Minimizing Immunogenicity Testing for Development of Oligonucleotide Therapeutics: A Risk-Based Strategy

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Agenda

01 Background: Oligonucleotides therapeutics (ONTs)

02 Immunogenicity risk assessment for GalNAc-siRNA and ASO

- Product related factors
- Immunogenicity data from approved drugs

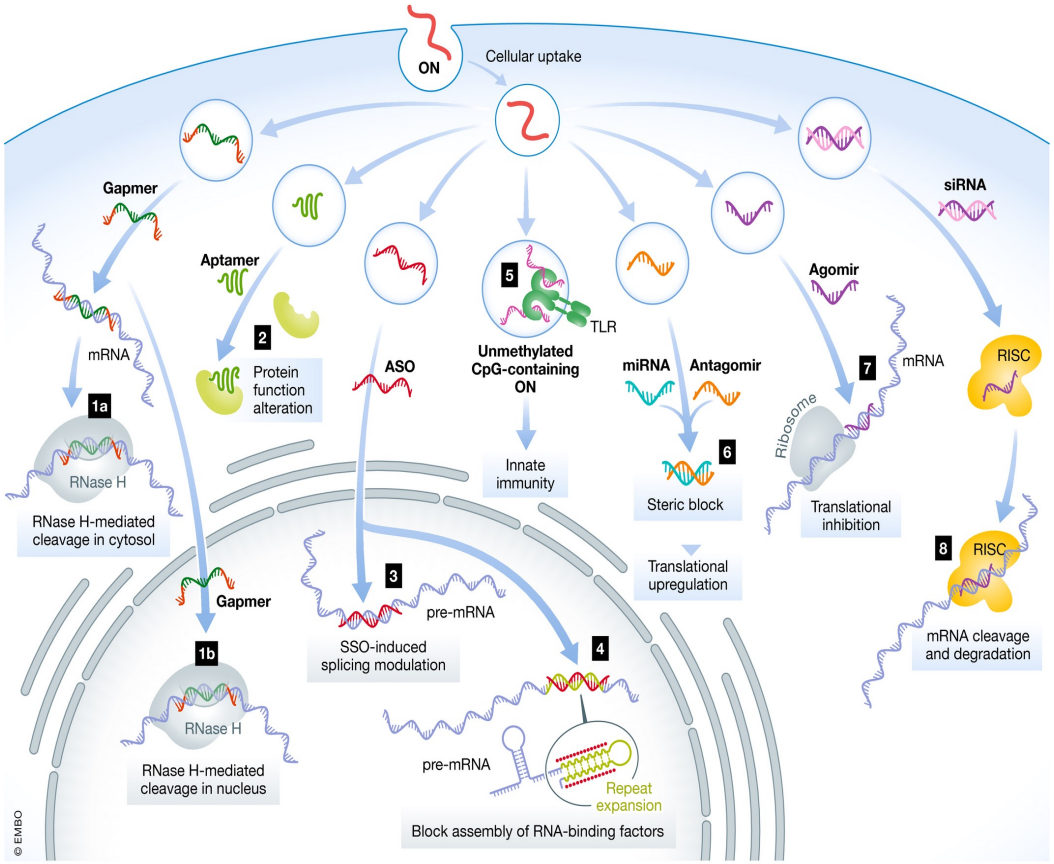
03 Streamlined Immunogenicity assessment strategy

- A risk-based ADA assessment process
- 1-tiered ADA testing approach

04 Forward looking proposal

Oligonucleotide-Based Therapeutics (ONTs)

- ONTs are short single or double stranded sequences, or oligomers of synthetic RNA and DNA that are function to regulate gene expression
- Different types of ONTs:
  - Antisense oligonucleotides (ASOs)
  - Small interfering RNAs (siRNAs)
  - Aptamers
  - MicroRNAs (miRNAs), etc
- Synthetic oligonucleotides are regulated as drugs by CDER



EMBO Mol Med, Volume: 13, Issue: 4, First published: 6 April 2021, DOI: (10.15252/emmm.202013243)

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24 FDA- and/or EMA-approved oligonucleotides therapeutics

These therapeutics manage diseases by targeting genes previously considered undruggable.

Type	Drug	Brand Name	FDA Approval	Company	Indication
ASO	Fomivirsen	Vitravene	1998 (Withdrawn)	Ionis/Novartis	CMV
	Mipomersen	Kynamro	2013 (Withdrawn)	Kastle/Ionis	HoFH
	Nusinersen	Spinraza	2016	Ionis/Biogen	SMA
	Eteplirsen	Exondys	2016	Sarepta	DMD
	Inotersen	Tegsedi	2018	Ionis	hATTR-PN
	Volanesorsen	Waylivra	2019	Ionis	FCS
	Golodirsen	Vyondys 53	2019	Sarepta	DMD
	Viltolarsen	Viltepso	2020	Nippon Shinyaku	DMD
	Casimersen	Amondys 45	2021	Sarepta	DMD
	Tofersen	Qalsody	2023	Ionis/Biogen	SOD1-ALS
	Eplontersen	Wainua	2023	AstraZeneca/Ionis	hATTR-PN
	Imetelstat	Rytelo	2024	Geron Corporation	MDS
	Olezarsen	Tryngolza	2024	Ionis	FCS
	Donidalorsen	Dawnzera	2025	Ionis	HAE

