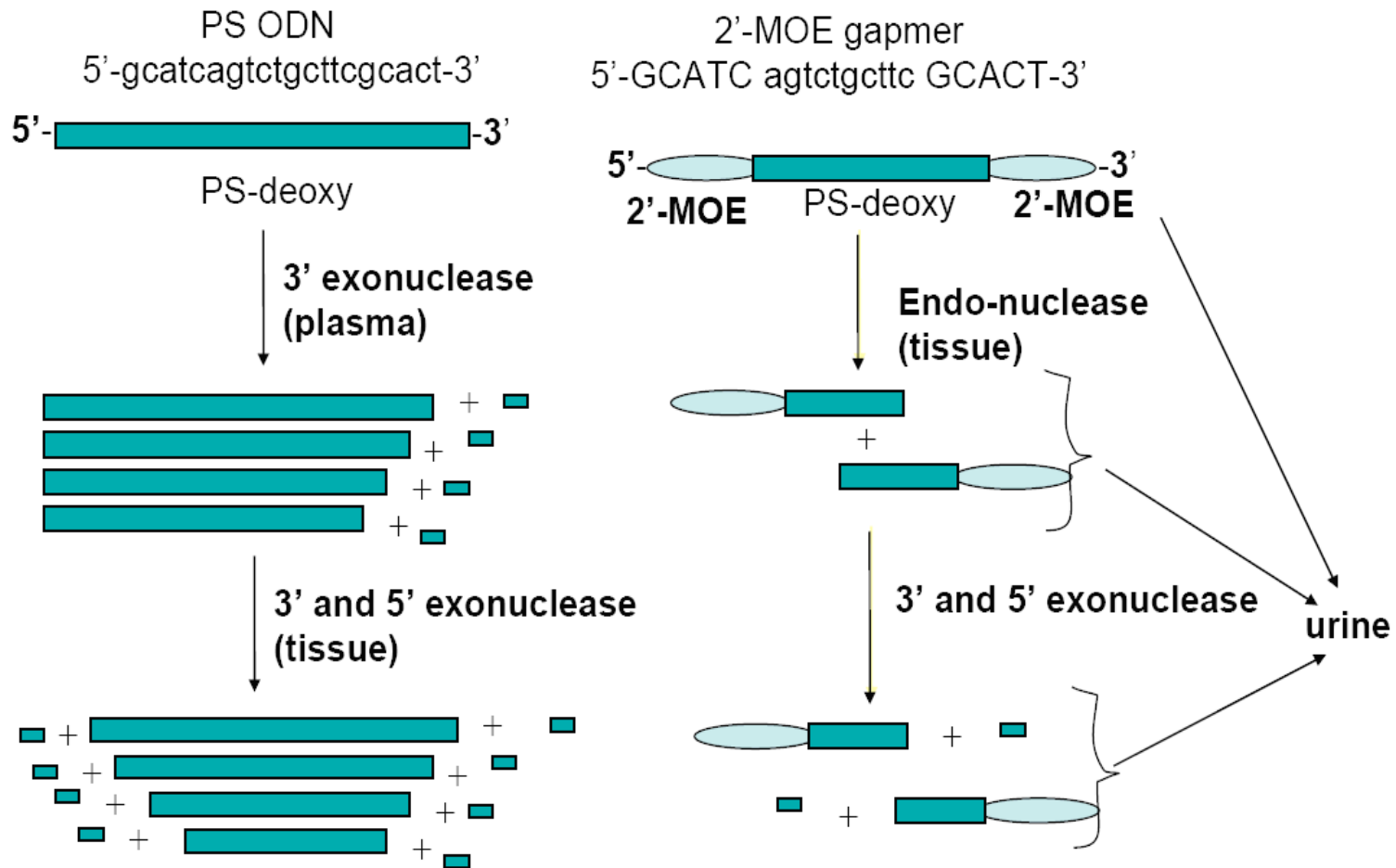
# DMPK Considerations and Optimization

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- A historical limitation of oligonucleotide therapeutics is short half-life
  - Metabolic instability – nucleases degradation
  - Renal clearance – elimination by glomerular filtration
- The vast majority of OTs are chemically modified to block nuclease based metabolism and prolong duration of action
- Many OTs are chemically conjugated or encapsulated to facilitate delivery, block renal filtration, or otherwise modulate pharmaceutical properties
  - e.g. cholesterol, lipid nanoparticles, HDLs, PEG, another oligo, small molecule functional group, etc. (limited only by imagination)
- The final properties of modified oligonucleotides are highly “tunable” by site-directed chemical modifications, many of which are now well characterized

