Immunosafety Training Course

Safety Considerations for Oligonucleotides

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Forward looking statements

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Learning Objectives

- Explain the key designs/features and mechanisms that oligonucleotide-based therapies use to modulate targets inaccessible to small and large molecule therapeutics.
- Explain and analyze impact of specific oligonucleotide chemistry and design on immunomodulatory/pro-inflammatory potential of oligonucleotides
- Evaluate the regulatory perspectives involved in oligonucleotide development for, widely prevalent to rare to ultrarare, genetic diseases

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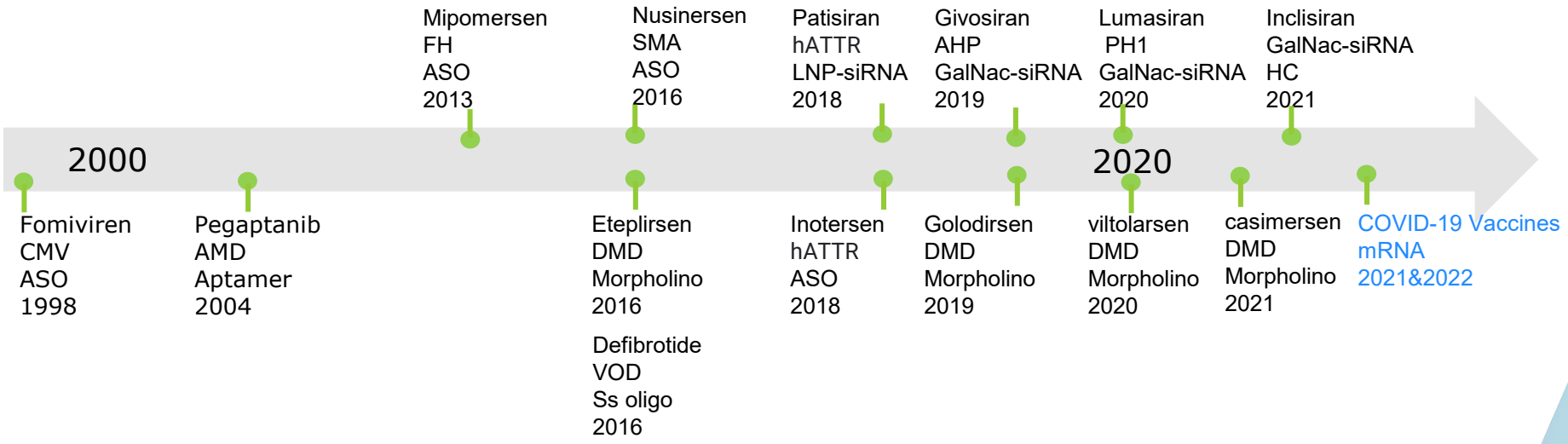
Approved Oligonucleotides



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Outline

- Introduction & characteristics of RNA therapeutics
- Regulatory guidance and study design considerations
- Sequence-dependent versus sequence-independent toxicities
- Toxicological effects of systemically and central nervous system administered antisense oligonucleotides (ASOs)
- siRNA
- Conclusions

Not included: Nucleosides/nucleoside analogs, Gene therapy, small molecule transcriptional regulators, aptamers, synthetic RNAs, guide RNA for CRISPR-Cas9

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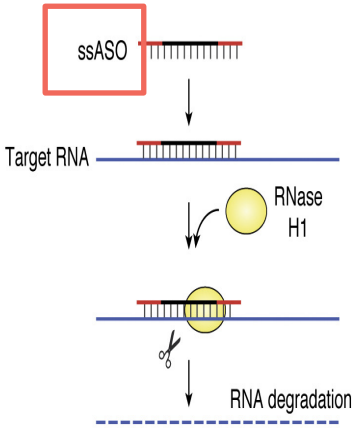
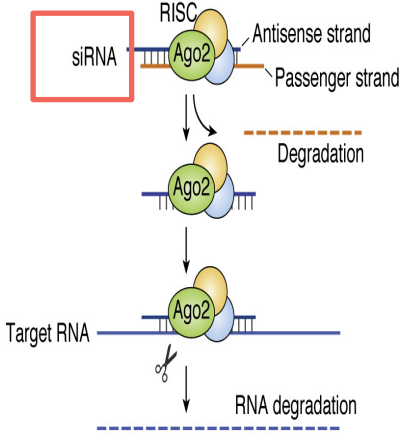
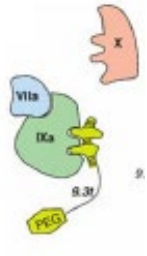
Diverse mechanisms of oligonucleotide therapeutics

Commonly used oligonucleotides

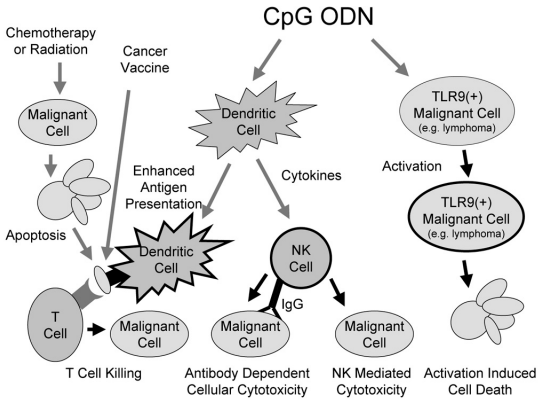




aptamer



Immunostimulatory



Crooke et al., 2021 J Biol Chem DOI:10.1016/j.jbc.2021.100416; Que-Gewirth and Sullenger, 2007 *Gene Ther.*14:283–291; Krieg and Kline, 2000 *Immunopharmacology* 48:303–305

RNA therapeutics: Major classes and general characteristics

Class	~ MW (unconjugated)	MOA
Antisense oligonucleotides (ASO)	**4-10 kDa**	- **RNA degrading** - **Splice switching** - **Steric blocking**
siRNA	**12-14 kDa**	**RNA degrading**
A-to-I RNA base editing	Broad range	endogenous ADAR recruitment
miRNA	7-8 kDa	RNA degrading
Aptamer	6-20 kDa	Protein binding (agonist/antagonist)
Synthetic mRNA	>500 kDa	Production of protein
Guide RNA for CRISPR-Cas9	30-40 kDa	Gene editing

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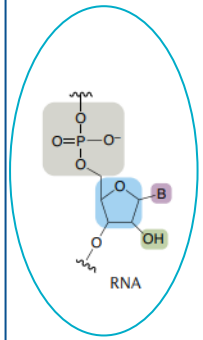
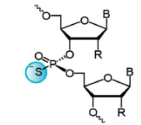
Oligonucleotide Modifications: Addressing the Vulnerabilities

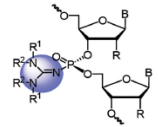
Modifications to improve metabolic stability, duration of action, binding affinity, off-target effects, cellular/tissue uptake

- Backbone modifications
  - phosphorothioate (PS), Phosphoryl guanidine (PN)
- Sugar modifications
  - 2'-O-methyl (2'-OMe), 2'-methoxyethyl (2'-MOE), 2-fluoro(2'F), Locked nucleic acids (LNAs)….
- Conjugation, chiral configurations, other chemical modifications
  - Morpholinos (PMO), chimeras, antibody conjugates, targeting conjugates (e.g. GalNAc)….

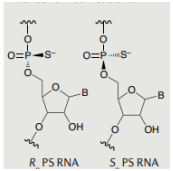
Backbone modifications



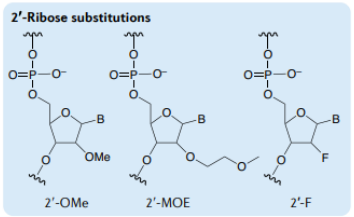

Phosphorothioate diester (PS)

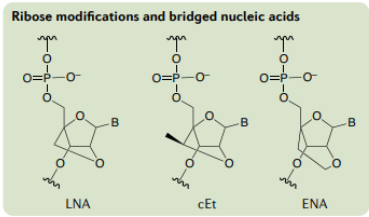

Phosphoryl guanidine diester (PN)