siRNA	Patisiran	Onpattro	2018	Alnylam	hATTR-PN
	Givosiran	Givlaari	2019	Alnylam	AHP
	Lumasiran	Oxlumo	2020	Alnylam	PH1
	Inclisiran	Leqvio	2021	Novartis	HeFH
	Vutrisiran	Amvuttra	2022	Alnylam	hATTR-PN
	Nedosiran	Rivfloza	2023	Novo Nordisk	PH1
	Fitusiran	Qfitlia	2025	Sanofi	Hemophilia A or B
	Plozasiran	Redemplo	2025	Arrowhead	FCS
Aptamer	Pegaptanib	Macugen	2004 (Withdrawn)	Pfizer/Eyetech	wAMD
	Avacincaptad pegol	IZERVAY	2023	Archemix/Iveric Bio	GA

- 14 ASO drugs
- 8 siRNA drugs
- 2 Aptamer drugs

Nucleic Acid Therapeutics: Approvals and Potential Blockbusters | Biopharma PEG

Oligonucleotide immunogenicity FDA and EMA guidelines

1. Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics (2024)
   - https://www.fda.gov/media/159414/download
   - **Section II B: Performing Immunogenicity Risk Assessments**
2. Nonclinical Safety Assessment of Oligonucleotide-Based Therapeutics (2024)
   - https://www.fda.gov/media/183496/download
3. Immunogenicity Assessment for Therapeutic Protein Products (2014)
   - https://www.fda.gov/media/85017/download
4. Immunogenicity Testing of Therapeutic Protein Products - Developing and Validating Assays for Anti-Drug Antibody Detection (2019)
   - https://www.fda.gov/media/119788/download
5. Guideline on the Development and Manufacture of Oligonucleotides (2024)
   - https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-developmentmanufacture-oligonucleotides_en.pdf
6. Guideline on Immunogenicity Assessment of Therapeutic Proteins (2017)
   - https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-immunogenicityassessment-therapeutic-proteins-revision-1_en.pdf

2024 FDA Guidance on development of ONTs

Section IIB: Performing immunogenicity risk assessments

"An unwanted immune response to an oligonucleotide therapeutic can be generated to the carrier, backbone, oligonucleotide sequence, or any novel epitopes created from the whole drug (carrier plus oligonucleotide). The development of oligonucleotide therapeutics is rapidly evolving, and new chemical modifications and delivery approaches, for example, can significantly affect the immunogenicity risk and approach to clinical immunogenicity assessment of a particular product.

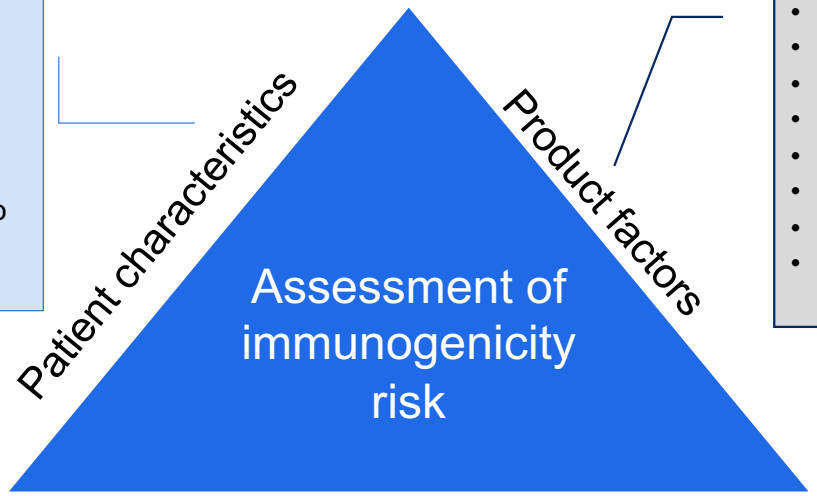
The clinical and nonclinical immunogenicity assessment for an oligonucleotide therapeutic should follow a risk-based approach and be included in a product-specific immunogenicity risk assessment as outlined in the FDA guidance entitled *Immunogenicity Assessment for Therapeutic Protein Products* (August 2014). Some considerations when determining the immunogenicity risk of an oligonucleotide therapeutic include…"

"For clinical immunogenicity assessments, where applicable, immunogenicity sample collection should coincide with pharmacokinetic and pharmacodynamic sampling time points to evaluate whether ADAs impact the pharmacokinetics, pharmacodynamics, and any immune-mediated adverse events of the oligonucleotide therapeutic. It is also important to evaluate samples to determine if the oligonucleotide therapeutic interferes with ADA testing. Of note, as determined by the immunogenicity risk assessment, it may be adequate to bank samples in early development (e.g., Phase 1/ first-in-human studies) for later testing in case evidence emerges of altered pharmacokinetics, pharmacodynamics, or immune-mediated adverse events. Sponsors should discuss their immunogenicity risk assessment and how it informs their clinical immunogenicity assessment for a particular product with the Agency.