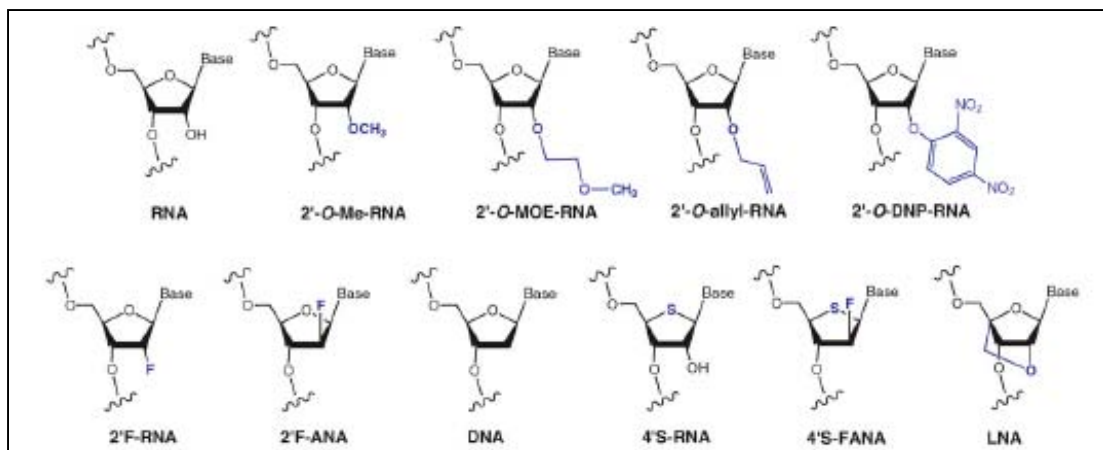
# Metabolism of Oligonucleotides

## Nuclease Mediated Metabolic Pathway

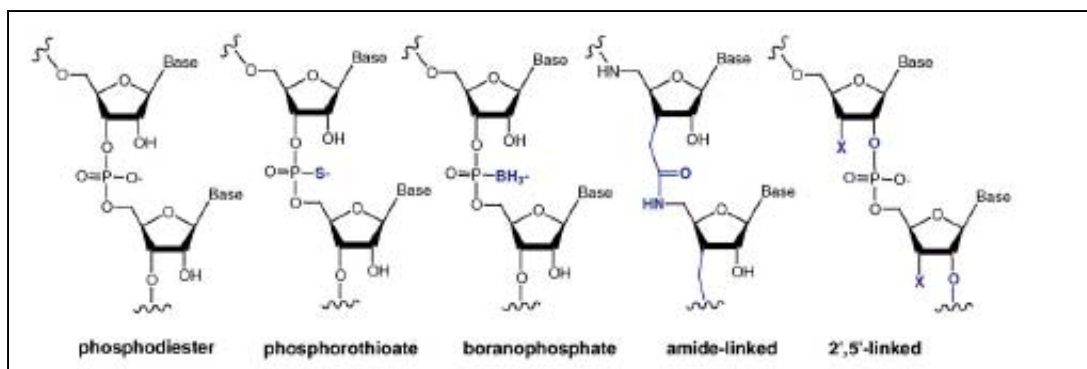


# Oligonucleotide-Based Therapeutics Chemical Modifications

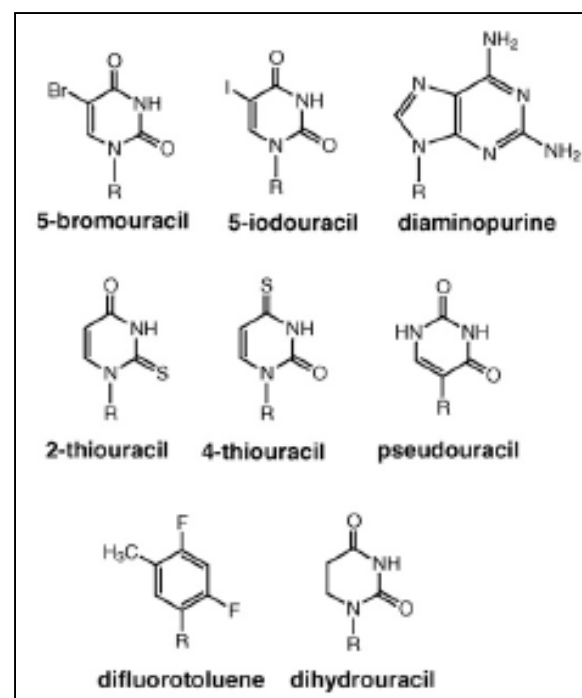
Sugar modifications: target binding affinity, nuclease stability, potency, duplex binding affinity (siRNA), immunostimulation



Phosphate linkage modifications: nuclease stability, potency, immunostimulation



Base modifications: target binding affinity, potency

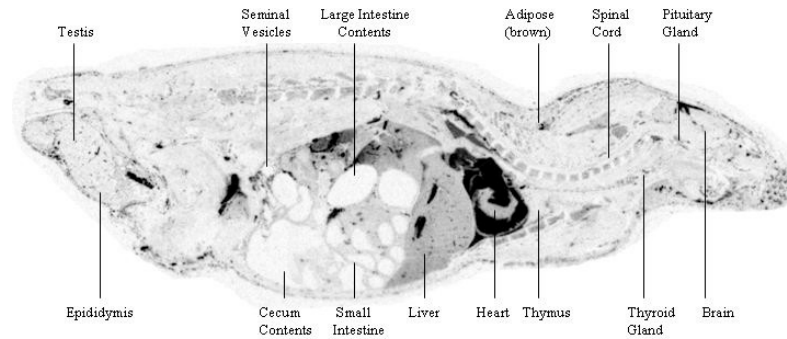


Adapted from Watts et al. 2008, Drug Disc Today 13(19-20), 842-855.

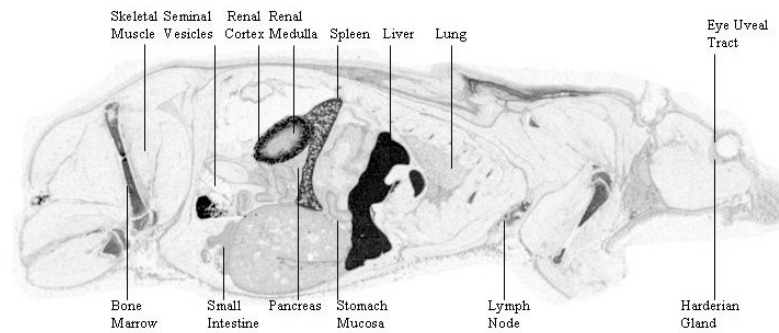
# Bioanalytical Techniques for Oligonucleotide Therapeutics

Parameter	Bioanalytical Method	LLOQ (ng/mL)	Aptamer	Specificity
Charge	CGE-UV	1000	Oligo and metabolite	++++
Charge	SAX (IEX)-HPLC-UV	100	Oligo, PEGoligo and metabolite	++++
Mass	RP-ESI-LC-MS	10	Oligo and metabolite	++++
Mass	MALDI-TOF-MS	10	Oligo and PEGoligo	++++
Binding	Oligreen	520	Oligo, PEGoligo and metabolite	-
Binding	Hybridization ELISA	1	Oligo, PEGoligo, and metabolite	++
Radiolabeled	LSC	<0.1	Oligo, PEGoligo, and metabolite	++
Radiolabeled	Autoradiography (QWBA or MARG)	<0.1	Oligo, PEGoligo, and metabolite	++

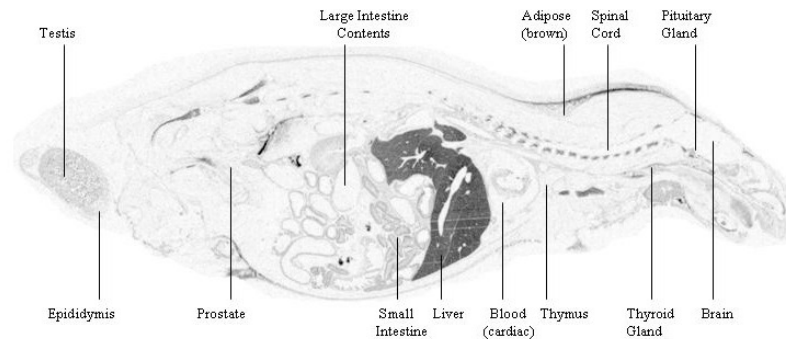
# Typical Biodistribution of Oligonucleotides at the Whole Body Level