Stereochemistry

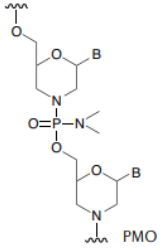


Sugar Modifications





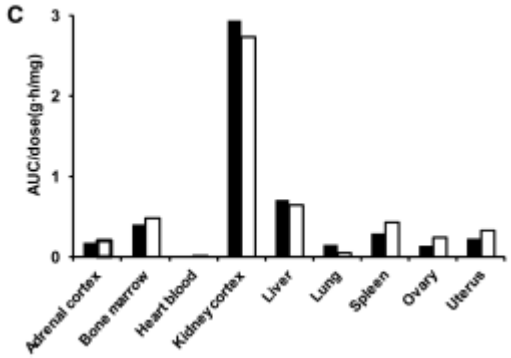
Alternative chemistries

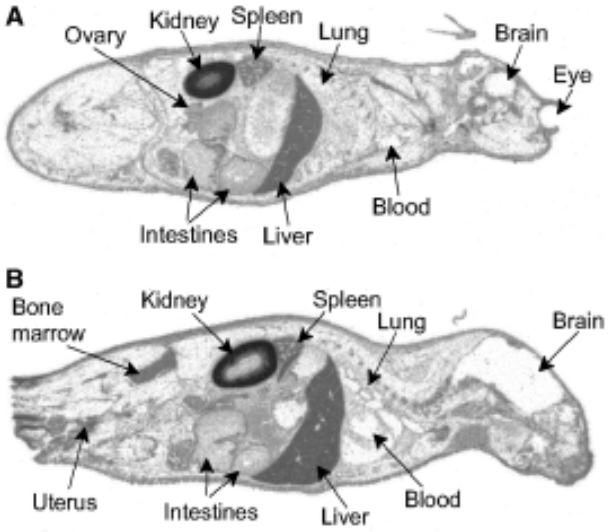


Images from: Nucleic Acids Research, 2022; Nat Rev Drug Disc, 2020

Biodistribution of oligonucleotides: Systemic ROA

Tissue distribution (quantitative autoradiography) in mice 24 hr following IV administration of LNA oligonucleotides





- Distribute broadly: highest concentrations typically kidney & liver followed by bone marrow, adipocytes, lymph nodes & spleen
- Low BBB penetration
- Secreted renally

Data from: Straarup et al, Nucleic Acids Research, 2010, Vol. 38 (20)

Nonclinical Regulatory Guidance

Synthetic oligonucleotides have been reviewed as small molecules but with influence from biologics:

- Small molecule-like: Chemically synthesized
- Biological-like: Species specificity, duration of effect

- Nonclinical regulatory guidance documents for evaluation of small molecules are generally applicable but not specific for synthetic oligonucleotides. Examples include:
  - ICH M3(R2) –Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals
  - ICH S2(R1) –Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use
  - ICH S5A and S5B –Detection of Toxicity to Reproduction for Medicinal Products
  - ICH S6(R1) –Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
  - ICH S7A –Safety Pharmacology Studies for Human Pharmaceuticals
- European Medicines Agency. EMEA/CHMP/SWP/199726/2004CHMP: SWP reflection paper on the assessment of the genotoxic potential of anti-sense oligodeoxynucleotides. 2005
- MHLW/PMDA, Japan. Guideline for preclinical safety assessment of oligonucleotide therapeutics (PSEHB/PED Notification No . 330-1). March 2020; English translated version, August, 2020

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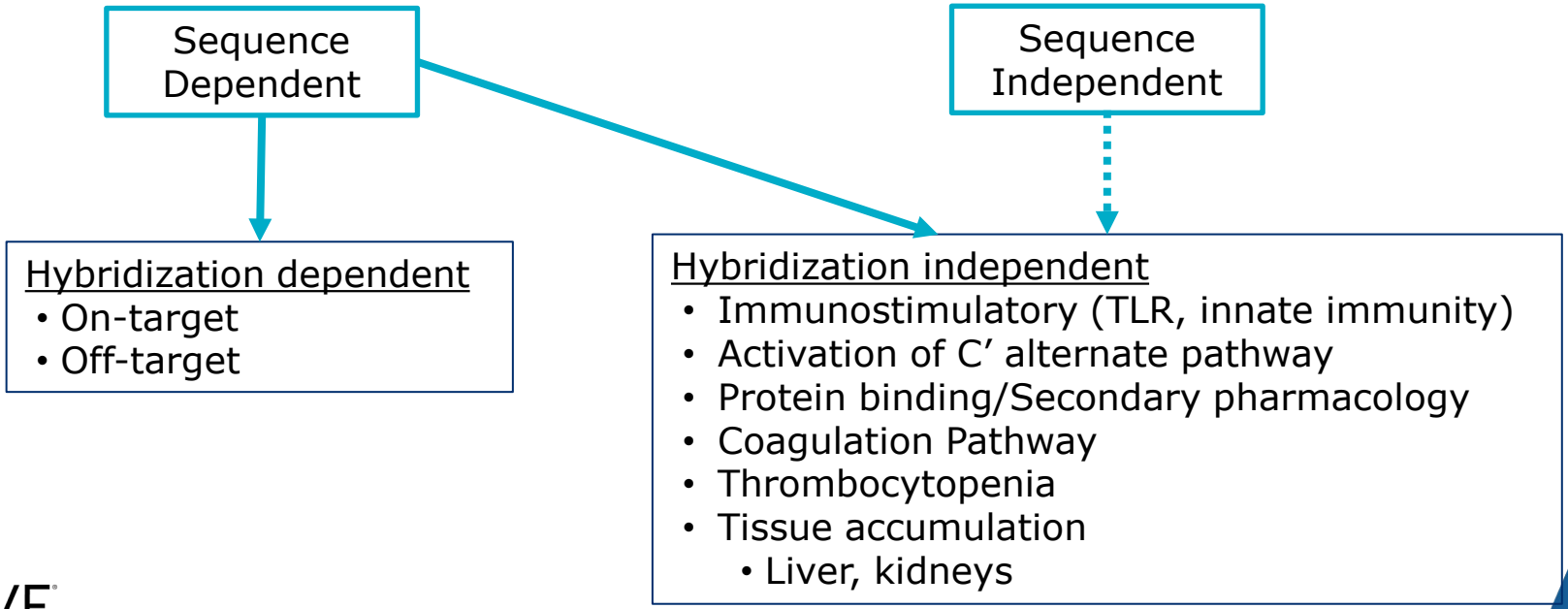
Overview of sequence-dependent or independent toxicity of oligonucleotides

- Class-wide toxicities are known - standardized mitigation steps available
- Seen across most chemistries



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Evaluation of potential on-target effects

Sequence homology: Biological relevance of animal models

- Depending on the clinical development candidate, homology may be:
  - Identical or similar (and active) between human and both tox species target sequences
  - Active in only one of the two tox species
  - Not active in either species or no target
- Regulatory Guidance:
  - Studies should be conducted in two mammalian species (one non-rodent), ICH M3(R2)
  - However, use of one species may be justified when a biologic is only pharmacologically active in one species, ICH S6(R1)

Sequence Homology: Considerations for Nonclinical Study Designs

- Options when human sequence is homologous to *only one* tox species
  - Conduct toxicology study in one species with clinical candidate [consistent with ICH S6(R1)]
  - Conduct toxicology studies in two species with clinical candidate
    - Exaggerated pharmacology evaluated in one species
    - Class/chemistry effects evaluated in both species
  - Conduct toxicology studies in two species with clinical candidate and active animal analog
    - Exaggerated pharmacology evaluated in two species, but with two molecular entities
    - Class effects evaluated in both species
- Option when human sequence is *not homologous* to either tox animal species
  - Consider using active animal analog

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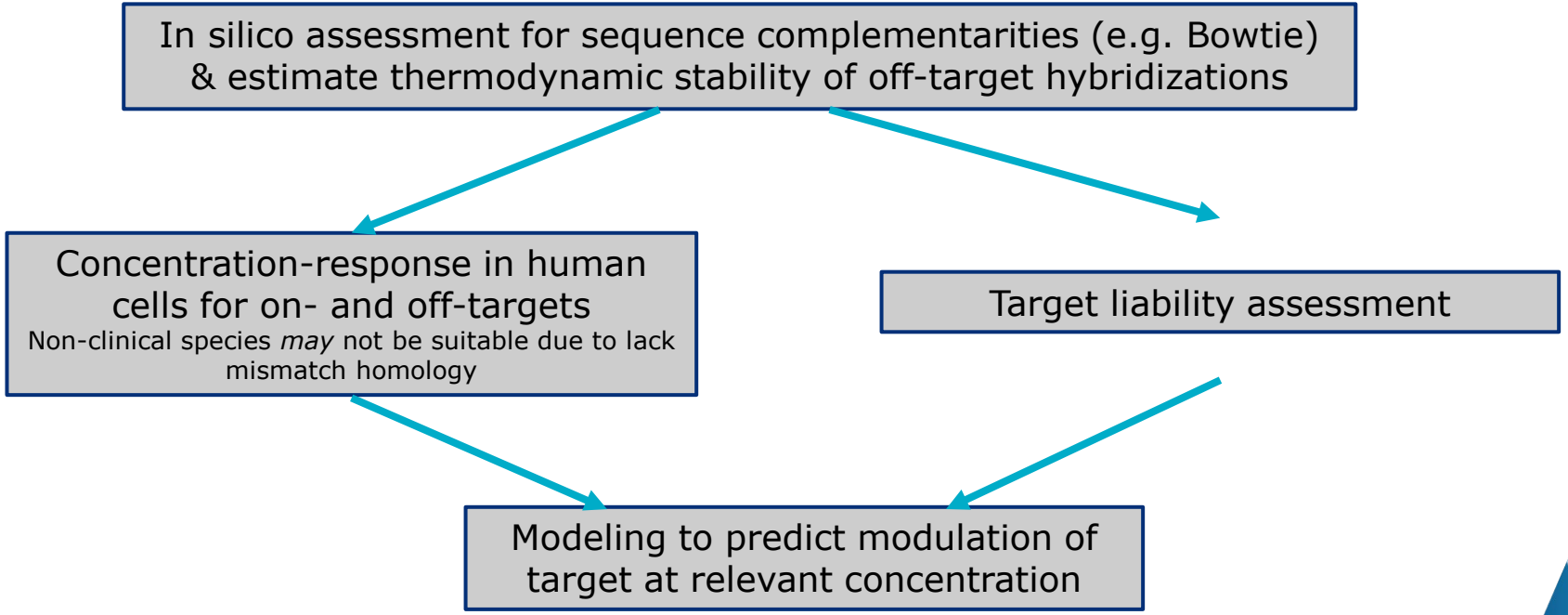
See: Kornbrust et al. Oligo safety working group exaggerated pharmacology subcommittee consensus document. *Nucleic Acid Ther* 2013, 23(1):21-8