FDA Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics (Jun 2024)

Factors impact on observed immunogenicity

- Immune activation status of the population (autoimmune or inflammatory conditions)
- Concomitant medications (e.g., immunosuppressants such as chemotherapy) that have an ability to influence the incidence or clinical impact of ADA



- Mechanism of functions
- Base sequence,
- Base modification,
- Backbone modification
- Strangeness
- Purity
- Modified nucleotides
- Secondary and tertiary structures
- Carrier components (e.g., PEGylated lipid nanoparticles) and conjugates

- Cell/tissue target,
- Expression profile,
- Route of administration
- Dosing regimen (chronic versus acute

FDA Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics (Jun 2024)
FDA Immunogenicity Assessment for Therapeutic Protein Products (2014)

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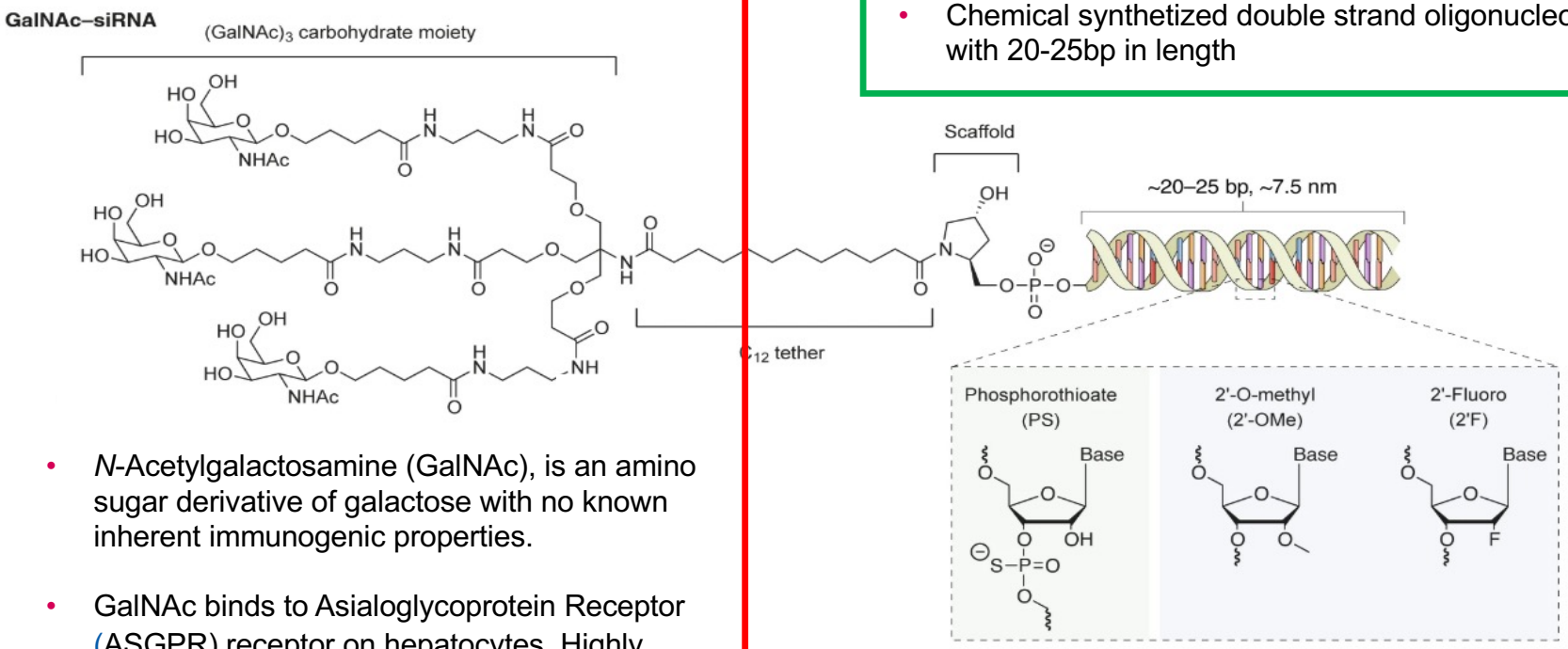
Case study: GalNAc-siRNA therapeutics

Product-related factors



- *N*-Acetylgalactosamine (GalNAc), is an amino sugar derivative of galactose with no known inherent immunogenic properties.
- GalNAc binds to Asialoglycoprotein Receptor (ASGPR) receptor on hepatocytes. Highly specific for targeted delivery to liver.
- Administrated subcutaneously (SC)

- Chemical synthetized double strand oligonucleotides with 20-25bp in length

- Modifications 2'-O-methylation and 2'-fluorination are effective strategies to minimize immunogenicity.