5 min post dose



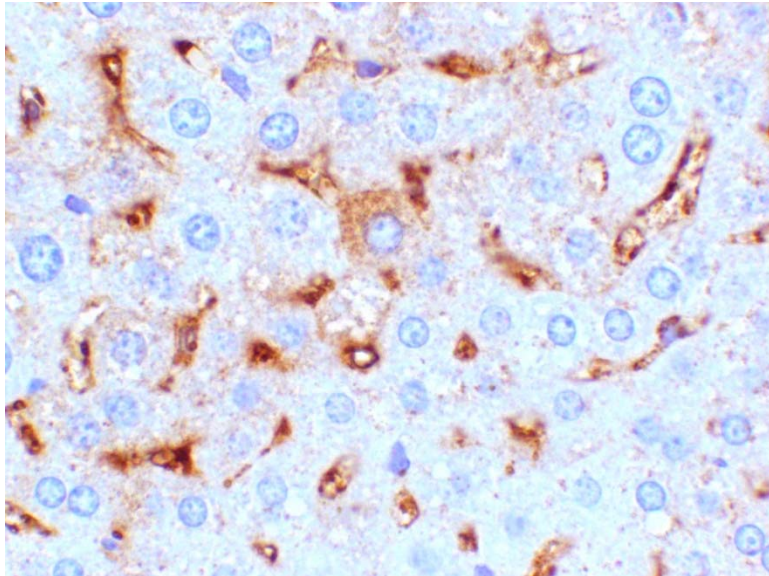
8 hour post dose



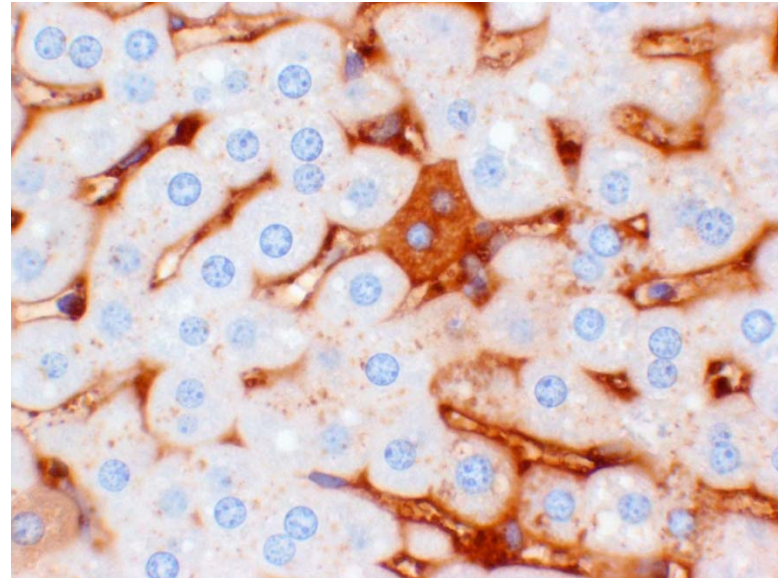
336 hour post dose

# Biodistribution at the Tissue Level

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Liver: Anti-PEG antibody 60x



Liver: anti-apptamer ISH 60x

# Historical Review of Toxicity of Oligonucleotides

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- Oligonucleotide “class effects” were initially established with ASOs
  - **Polyanion effects** (non-specific protein interactions)
    - Activation of C' alternative pathway
    - Inhibition of coagulation cascade
  - **Immune stimulation**
    - Somewhat dependent on sequence, independent of target
    - CpG motif/TLR-9 interaction
  - **Tissue accumulation**
    - kidney – proximal tubule
    - tissue macrophages – liver, spleen, lymph nodes, others
    - There is a threshold [ ] of oligo for cytotoxicity and tissue injury

# Regulatory Considerations for Development Oligonucleotide Therapeutics

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- There are no specific guidance for oligos, therefore standard NME guidance's need to be adapted
- Oligonucleotides are at the intersection of small molecules and biotherapeutics when considering program design
  - Chemically synthesized and derived from solid phase synthesis
  - Metabolized by endogenous metabolic processes and metabolites can be bioanalytically defined
  - Composed of endogenous (or modified endogenous) building blocks
  - ~5-50 kDa molecular weight
  - Pharmacological species specificity is typical
  - Tend to behave in “platform” specific patterns in terms of general pharmaceutical properties

# Specific Considerations for Antisense Oligonucleotides (ASOs)

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- **General Properties**

- ssDNA, 2'modified, P=S backbone, ~20 mer (classical design)
- Generally administered by intermittent SC or IV routes
- High degree of protein binding (mostly albumin)
- Highly stable to nucleases
- Rapid immediate distribution from the plasma into certain tissues and cells and long persistence in those sites

- **Key considerations in preclinical testing**

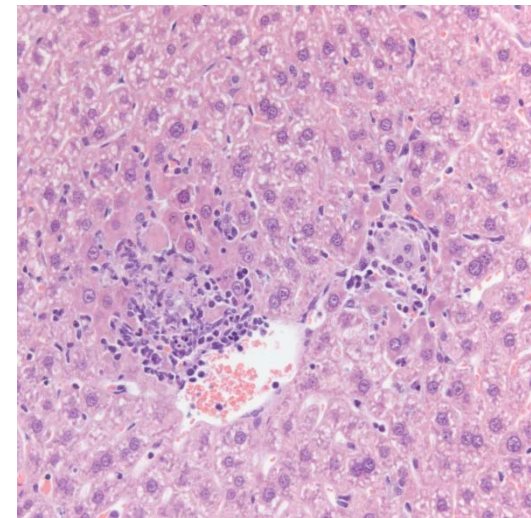
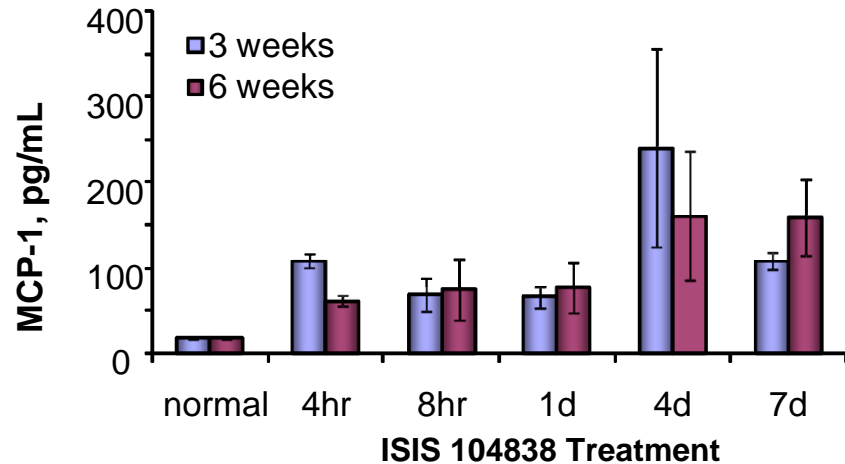
- Pharmacological species restriction – Use of murine surrogate of the same composition and target mRNA for pharmacology and toxicology is typical