Assessing hybridization-dependent off-target effects

Example of weight of evidence approach



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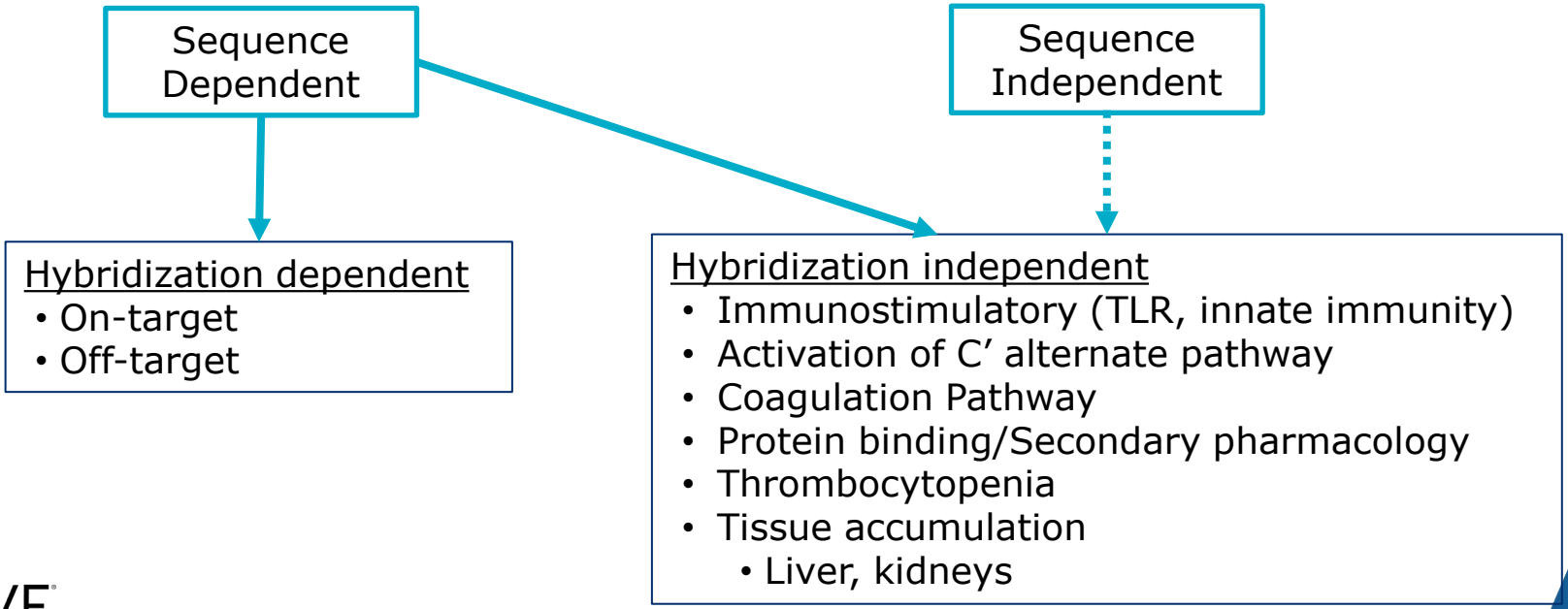

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Immune effects: CpG and non-CpG

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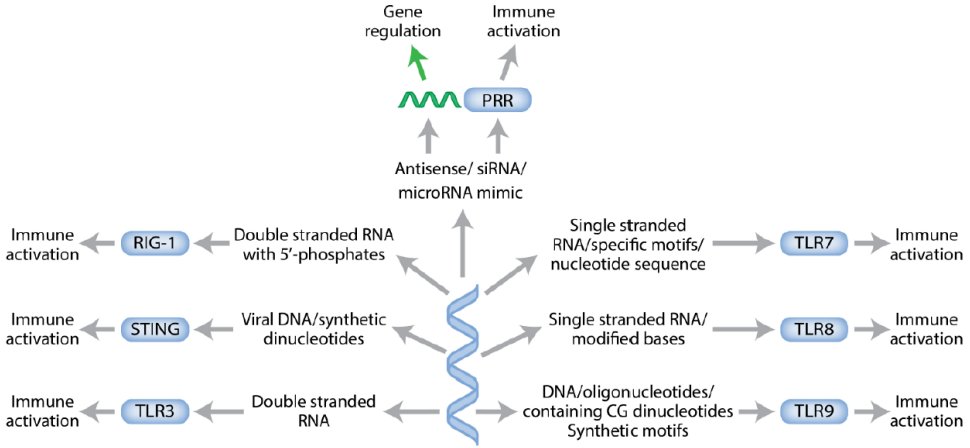
CpG oligonucleotides modulate pro-inflammatory response

Immune modulatory oligonucleotides

- CpG oligonucleotide
  - 5’-CpG-3’ in a sequence context
  - Mimic natural TLR9 receptor agonist (bacterial/viral DNA)
- Sequence-dependent and independent backbone-related effects
  - Species specificity
  - TLR9 responses most prominent in rodent
    - Rats are very sensitive
    - Monkeys and humans less responsive
  - No functional TLR8 in rodents
- Causes dose-dependent cytokine/chemokine expression
- Transient broad spectrum immune activation
  - Injection site reactions, flu-like symptoms

Pattern recognition receptors (PRR) that interact with exogeneous nucleic acids

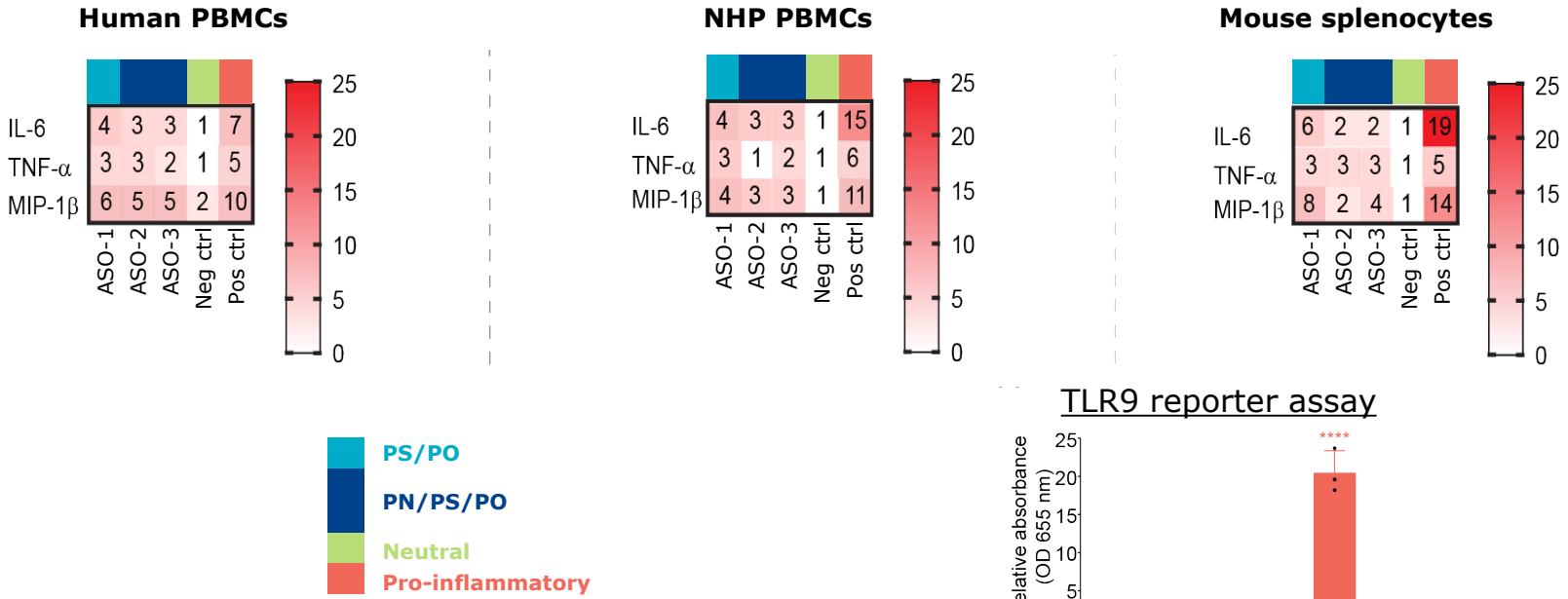


Agrawal. 2021 Biomedicines 9:503. Vollmer and Krieg. 2007 *Antisense Drug Technology* 747-772 2nd Ed.
TLR9, Toll-like receptor 9