Liu M et al., Signal Transduction and Targeted Therapy, 10(1), 73

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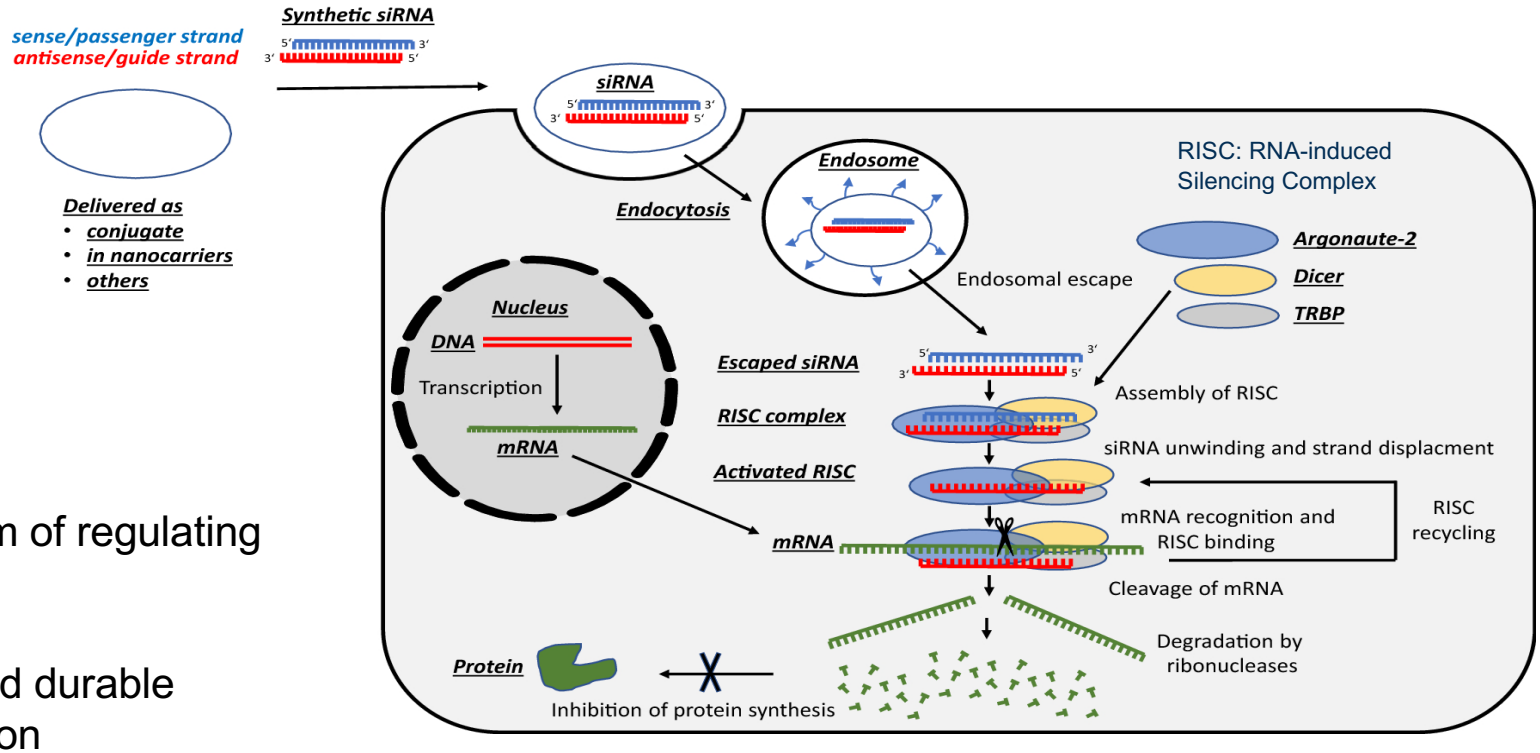
GalNAc-siRNA therapeutics

Mechanism of RNA interference (RNAi)



- Natural mechanism of regulating gene expression
- Potent, specific and durable mechanism of action

Fire A et al., Nature 391: 806-11 (1998)
Elbashir et al., Nature 411: 494-98 (2001)
Hannon, G. RNA interference. Nature 418, 244–251 (2002).

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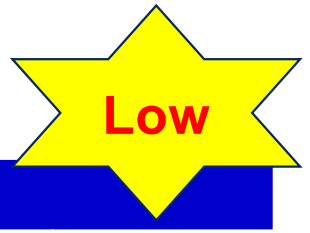
Immunogenicity of 8 Approved siRNA Therapeutics

Low immunogenicity incidence rates without meaningful impact on PK, efficacy, or safety



Information of Interest		Drug (Year of Approval)							
		Patisiran (2018)	Givosiran (2019)	Lumasiran (2020)	Inclisiran (2021)	Vutrisiran (2022)	Nedosiran (2023)	Fitusiran(2025)	Plozasiran (2025)
Indication		hATTR-PN[1]	AHP[2]	PH1[3]	HeFH[4]	hATTR-PN[1]	PH1[3]	Hemophilia A or B	FCS[5]
Route of administration		Intravenous	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous
Delivery		LNP	GalNAc	GalNAc	GalNAc	GalNAc	GalNAc	GalNAc	GalNAc
Sense/antisense length (nt)		21/21	21/23	21/23	21/23	21/23	36/22	21/23	21/21
Chemical modification		2'-O-methyl	2'-O-methyl and 2’-fluro	2'-O-methyl and 2’-fluro	2'-O-methyl and 2’-fluro	2'-O-methyl and 2’-fluro	2'-O-methyl (19) and 2’-fluro (35)	2'-O-methyl and 2’-fluro	2'-O-methyl or 2’-fluoro
Site of action		Hepatocyte	Hepatocyte	Hepatocyte	Hepatocyte	Hepatocyte	Hepatocyte	Hepatocyte	Hepatocyte
ADA	**ADA incidence rate**	3.6% (7/194)	0.9% (1/111)	6% (7/120)	4.9% (90/1830)	2.5% (3/120)	0% (0/79)	3.4% (10/290)	0% (0/50)
	Clinical Impact	Impact of ADA on efficacy/safety is not identified but not conclusive due to limited data	No clinically meaningful impact on PK, PD, efficacy, or safety	No clinically meaningful impact on PK, PD, or safety	No clinically meaningful impact on PD, efficacy, or safety, but long-term consequences are unknown	Impact of ADA on PK, efficacy/safety is not identified, but not conclusive due to limited data	No Treatment-emergent ADA response observed	There was no identified clinically significant effect of ADAs on PK, PD, safety, or effectiveness of QFITLIA	The effect of ADAs on the PK, PD, safety, and/or effectiveness is unknown.