- **Key challenges for the technology**

- Effective intracellular delivery to desired target cells
- Potency (on a delivered dose basis)

# Immune Stimulation in Rodents with ASOs

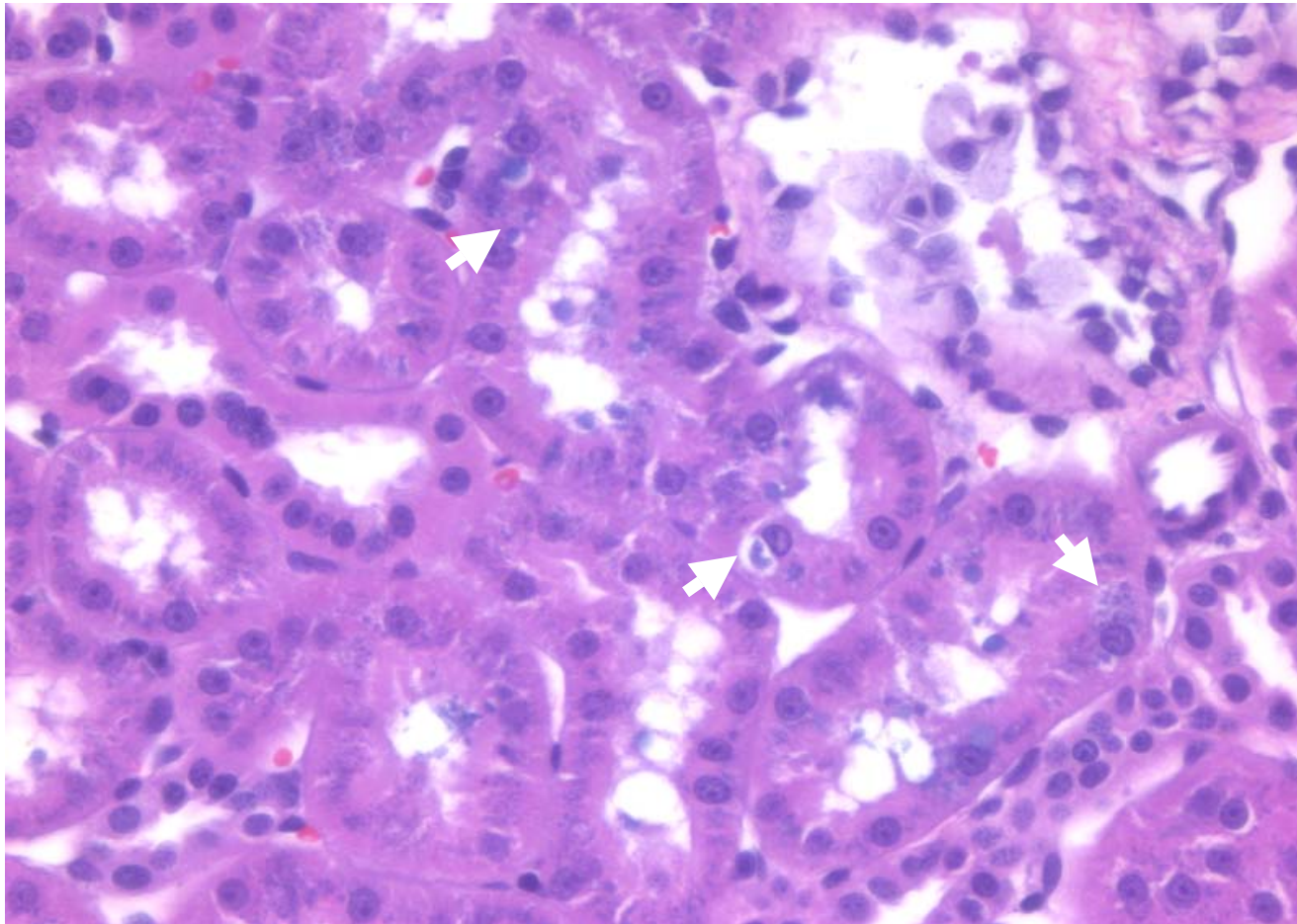
- Design
  - C57BL/6 Mice
  - 100 mg/kg 104838 by SC
  - Q2d Load/q1w Maintain
  - 3 and 6 weeks of Tx
- Results
  - MCP-1, MIP-1a, and IP-10 levels increased 4 hr after dose
  - No increase in IL-6 or IL-12
  - Increase sustained throughout dose interval
  - No difference in MCP-1 between 3 and 6 weeks of Tx
  - Variable incidence of mild mononuclear cell infiltrates in liver