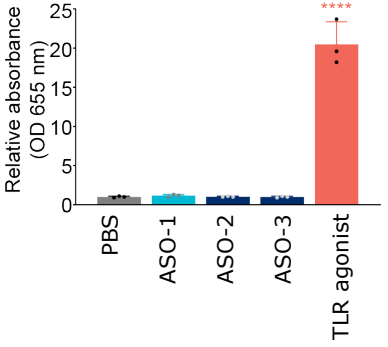
CpG and non-CpG containing oligonucleotides can be pro-inflammatory

Screening tools to de-select ASOs eliciting pro-inflammatory response

In vitro cytokine release assays



TLR9 reporter assay



Fold increase over control after cells cultured with ASO (30 uM) for 24 hr
Data from Kandasamy *et al.*, 2022 *Nuc Acids Res*

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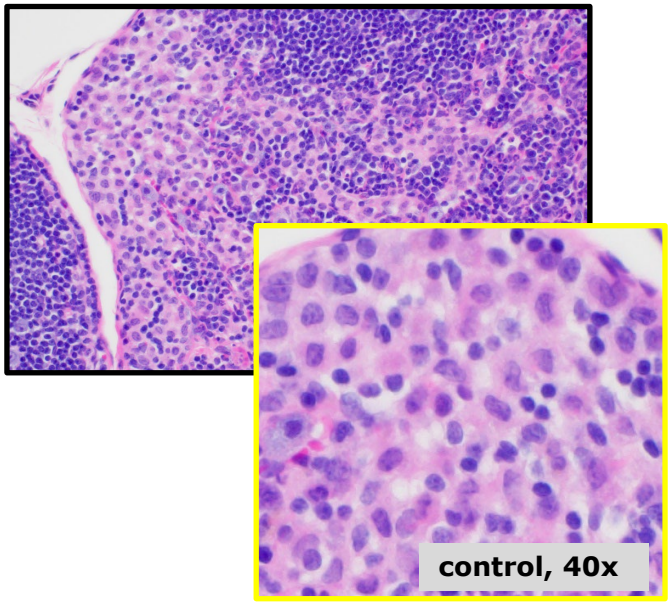
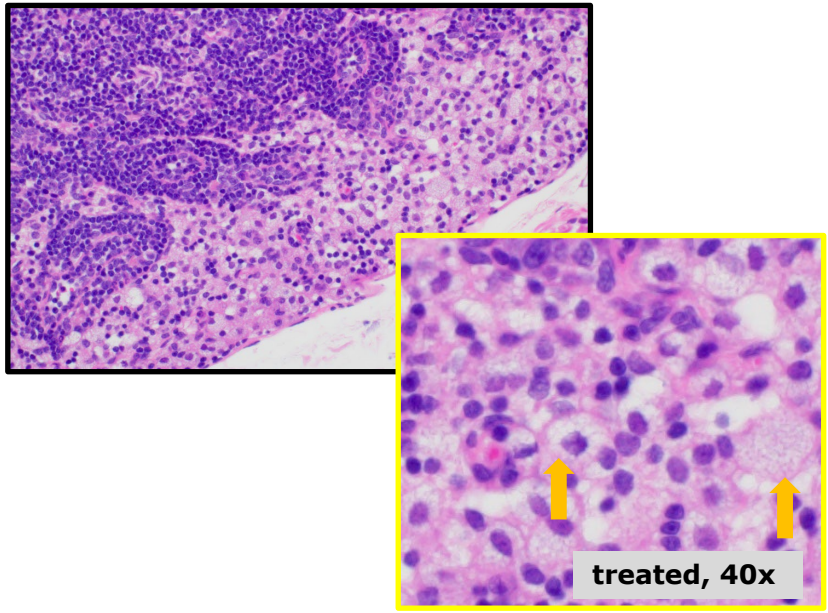
Long half-life and macrophage activation by ASO can result in vacuolated macrophages in several organs

Example: Vacuolated macrophages (orange arrows) in cervical lymph node in mice (4 monthly IV doses)





Clinical relevance: Mild injection site reactions are common in patients after subcutaneous administration

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Complement

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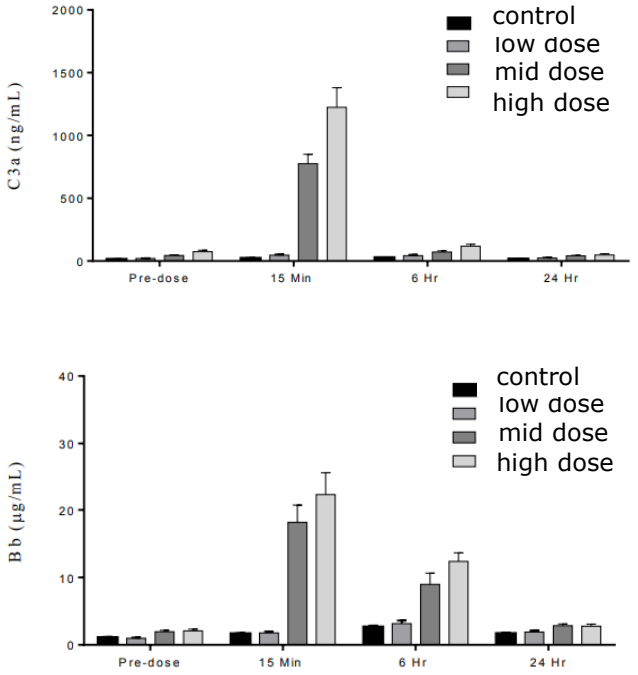
ASOs can activate alternate complement pathway resulting in increased anaphylactic split products (C3a,Bb)

- Impacts all chemistries
- Transient, dose-dependent, Cmax driven
  - Threshold for phosphorothiates ~40-50 ug/mL[1]
- Direct interaction with complement Factor H (regulates alternate complement pathway)[2]
- Monkeys are <u>much more sensitive</u> to these effects than humans *in vitro* & *in vivo*
  - Requires chronic administration <u>≥</u> 2-3 months to develop idiosyncratically in predisposed individuals
  - Can result in cardiovascular collapse, platelet count reductions, & vascular/ perivascular inflammation in various tissues

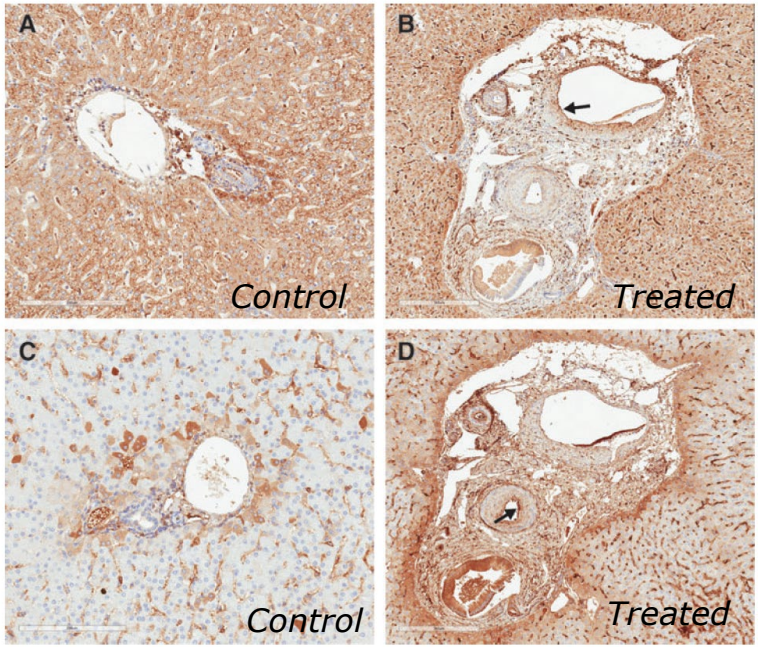
Transient elevations in plasma C3a and Factor Bb levels in monkeys (twice weekly IV doses for 13 weeks; after last dose)



1Crooke, ST. **Antisense Drug Technology: Principles: Strategies, and Applications, CRC pres, 2008**

2 Henry *et al*., 2014 *Nucleic Acid Ther*, 24(5) ; Henry et al., 2008 *Antisense Drug Technology* 2nd Ed, Ch12; Engelhardt *et al*, 2015 *Toxicol Pathol* 43:935-44

Complement and immunoglobulin deposition confirmed in monkeys with severe C3 depletion by IHC staining



scalebar=200 µm

scale bar=300 µm

- Endarteritis in small- & medium-sized arteries/arterioles in monkeys
- Liver sections from selected animals stained for the presence of C3 and IgM
  - Lack of immunopositive C3 or IgM staining of the vasculature from one representative control animal
  - Strong C3 and IgM staining of the vasculatures accompanied by thickening of the intima (**arrow**) observed in one monkey given ISIS 104838 (30 mg/kg, weekly s.c.)