1. Hereditary transthyretin amyloidosis polyneuropathy; 2. Acute hepatic porphyria; 3. Primary hyperoxaluria type 1; 4. Heterozygous familial hypercholesterolemia 5. Familial Chylomicronemia Syndrome

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GalNAc-siRNA immunogenicity risk assessment

Unique properties of oligonucleotides need to consider

Low

Immunogenicity Risk Factor		Brief description
product related factors	Mechanism of action	RNA interference (RNAi) pathway specifically silence target genes within cells
	Structure	Double-stranded RNAs generally are less immunogenic than single-stranded RNA.
	Modifications	2'-O-me, 2'-F and PS are effective strategies to minimize immunogenicity
	Delivery Vehicle	GalNAc is a sugar molecule with no known inherent immunogenic properties.
	Sequence liabilities	Certain sequence motif (e.g GU motif) in the siRNA sequence are associated with a higher risk of triggering immune response.
	Length	20-25bp, longer sequence tend to be more potent immune stimulator compared to the short ones.
	Source	Chemical synthetic
	Impurity	Contains low level impurities and no evidence that these impurities could impact on immunogenicity
	aggregation	Aggregation is generally well-controlled during synthesis. Minimizing aggregation helps reduce potential immune responses.

Immunogenicity Risk Factor		Brief description
Patient related factors	Immune status	Immuno-compromised individuals may exhibit a lower immunogenic response compared to those with an active immune system
	Diseases	Immune status of a disease population may affect the immunogenic response in that population
	Concomitant medications	Immunosuppressive medications generally decrease immunogenicity risk. Immunostimulatory medications generally increase immunogenicity risk.
Treatment related factors	Dose frequency	A single dose administration is associated with a lower immunogenicity risk compared to multiple dosing regimens
	Dose amount	Higher average doses can sometimes lower immunogenicity
	Route of administration	Systematic administration may provoke a stronger immune response compared to administration into immune-privileged sites

Rajeev, K.G., et al Chembiochem, 2015. ***16****(6): p. 903-8*
Watts, J.K. et al Drug Discov Today, 2008. ***13****(19-20)*
Sioud, M. et al J Mol Biol, 2005. ***348****(5): p. 1079-90.*
Judge, A.D. Nat Biotechnol, 2005. ***23****(4): p. 457-62*
Mora, J.R. et al. AAPS J, 2023. ***25****(3): p. 43*
Grudzinska-Goebel, J., et al. Front Immunol, 2025. ***16****: p. 1581153.*

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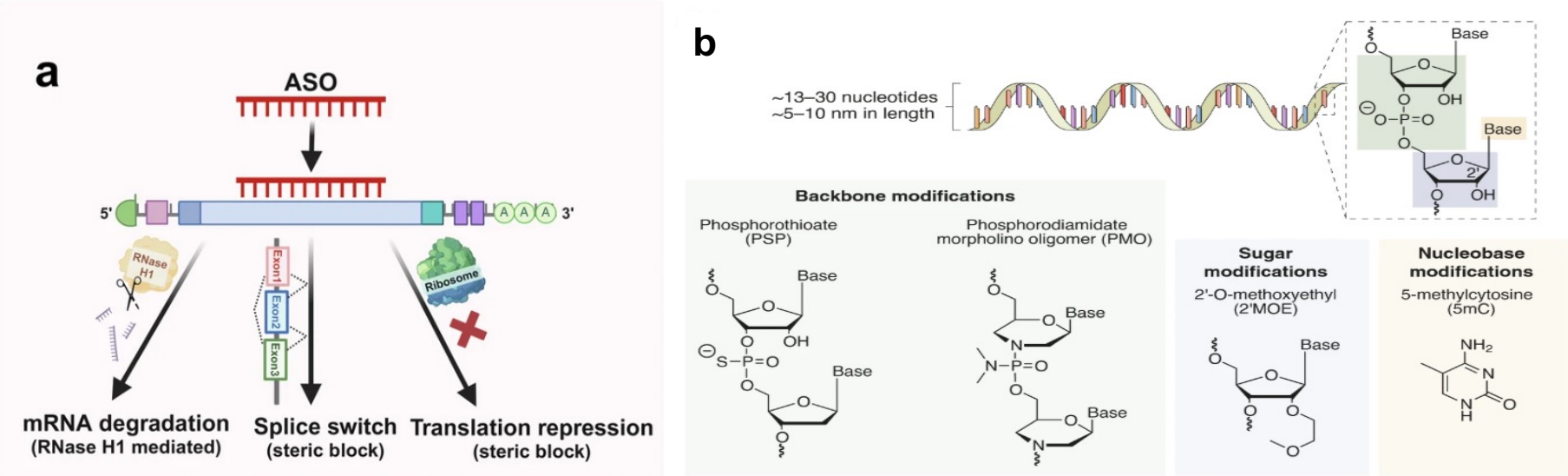
Case study: Anti-Sense Oligonucleotides (ASO) therapeutics