# Basophilic Granules in Renal Proximal Tubules in the Cynomolgus Monkey

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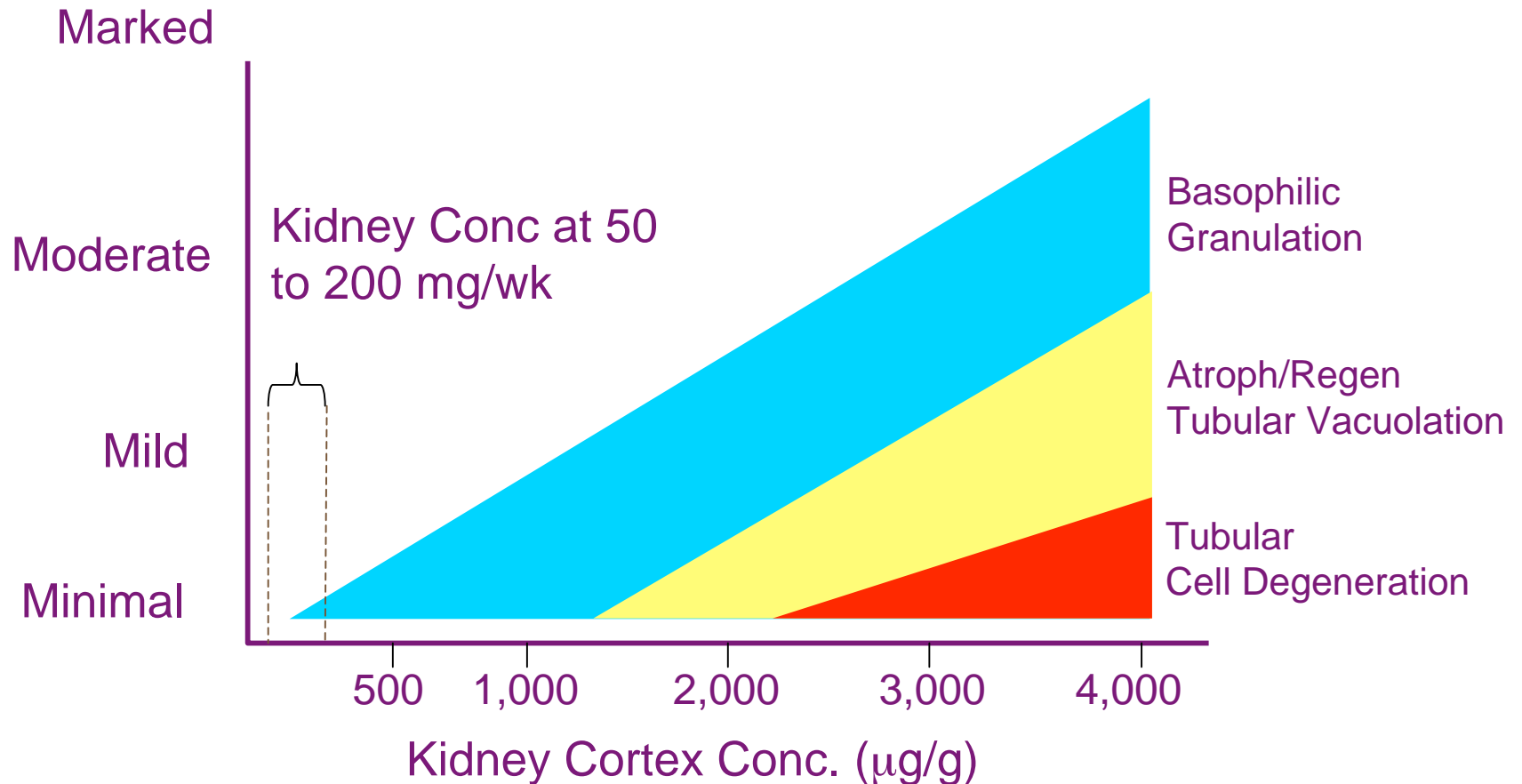
ISIS 353512 administered at 40mg/kg/wk



From: Scott Henry, ISIS Pharmaceuticals

# Monkey Kidney Cortex

## Concentration & Histologic Changes in Proximal Tubules



doses of 20 mg/kg/wk produce up to 3,500 to 4,500 µg/g cortex conc.

# Specific Considerations for Immunostimulatory Oligonucleotides (ISOs)

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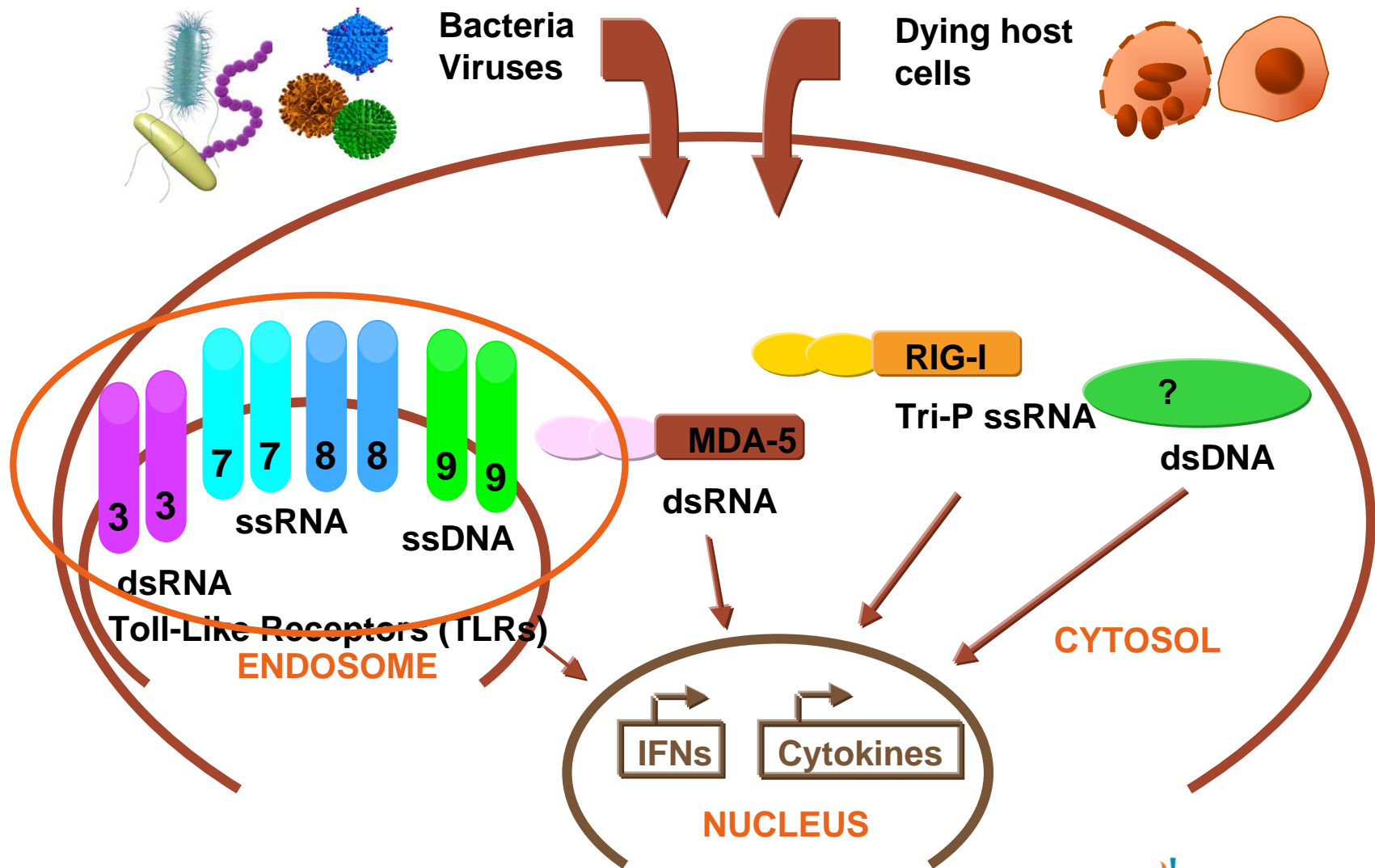
- General properties
  - ss or dsDNA, usually partially 2' modified, ~  $\geq 20$  mer
  - Endosomal → intracellular site of action
  - Agonist MOA inducing a cascade of biological response
  - Potent ( $\mu\text{g}/\text{kg}$  doses)
  - Clinical applications: vaccine adjuvant, cancer immunotherapy, chronic viral disease, and immune modulation in allergic diseases
- Key considerations for preclinical testing
  - Nature of immune response differs between species
  - Optimal molecule design differs between species
  - Use of surrogates for development?
- Key challenges
  - Harnessing the positive effect without intolerable side effects