Vascular injury in human patients has never been documented with any ASO*

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data from Shen *et al.,* 2016 *Nucleic Acid Ther* 26:236-49
*Engelhardt *et al*, 2015 *Toxicol Pathol* 43:935-44; Frazier and Obert, 2018 *Toxicol Pathol* 46: 904-917.
s.c. subcutaneous

Coagulation Pathway

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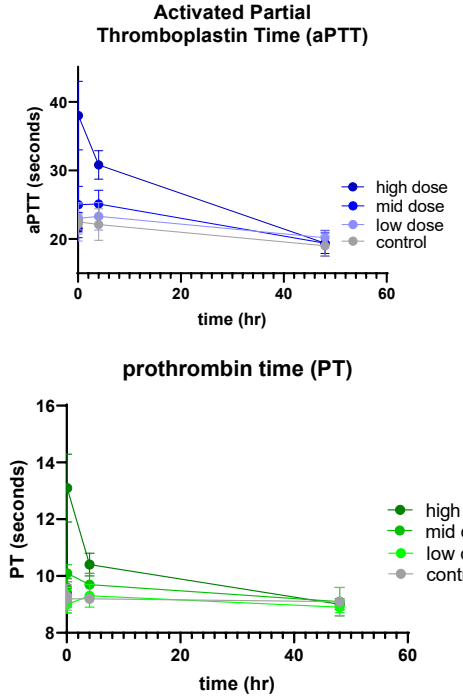
Prolongation of Clotting Cascade

- Hybridization-independent effects characterized for PS oligodeoxynucleotides
- Transient prolongation of clotting times following high-dose IV
- Intrinsic pathway (aPTT) usually more sensitive to inhibition than the extrinsic pathway (PT)
- In both clinical trials and nonclinical studies, there is <1 sec per µg/mL increase in aPTT
- After IV infusion of 3 mg/kg in monkeys or humans, typical concentrations of PS ODNs are in the range of 10-20 µg/mL
  - At Cmax there is a transient increase in aPTT of 10-20 sec
  - Increase is directly proportional to plasma concentrations; as PS ODN clears, the inhibition reverses (within 3 h)
  - Cmax is blunted, and typical peak plasma concentrations are in the range of 3-5 µg/mL, for SC administered PS ODNs (3 mg/kg) not clinically significant
  - In monkey, there is no indication of hemorrhage; at very high doses (~50 mg/kg) bruising has been observed
  - No significant AEs have been reported related to prolongation of aPTT in the clinic

Increased aPTT and PT in monkeys (twice weekly IV doses for 13 weeks; after last dose)



PS, phosphorothioate; IV intranenous; aPTT activated partial thrombin time; PT prothrombin time; ODN, oligonucleotide; SC subcutaneous; AEs adverse events

Platelet effects

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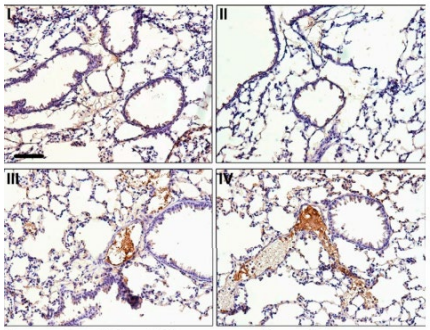
Phenotype 1: ASOs can cause moderate decreases in platelet count

- Dose-dependent, reproducible, moderate decline in platelets - reversible
- Translatable to humans (not considered SAE)

- PS-modification dependent
- ASOs bind to platelets; elicit platelet activation & aggregation
- Mediated by platelet-specific receptor glycoprotein VI (GPVI)



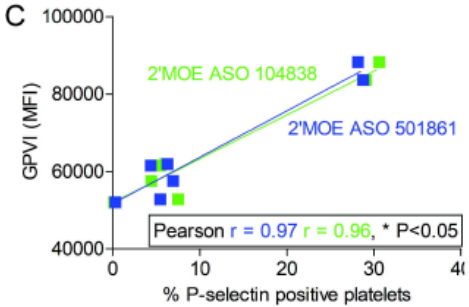
Average thrombi from 10 transverse sections of lobe

Treatment	Left superior	Right superior	Right middle	Total thrombi
PBS (n=3)	1	0	1	2
ODN (n=5)	37	44	26	107
ODN nonmod (n=4)	1	0	1	2

Thrombus formation in the lung vessels of C57BL/6 mice induced by ODN2395

Data from: JEM, 2015; 212 (2); 129-137

- ASO showed ~50% PLT decrease in monkeys; thrombocytopenia was likely due to ↑ PLT destruction or splenic sequestration (not diminished platelet production)*
  - No effect on proplatelet production
  - Platelet activation and platelet-leukocyte aggregates – correlated with GPVI expression



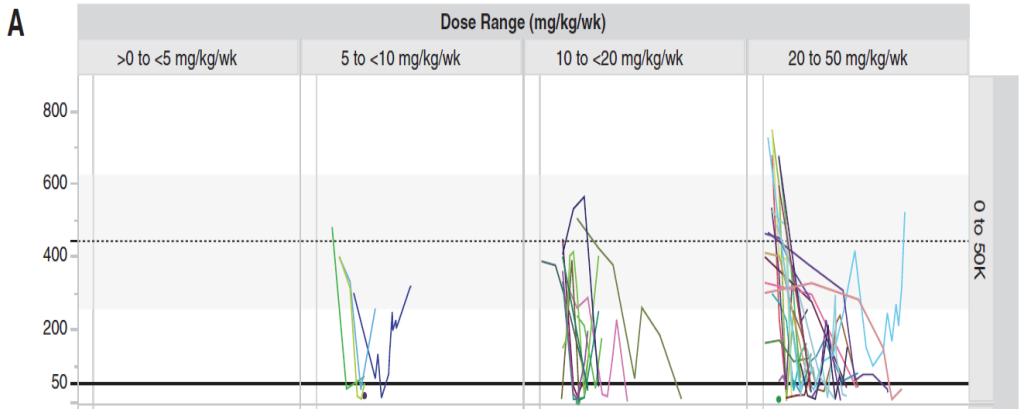
Individual donor platelet GPVI receptor levels correlated with platelet activation

Data from: Hematalogica, 2022;107(2):519-531

*Narayanan P, Toxicol Sci. 2018;164(2):613-626.

Phenotype 2: Reduced platelet count in monkey toxicology studies

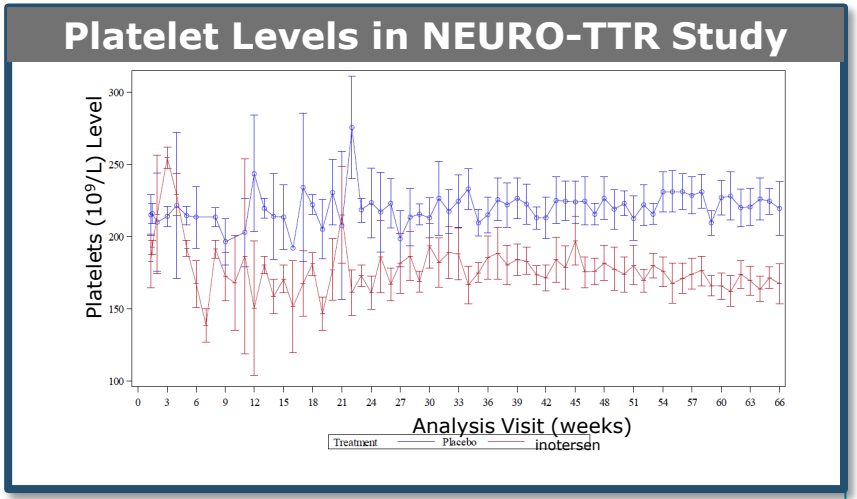
- Sporadic incidence of severe decrease in platelet count
  - Occurs at doses ≥5 mg/kg/wk at a 2.8 to 4% incidence in monkey
  - Adverse, with PLT nadir <50 K/µL
  - Monkey predicted clinical thrombocytopenia*
  - More prevalent in Mauritian-sourced cynomolgus monkeys
  - Common phenotype in drisapersen (DMD), Tegsedi® (TTR) and Waylivra ® (APOC3) clinical trials



Reversible upon discontinuation of treatment and steroid treatment

PLT, platelet count
*Inotersen Summary Basis of Approval, October 5, 2018 (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/211172Orig1s000TOC.cfm)

Clinical case study



- Results suggest antibody-mediated mechanism in the 3 severe cases
  - Anti-PLT IgG in three Grade 4 cases (2 of 3 were GPIIb/IIIa+)
- Baseline cytokines suggest underlying immune dysregulation in TTR amyloidosis patients