1. ASO is a synthetic single-stranded nucleic acid polymer of about 13-30 nt.
2. ASO often uses DNA bases or modified nucleobases (like 5-methylcytosine (5mC)) to achieve hybridization. The RNase H-dependent ASOs, for example, typically require a DNA gap region for RNase H to cleave the RNA.
3. The Phosphorothioate (PS) backbone modification (shown in panel b) is a key feature that increases nuclease resistance and improves cellular uptake, allowing some ASOs (often called "naked") to be administered systemically without delivery vehicles.

Liu M et al., Signal Transduction and Targeted Therapy, 10(1), 73

Immunogenicity of 8 Approved ASO Therapeutics (14 Approved)

Relatively high immunogenicity incidence rates with minimal clinical impact

Information of Interest		Drug (Year of Approval)							
		Mipomersen(2013, discontinued)	Viltolarsen(2020)	Casimersen (2021)	Eplontersen (2023)	Tofersen (2023)	Imetelstat* (2024)	Olezarsen (2024)	Donidalorsen (2025)
Indication		HoFH[1]	DMD[2]	DMD[2]	hATTR-PN[3]	SOD1-ALS[4]	MDS[5]	FCS[6]	HAE[7]
Route of administration		Subcutaneous	Intravenous	Intravenous	Subcutaneous	Intrathecal	Subcutaneous	Subcutaneous	Subcutaneous
Delivery		Naked	Naked	Naked	GalNAc	Naked	Naked	GalNAc	GalNAc
antisense length (mer)		20	21	22	20	20	13	20	20
Site of action		Hepatocyte	Hepatocyte	Hepatocyte	Hepatocyte	CNS	Hepatocyte	Hepatocyte	Hepatocyte
ADA	**ADA incidence rate**	38% in 6-month phase 3 trials 72% in open-label extension trial	6.25% (1/16) at weeks 13 & 24, all other weeks 0%	0%(0/12)	37% (53/144) developed ADA during 85 week treatment	58.4% (97/166)	7%(28/166)	42% (18/43)	20% - 36%
	Clinical Impact	Incidence of flu-like symptoms higher in ADA positive patients	**None**	**No** ADA	Increase C_{trough} but did **not** affect anything else	Decreases plasma clearance by 32% Safety & efficacy effects **unknown**	**no** clinically significant effect of ADA on the PK, safety, or efficacy	Increased C_{trough}, and **not** found to affect the PD, safety and efficacy	Increased C_{trough}, and **not** found to affect the PD, safety and efficacy

1. Familial hypercholesterolemia; 2. Duchenne Muscular Dystrophy; 3. Hereditary Transthyretin Amyloidosis; 4. superoxide dismutase 1-amyotrophic lateral sclerosis;
5. low- to intermediate-1 risk myelodysplastic syndromes; 6. familial chylomicronemia syndrome; 7. hereditary angioedema
* Lipid-conjugated ASO

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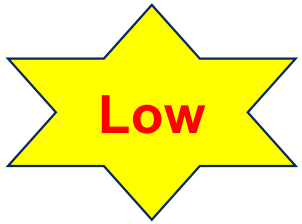
ASO Immunogenicity risk assessment



The incidence of ADA is relatively high (up to 72%)

- DNA-based ASOs (e.g., Mipomersen, Nusinersen, Eteplirsen, Inotersen) are inherently more immunogenic than RNA.
- Single stranded nature of ASOs make them more prone to interacting with pattern recognition receptors (PRRs), increasing immunogenic potential compared to double stranded siRNA.
- Many ASO drugs are administrated without a specialized delivery vehicle, relying on passive uptake or nonspecific receptor-mediated endocytosis.

Clinical impact appear to be limited

- Increased C_{trough} (or decreased clearance) level has been observed in the presence of ADA for ASOs (e.g. Eplontersen, Tofersen, Olezarsen, Donidalorsen).
- Presence of ADA has been non-conclusive or shown no impact on efficacy and safety of these therapies, likely because plasma is not the primary site of ASO action.
- Flu-like adverse effects have been observed with ASO drug Mipomersen, which is potentially linked to its immunogenicity.

Risk-based Immunogenicity assessment strategy

Immunogenicity risk assessment (pre-phase 1)	Early phase studies (phase 1&2)	Registrational studies (phase 3)
All related components: - Product-related factors - Patient-related factors - Treatment related factors - Prior experience of the same or similar drug	• **Low risk:** - Collect & bank samples. - ADA sample analysis triggered by atypical PK/PD or safety event • **Increased risk:** - Collect & analyze samples (e.g. protein-siRNA conjugate; peptide-siRNA conjugates)	• Collect & analyze ADA samples • • No NAb

Pre-IND

Phase 3

Communication with health authorities

Potentially collect and bank strategy can be applied throughout the clinical development for low-risk molecules.