# Key Considerations – Immune effects

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- The immune system evolved Toll-like receptors and other pathways to detect RNA (TLR3,7,8) and DNA (TLR9) from pathogens, and to trigger protective responses
  - These pathways are likely activated many times during life
    - Mice with TLR deficiencies are more susceptible to many types of infections
  - There are many checks and balances to prevent excessive or inappropriate responses
  - TLR responses differ between primates and rodents
    - no functional TLR8 in rodents
    - Differential expression of TLR9 between rodents and primates
- These pathways are readily activated by administration of exogenous RNA or DNA (intentionally or unintentionally)
  - Oligos are concentrated in endosomes: not cytoplasm/nucleus!
  - Cause dose-dependent systemic cytokine/chemokine expression
  - Transient broad spectrum immune activation
    - Protects against infectious challenge
    - Rejects tumors

# Immune Recognition of Nucleic Acids (PRRs)



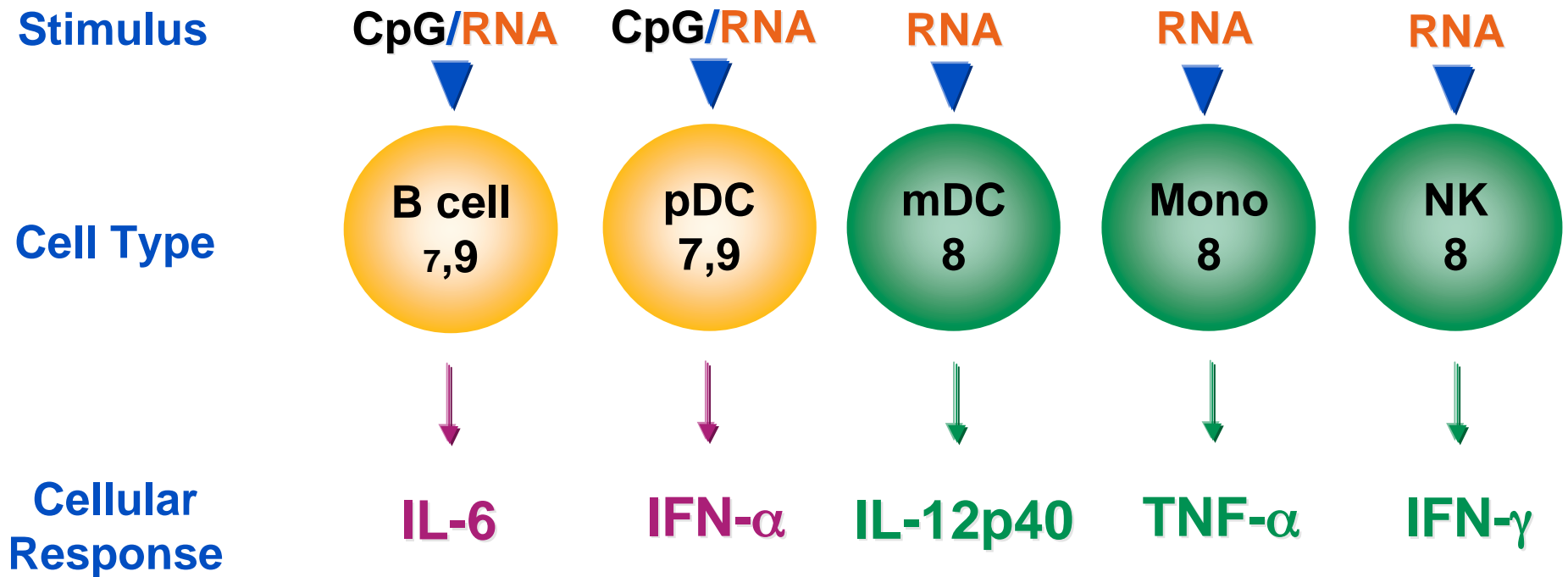
From: Art Krieg, Pfizer

# Safety Findings From TLR9 Activation Differ In Rodents And Primates

	Rodents	NHP	Humans
<b>extramedullary hematopoiesis, splenomegaly</b>	Yes	No	No
<b>multi-organ lymphoid infiltrates</b>	Yes	No	No
<b>Lethal SIRS</b>	Yes	No	No
<b>Lymphoid hyperplasia</b>	Yes	No	No
<b>Injection site reactions</b>	Rare	Yes	Yes
<b>Hypersensitivity reactions</b>	No	No	Rare
<b>Complement activation acute DLT</b>	No	Yes	Yes
<b>Transient leukopenia</b>	Yes	Yes	Yes

From: Art Krieg, Pfizer

# Stimulation of human TLR7, TLR8 or TLR9 leads to cell type specific cytokine secretion



– RNA oligos are less stable, require formulation for delivery

# Therapeutic effects of RNA resulting from immune activation

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- TLR-induced immune activation provides:
  - a transient but broad spectrum defense against many pathogen challenges
  - Anti-tumor activity
  - Strong vaccine adjuvant
  
- An RNAi could have the same effects from immune stimulation, not through RNAi MOA