TABLE 3. ANTIBODY STATUS IN SUBJECTS TREATED IN THE NEURO-TTR CLINICAL TRIALS

	NEURO-TTR			*OLE*		
	Placebo (n = 17[a]), n (%)	*Inotersen (n = 32[a]), n (%)*	*Total (N = 49)*	*Placebo (NEURO-TTR)/ inotersen (n = 10), n (%)*	*Inotersen (NEURO-TTR)/ inotersen (n = 23), n (%)*	*Total OLE (N = 33)*
Baseline antiplatelet IgM						
Drug independent	1/16 (6.3)	0	1/47 (2.1)	1/9 (11.1)	0	1/28 (3.6)
Drug dependent	0	1/31 (3.2)	1/47 (2.1)	0	1/19 (5.3)	0
Treatment emergent[b] antiplatelet IgM						
Drug independent	0	0	0	0	1/19 (5.3)	1/28 (3.6)
Drug dependent	0	1/30 (3.3)	1/45 (2.2)	3/9 (33.3)	2/19 (10.5)	5/28 (17.9)
Baseline antiplatelet IgG						
Drug independent	0	4/31 (12.9)	4/47 (8.5)	0	2/19 (10.5)	2/28 (7.1)
Drug dependent	0	1/31 (3.2)	1/47 (2.1)	0	1/19 (5.3)	1/28 (3.6)
Treatment emergent antiplatelet IgG						
Drug independent	0	5/30 (16.7)	5/45 (11.1)	4/9 (44.4)	3/19 (15.8)	7/28 (25)
Drug dependent	0	0	0	3/9 (33.3)	6/19 (31.6)	9/28 (32.1)
Anti-PF4 IgA						
Baseline	0	0	0	0	0	0
Treatment emergent	0	1/30 (3.3)	1/45 (2.2)	0	0	0
Anti-PF4 IgM						
Baseline	1/16 (6.3)	0	1/47 (2.1)	1/9(11.1)	0	1/28 (3.6)
Treatment emergent	0	6/30 (20)	6/45 (13.3)	0	5/19 (26.3)	5/28 (17.9)
Anti-PF4 IgG						
Baseline	0	0	0	0	0	0
Treatment emergent	1/15 (6.7)	0	1/45 (2.2)	0	1/19 (5.3)	1/28 (3.6)
Total antibody positive subjects						
Baseline	2/16 (12.5)	6/31 (19.4)	8/47 (17.0)	2/9 (22.2)	4/19 (21.1)	6/28 (21.4)
Treatment emergent	1/16 (6.3)	9/31 (29.0)	10/47 (21)	5/9 (55.6)	10/19 (52.6)	15/28 (53.6)

[a]Patients with missing values were not included in the analysis.
[b]Treatment emergence was defined as negative baseline value followed by any postbaseline value. Positive postbaseline values for subjects whose baseline values were missing were not considered to be treatment emergent.
OLE, open-label extension.

- Not observed with GalNAc-conjugate (eplontersen)
  - Lower, less frequent SC dose improves drug safety profile
    - Reduces total systemic exposure
    - Provides more convenient and tolerable dosing regimen for patients

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Data from Narayanan *et al.*, 2020 *Nucleic Acid Ther* 30:94-103.
PLT, platelets; Ig, immunoglobulin; TTR, transthyretin; SC subcutaneous

Liver and Renal effects

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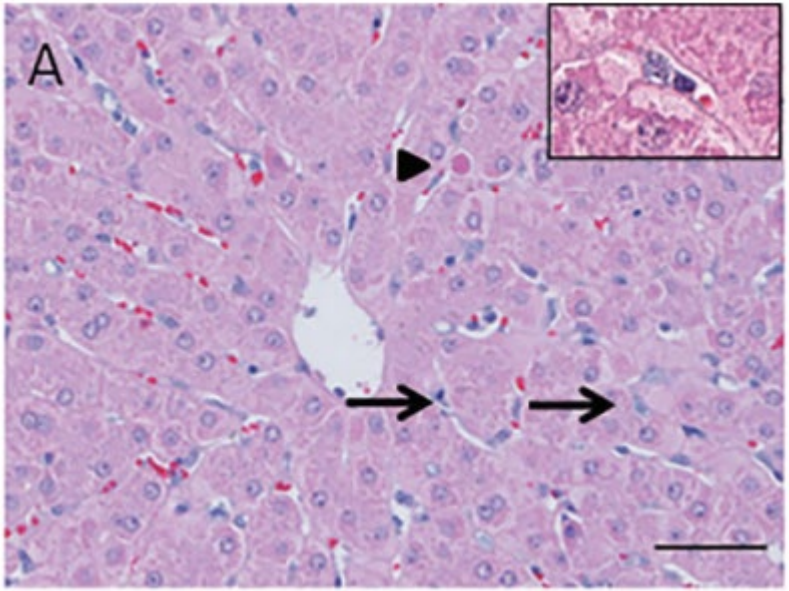
ASOs can induce specific class effects in the liver

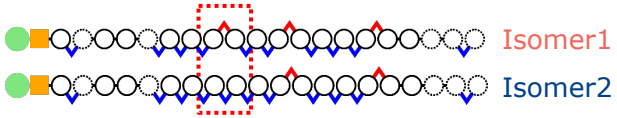
- Kupffer cell hypertrophy & basophilic granules
  - On its own, not adverse
  - ASO accumulation
  - Fully/partially eversible
- Liver enzyme elevations
  - Often mild & not associated with histologic correlate
  - Mouse most sensitive
    - Good screening tool
    - Severe liver elevations/histopathological changes (hepatocyte degeneration/necrosis) can be identified in 14-28 day studies



Histopathologic findings in minipigs: Liver, H&E. Basophilic granules in Kupffer cells (long arrowheads and insert) and apoptotic hepatocytes (short arrowheads); 45 mg/kg RTR2996.
Image from: Braendli-Baiocco, A., et al, *Tox Sci*, 157(1), 2017, 112–128

A single stereoisomeric change can dramatically alter the tolerability profile

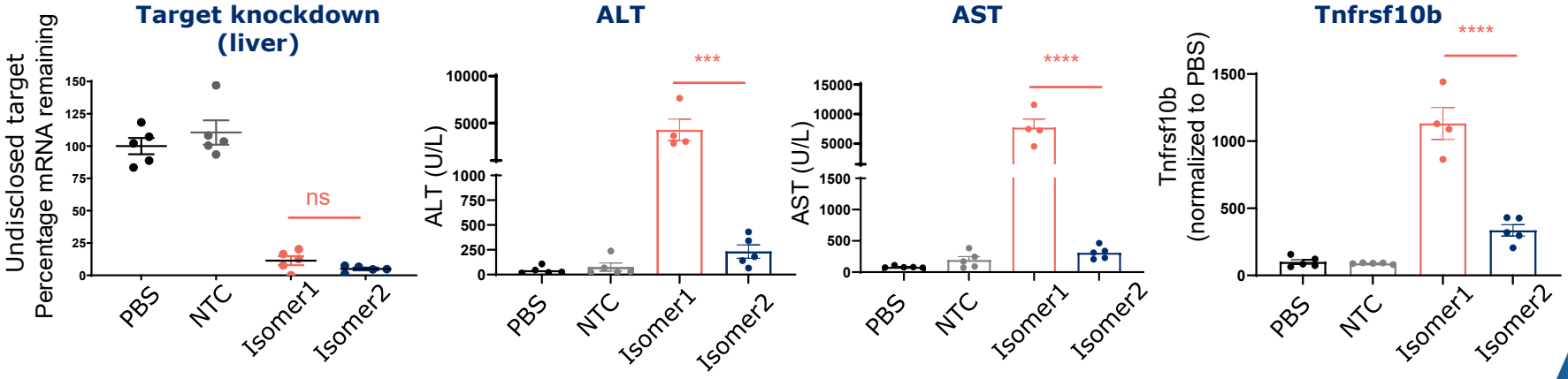
GalNAc conjugated oligonucleotide administered **subcutaneously**



Same sequence and chemical modifications, but different stereochemistry

Stereoisomers have **similar** pharmacodynamic effects

Changing backbone stereochemistry leads to **different** hepatotoxicity profiles *in vivo*



C57Bl/6 mice were administered 5 mg/kg oligonucleotide or PBS by subcutaneous injection on days 1, 3, 5 and 8. Liver tissue was collected on day 11. Target mRNA was normalized to Hprt1. Data are presented as mean ± sem (n=5). Stats: One-way ANOVA ns not significant, PBS phosphate buffered saline, NTC non-targeting control