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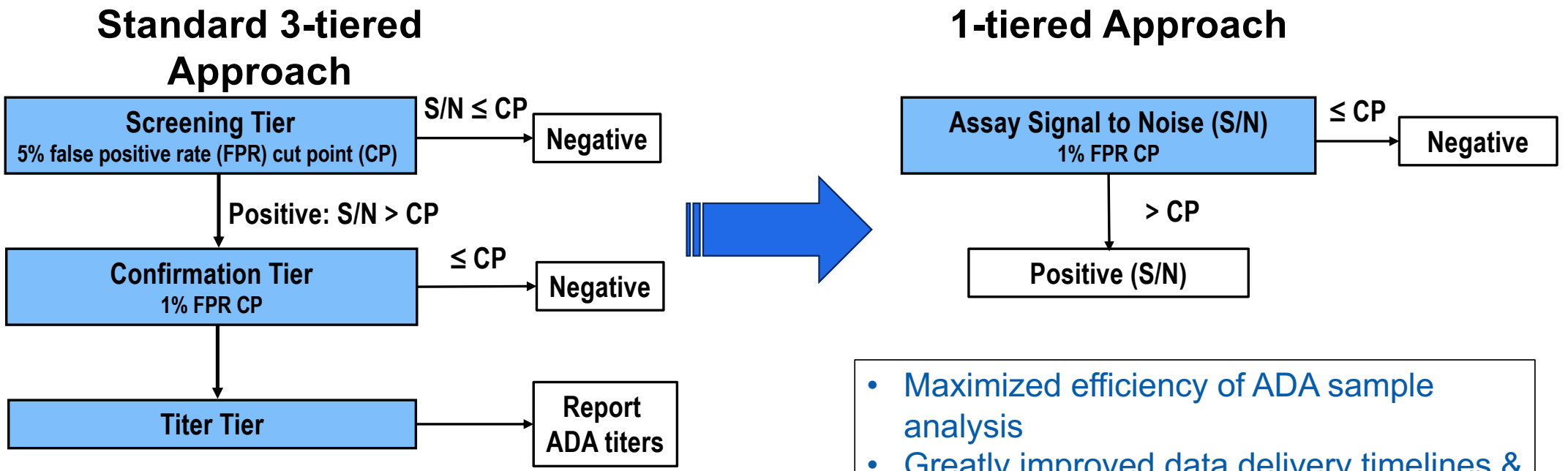
Implement 1-Tiered ADA Testing Approach



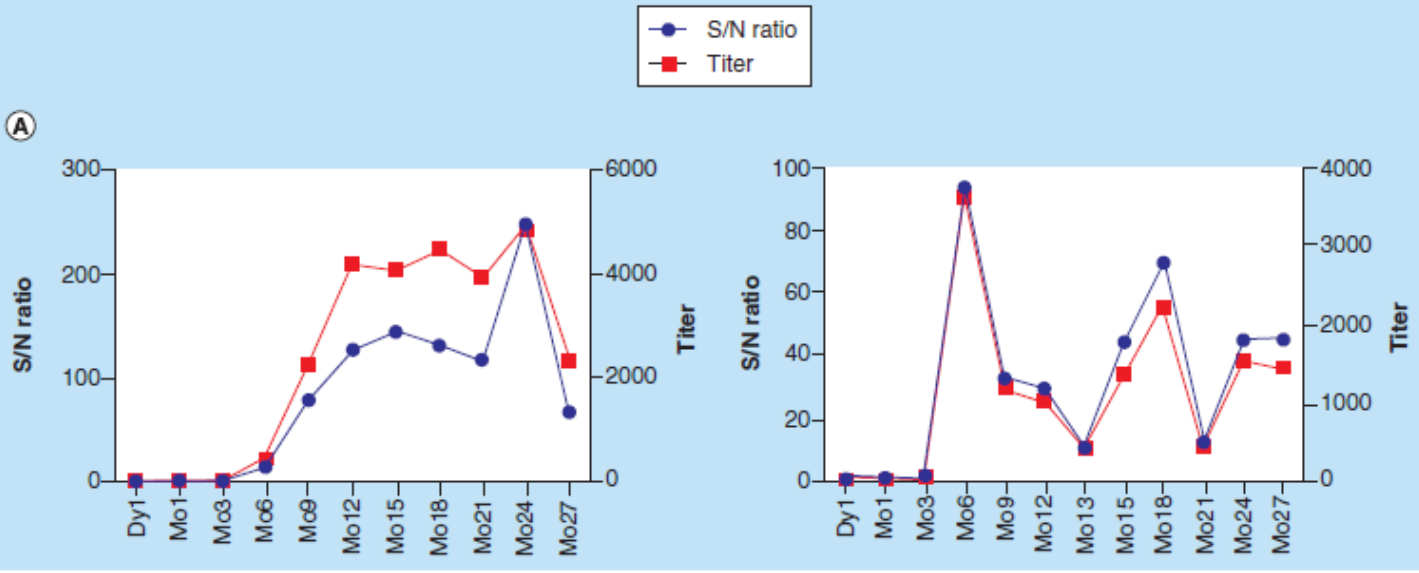
- Maximized efficiency of ADA sample analysis
- Greatly improved data delivery timelines & minimized resource utilization (~50% reduction)
- Simplified ADA data reporting
- Minimal/No impact on fidelity of data