# Specific Considerations for Aptamers

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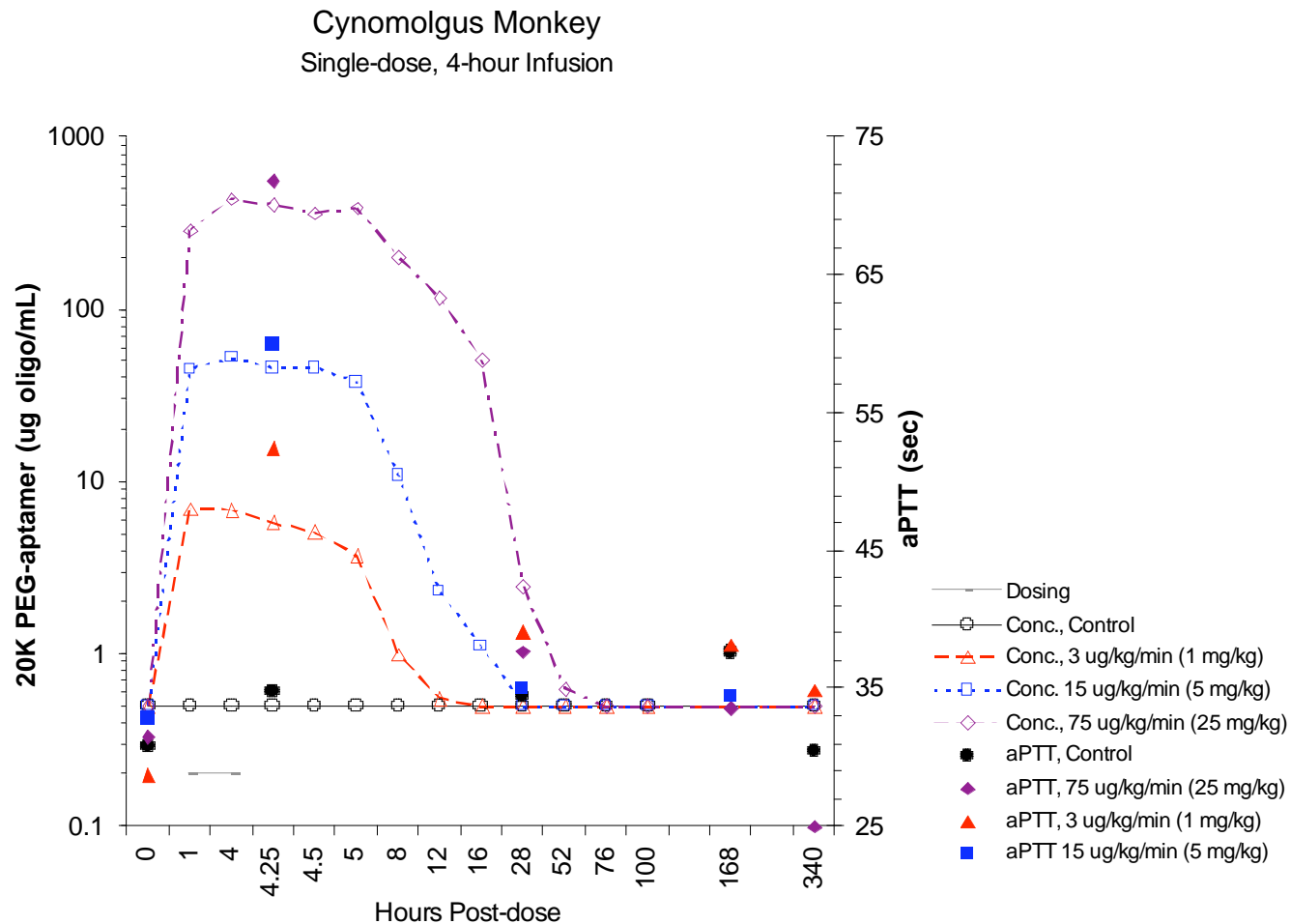
- General properties
  - Aptamers are single stranded structured oligonucleotides that bind to molecular (protein) targets with high affinity and specificity
    - Most therapeutic aptamers target extracellular proteins and disrupt protein:protein interactions
  - 15-40 mer, variety of 2' modifications, often PEG conjugated
  - Site of action in the plasma and interstitial fluid, outside of cells
  - Dose regimens vary widely depending on the aptamer compositions and the intended use (IV bolus, infusion, repeated bolus, SC bolus, etc...)
- Key considerations in preclinical testing
  - Species restriction is often observed; similar to mAbs
  - Two species toxicology testing, typically rat (off-target) and monkey (on-target)
    - Use of surrogates for development?
- Key challenges
  - Finding applications where properties of aptamers are uniquely advantageous
  - Limits to the systemic T1/2 without active recirculating mechanisms

# Typical Findings in General Toxicology Studies with Aptamers

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- Exaggerated pharmacology
  - ✓ Expected based on target biology
- Anticoagulation
  - ✓ Generally a modest effect with good safety margins
- C' activation
  - ✓ Rarely seen and only at very high concentrations with aptamers tested to date
- Hematopoietic bone marrow suppression
  - ✓ Seen in repeated-dose toxicity studies, modest effect with good safety margins
- Hemodilution (PEGylated oligos only)
  - ✓ Osmotic properties of PEG at high plasma concentrations
- Basophilic granulation and/or vacuolization
  - ✓ Mononuclear phagocytes and kidney tubule epithelial cells
  - ✓ Presence of drug-related material in these specific cells