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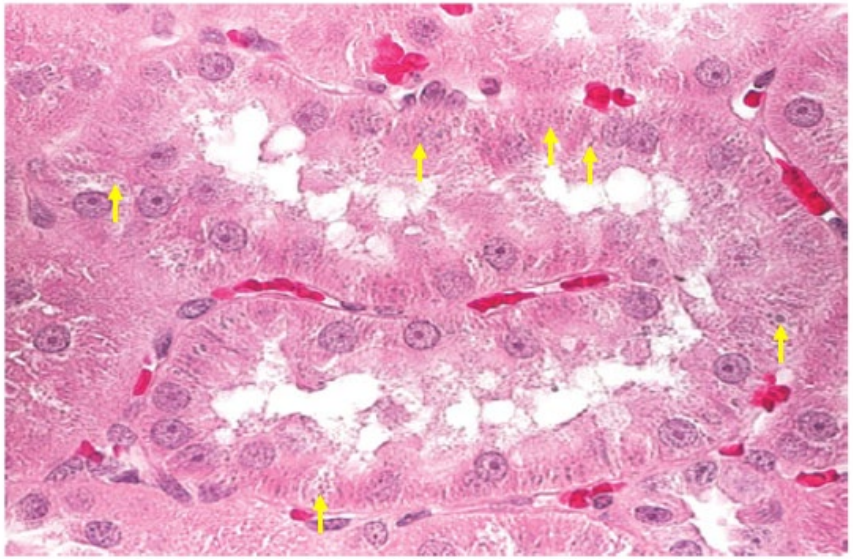
ASOs can induce specific class effects in the kidney

- **Tubule findings** (rodents, NHPs and humans)
  - Basophilic granules – ASO accumulation
  - Dose-dependent tubular degeneration
  - Clinical cases of tubular injury rare
- **Proteinuria** (rodents, NHPs and humans)
  - Associated with uptake in tubules after binding albumin (interferes with megalin/ cubilin receptor)
  - Transient, mild; generally does not pause dosing
- **Glomerulonephritis**
  - Can occur in studies longer than 3 months – not a class effect
  - Published clinical cases of ASO GN are rare: drisapersen and inotersen
  - Most clinical cases had no evidence in tox studies
  - Neither mice nor monkey GN are relevant for humans; pathogenesis is different*



Rat kidney. Basophilic granules (yellow arrows) are noted as dark grey spots within tubular epithelium. Image modified from: Frazier, K., *Tox Path*, 43: 78-89, 2015

*Frazier and Obert, 2018 *Toxicol Pathol* 46: 904-917.

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ASO class effects in animal studies: translation to the clinic

Effect in nonclinical studies	Clinical effect
Coagulation prolongation	Clinically not significant
Complement activation in monkeys – Consequential vasculitis, glomerulonephritis	Clinically not significant
Immune stimulation	Flu-like reactions and subcutaneous injection site reactions
Thrombocytopenia – Mild (~50% drop) – Severe phenotype	 – Translatable to humans – Low incidence
Hepatoxicity	Translates; screened out with preclinical assays/studies
Renal Toxicities – Tubular toxicity – proteinuria	Seen in preclinical species and in clinic – Does not pose a clinical hurdle – Generally transient &mild; does not pause dosing

Intrathecal administration

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Specific motor phenotypes observed after CNS-administration

- Observed in rodents and monkeys after ICV or IT administration
- Dose-dependent – adversity dependent on severity and recovery
  - Transient absence of reflexes or FOBs (recovery by 24-48 h)
    - Can be procedure-related
  - Acute findings (30 min- 4 h post-dose): ataxia, paresis, nystagmus, urinary incontinence, hypoactivity, tremor
    - Generally spontaneously resolve
  - Delayed hind limb paresis or paralysis (several days-weeks after dosing)
    - Generally result in moribundity

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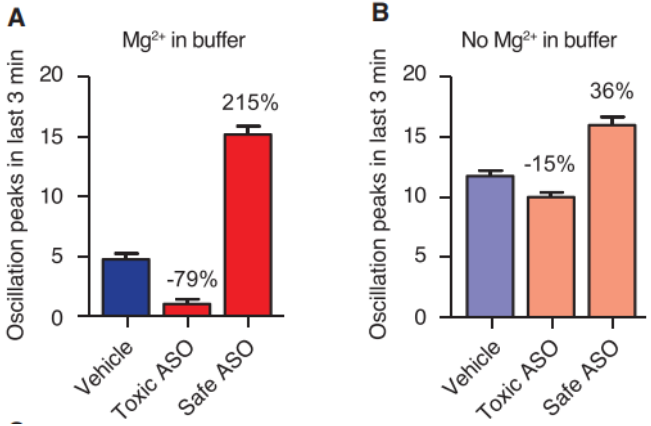
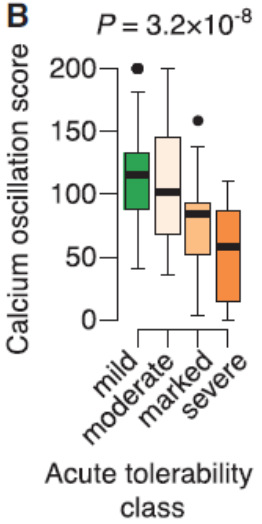
Korte, S, International Journal of Toxicology, 2020
ICV= Intracerebroventricular; IT = intrathecal; FOB = functional observation battery

Specific motor phenotypes observed after CNS-administration

- Acute tolerability in mice after ICV injection correlates with reductions in spontaneous calcium oscillations in neuronal cells
- Associations between sequence features and calcium oscillation scores



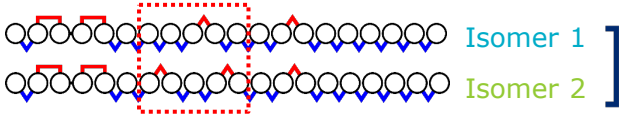


Data from: Nucleic Acid Ther, 2022 Feb 14, doi:10.1089/nat.2021.0071

ICV= Intracerebroventricular

Stereoisomeric changes can dramatically alter the tolerability profile in the CNS

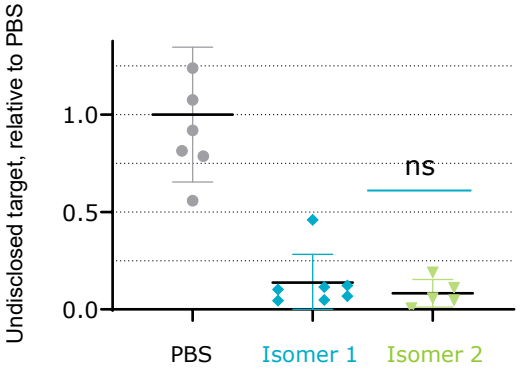
Unconjugated oligonucleotide administered **ICV**



Same sequence and chemical modifications, but different stereochemistry

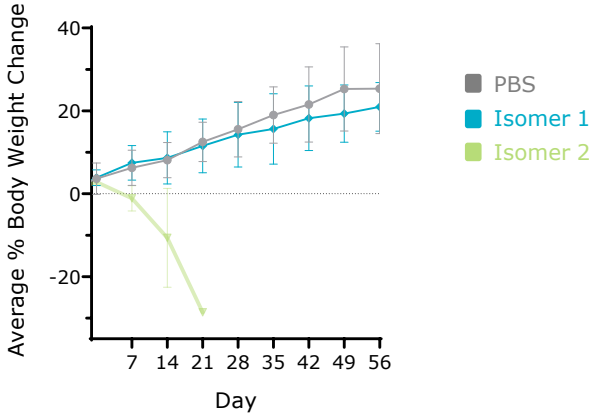
Stereoisomers have **similar** pharmacodynamic effects *in vivo*

CNS target knockdown *in vivo*



Changing backbone stereochemistry leads to **different** tolerability profiles *in vivo*

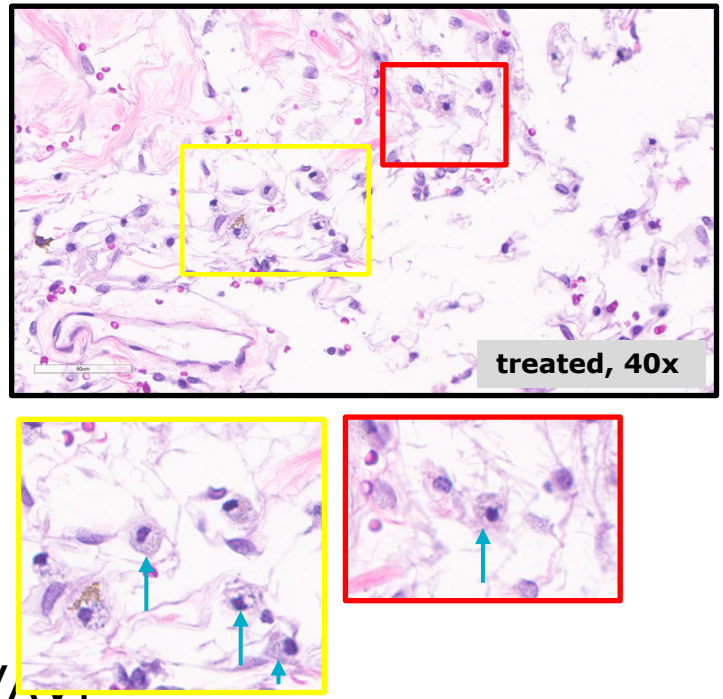
Percentage Body Weight Change



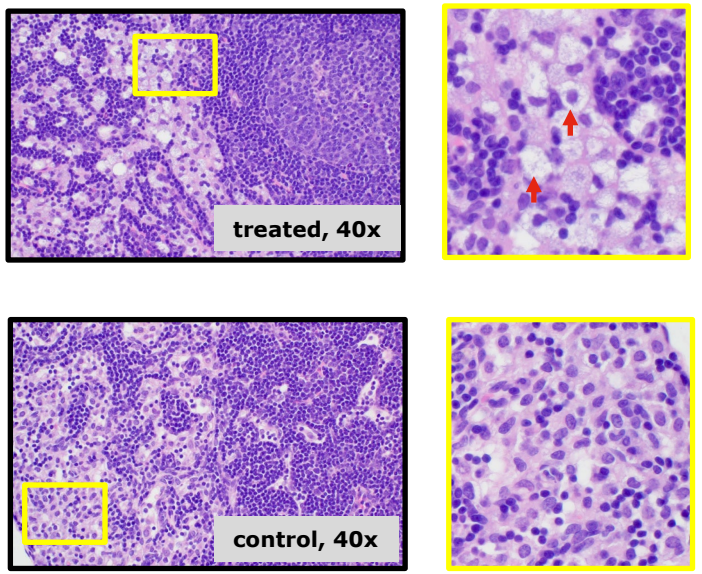
Left: In a target engagement study, 7 mice administered 2 x 50 ug oligonucleotide or PBS by ICV on days 0 and 7. Tissue collected on day 14. Target mRNA normalized to Tubb3 and plotted relative to PBS. Data presented as mean ± SD (n=7). Stats: One-way ANOVA ns not significant, PBS phosphate buffered saline. Right: wtmouse tolerability study, n=4 administered 100 ug oligonucleotide or PBS by ICV on day 0 and monitored for 8 weeks.

Histopathological findings after CNS delivery of oligonucleotides

Vacuolated macrophages (blue arrows) in brain meninges in NHP (2 weekly IT injections)



Vacuolated macrophages (red arrows) in lymph node in mice (4 monthly ICV injections)



ICV= Intracerebroventricular; IT = intrathecal

Small interfering RNAs (siRNAs)

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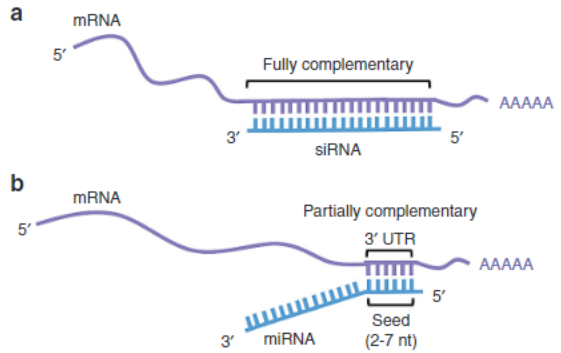
Safety considerations for siRNAs

Considerations for siRNAs

Highly polar molecules that do not cross cellular membranes by passive diffusion

- Delivery systems (eg, LNPs)[1]
- Targeting conjugates (e.g GalNAc for targeted delivery to hepatocytes via ASGR–mediated endocytosis[2])



Lam et al, Molecular Therapy—Nucleic Acids (2015) 4

Sequence-dependent/independent off-target effects

- Hybridization based off-target effects
  - eg, microRNA-like seed-based activity
  - Chemistry improvements (e.g.ESC+) mitigate seed-mediated off-target effects, improves specificity[3]
- Immunostimulatory effects
  - Injection site reactions
  - Severe thrombocytopenia avoided – only short stretches of PS needed for optimal stability of GalNAc-siRNAs
- Dysregulation of RNAi machinery
  - Competition with endogenous miRNAs

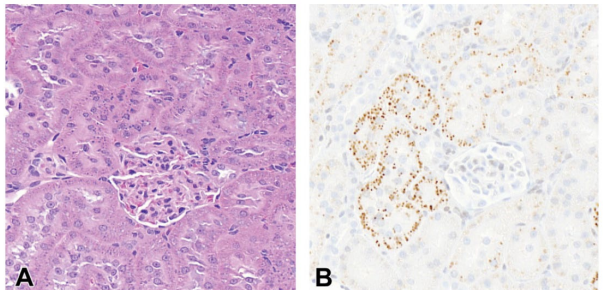
1: Jayaraman *et al*., 2012 *Angew Chem Int Ed Eng* 51:8529-33; Maier *et al*. 2013 *Mol Ther* 21:1570-8; 2: Nair *et al*., 2014 *J Am Chem Soc* 136:16958-61; 3: *Schlegel et al., 2021 Nucl Acids Res 49 (19) 10851–67;* Zatsepin, Kotelevtsev, and Koteliansky. 2016 *Int J Nanomedicine* 11:3077-86. Janas *et al.,* 2018 *Toxicol Pathol.* 46:735-745

LNPs, lipid nanoparticles; GalNAc, *N*-Acetylgalactosamine; PS phosphorothioate; ESC+ Enhanced Stability Chemistry+

GalNac-conjugated siRNA: nonadverse histologic manifestations of drug accumulation in liver, kidney, and lymph nodes

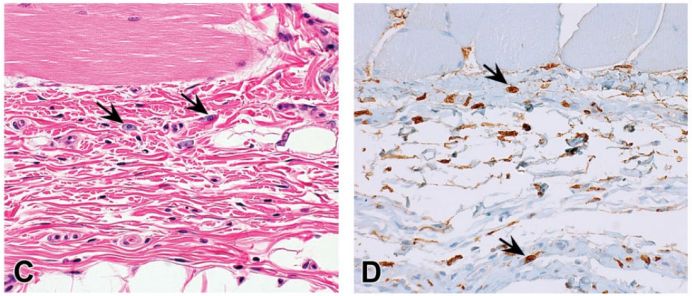
- 3-week repeat-dose toxicity studies in rat
  - Once weekly dosing up to 300 mg/kg

GalNAc-siRNA accumulation in proximal renal tubular cells in rats



- H&E: cytoplasmic basophilic granules in proximal renal tubular cells (A)
- IHC: confirms granules to be GalNAc-siRNA drug (B)

GalNAc-siRNA accumulation in subcutaneous injection site of rats



- H&E: vacuolated mononuclear cells (arrows) in the superficial dermis (C)
- IHC: confirms presence of GalNAc-siRNA drug in these cells (D)

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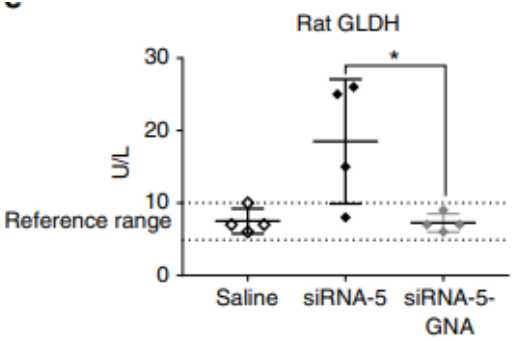
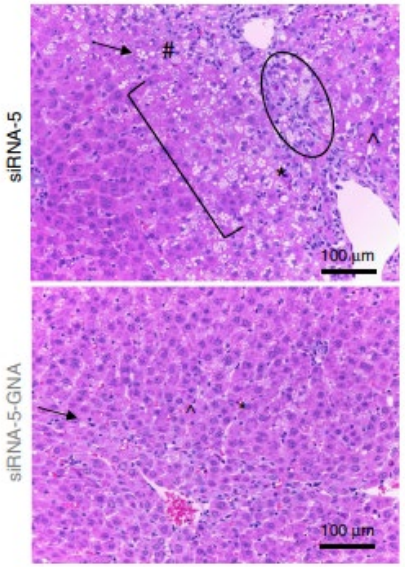
Janas, M et al Toxicologic Pathology 2018, Vol. 46(7) 735-745G

alNAc, *N*-Acetylgalactosamine

RNAi-mediated off-target effects are important drivers of hepatotoxicity in rodents

- Centrilobular hepatocellular degeneration and/or coagulative necrosis, associated with liver enzyme elevations seen with some GalNAc siRNAs
- Destabilizing seed-mediated base-pairing minimizes off-target effects and mitigates hepatotoxicity





- toxic parent siRNA-5: fibrosis (circle), hepatocellular degeneration (bracket), single cell necrosis (*), increased mitoses (^), Kupffer cell hyperplasia and/or infiltrating leukocytes (#), and hepatocellular vacuolation (arrow) with incr GLDH
- non-toxic siRNA had only minimal vacuolation and no elevated liver GLDH

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Janas, et a, Nat Comm, 2018; 9:723

siRNAs administered to rats at 30 mg/kg six times every other day

Conclusion

RNA-based therapeutics delivering on promise envisioned more than 30-years ago

- ~50 investigational RNA drugs in various phases of development
- Chemistry improvements have allowed improvements in duration of action, selectivity, off-target effects, & targeted tissue uptake
  - Overcome synsthesis hurdles
- Continuing to broaden spectrum of diseases & enable additional ROA
- Class-wide toxicities are known - standardized mitigation steps available
- Definition of the mechanisms of toxicities has facilitated solutions to many of the issues encountered in the past

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- Jigar Desai
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