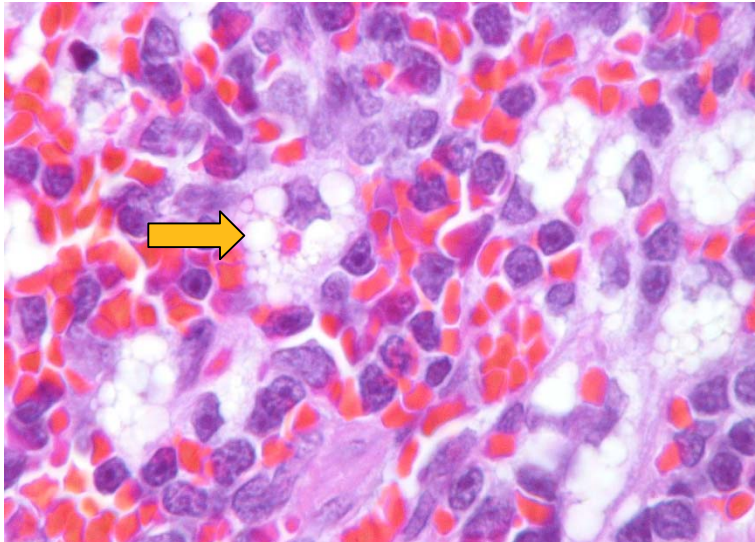
# Off-target Anti-coagulation



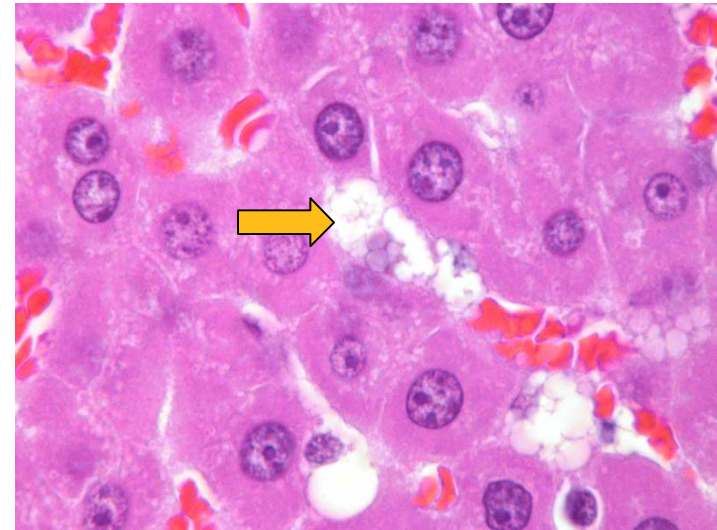
Concentration-dependent prolongation of aPTT

# Vacuolization, and/or Basophilic Granules Mostly in Mononuclear Phagocytes

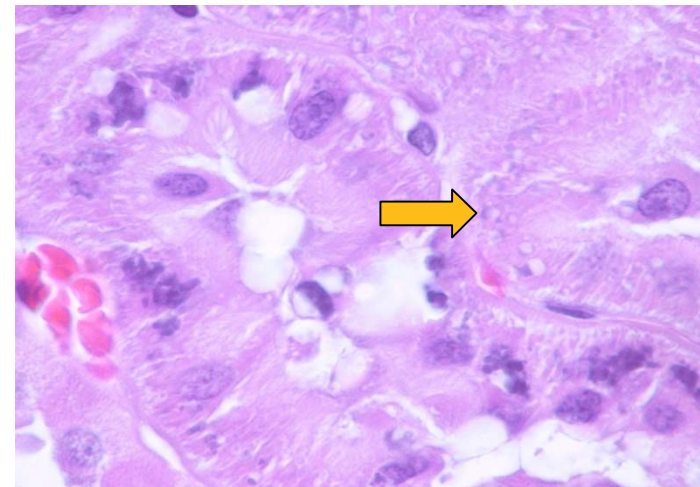
**Spleen; PAMS vacuolization**



**Liver; Kupffer cell vacuolization**



**Kidney; Basophilic granules in proximal tubular epithelium**



- Presence of test article-related material in cells has not been associated with apparent adverse effects on those cells or tissues
- Therefore, this finding alone is not considered to be an adverse effect (thus far)

# Specific Considerations for siRNAs

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- General Properties
  - dsRNA, 2' modified, ~2x21 mer (classical design)
  - Intracellular site of action
  - Generally administered by intermittent local or IV routes
  - Variable stability to nucleases depending on degree of 2' modification
  - Generally require carrier to access site of action (cytoplasm of target cells)
- Key considerations in preclinical testing
  - Pharmacological species restriction – Use of surrogates?
  - Poorly understood mechanisms of immunostimulation
  - Delivery systems often dominate the toxicity profile of the combined product candidate
- Key challenges for the technology
  - Delivery, delivery, delivery!
  - Specificity

# Potential siRNA Safety Issues to Consider

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## ■ Off-target RNA effects

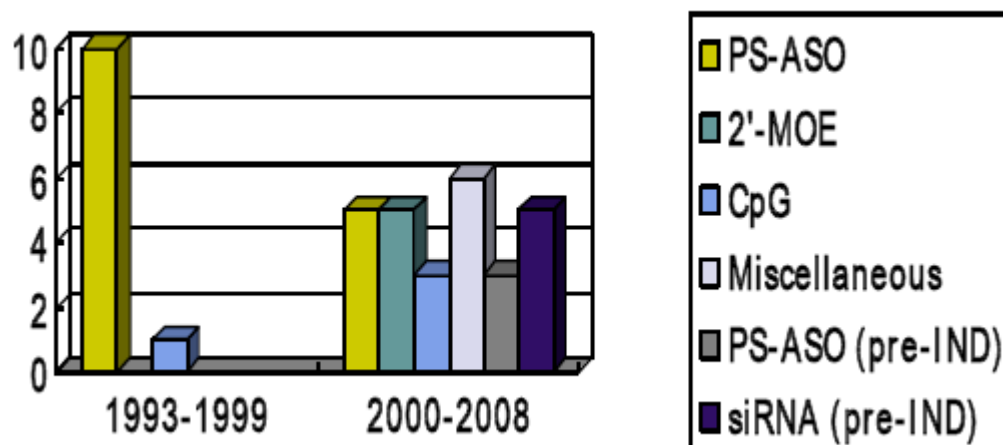
- immunostimulation (TLR activation & IFN response)
- “aptamer” effects (C’ activation, anticoagulation, other)
- non-specific knock down of mRNA
- perturbation of endogenous RNAi machinery

## ■ Carrier system effects

- Nanoparticles (various liposomes and others)
- mAb, aptamer, or small molecule targeted nanoparticles
- Lipid conjugates
- Folate receptor-mediated uptake

# Oligonucleotide Therapeutics are a Prominent Class of Products in Development; Be Ready!

- Oligo Therapeutics IND Submissions (n = 38)



- Only INDs of new molecular entities are shown
- Miscellaneous: siRNA, aptamer, LNA, decoy, DNAi, non-PS-ASO (one each)
- Clinical development : Phase 1 through 3
- New chemically modified oligonucleotides emerged after 2000
- Withdrawn: 4 (PS-ASO, 3 before 2000)

## Acknowledgments:

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- Scott Henry, Isis Pharmaceuticals
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- Jennifer Marlowe, Novartis