S/N: the ratio of the sample response divided by the negative control (or background) response

Published Data to Support the Use of S/N

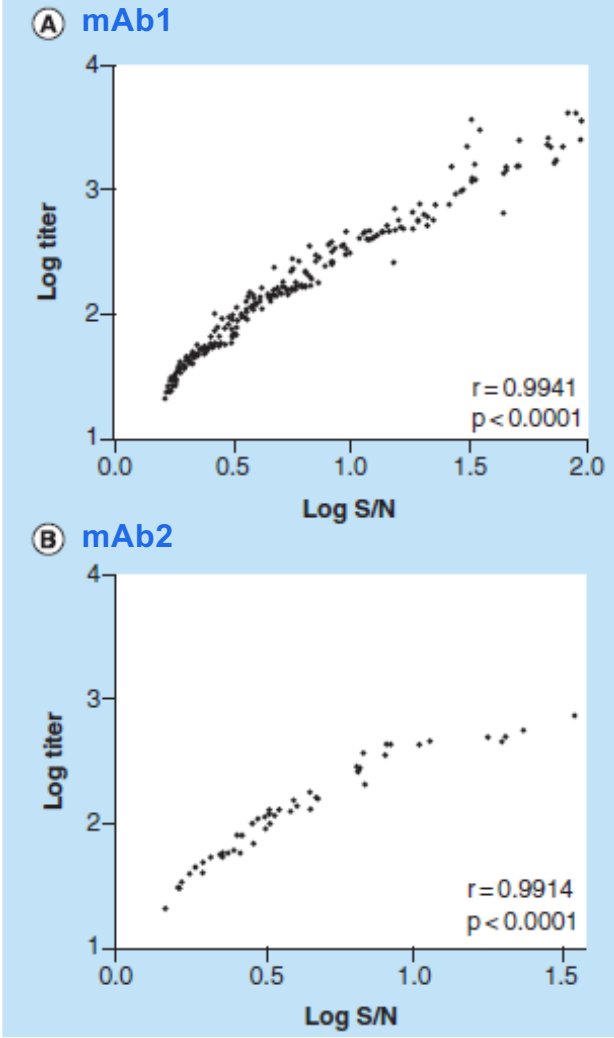
S/N and Titer Data are Strongly Correlated

Individual Subject Profiles for Subjects with the Highest S/N Values for mAb1



Conclusions: S/N correlated strongly with titer and produced similar subject ADA profiles.

Correlation of S/N and Titer in Samples from Phase II Clinical Studies



Manning, MS. *et al.* Bioanalysis (2017), 9(23), 1849-1858.

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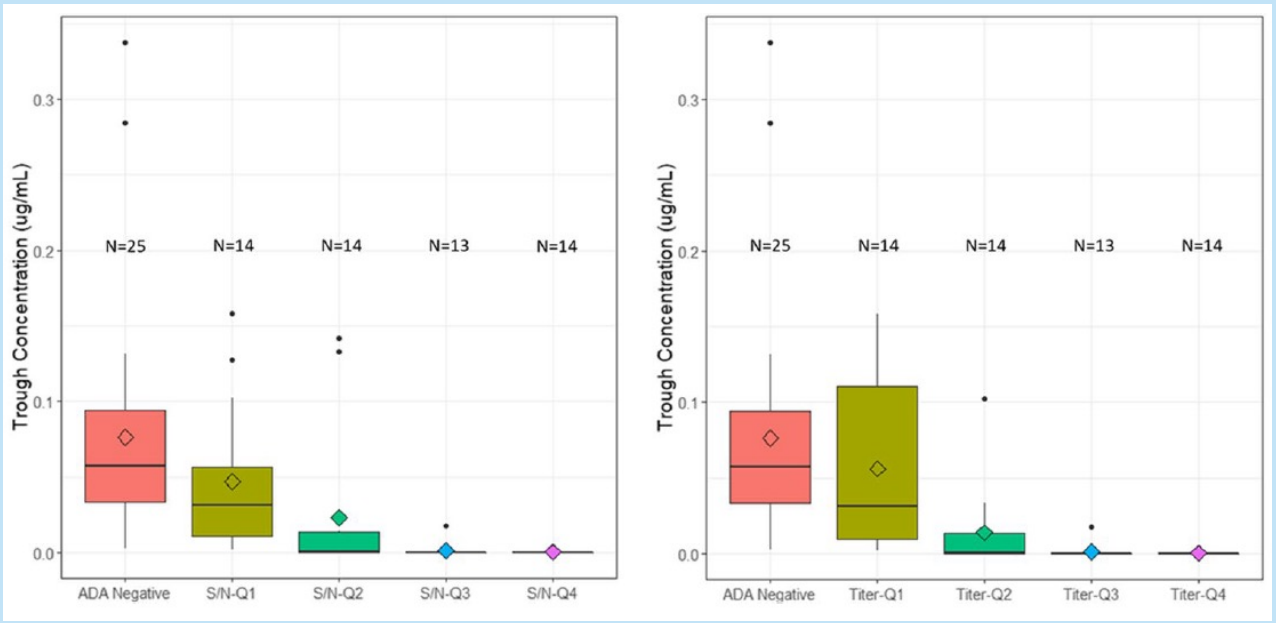
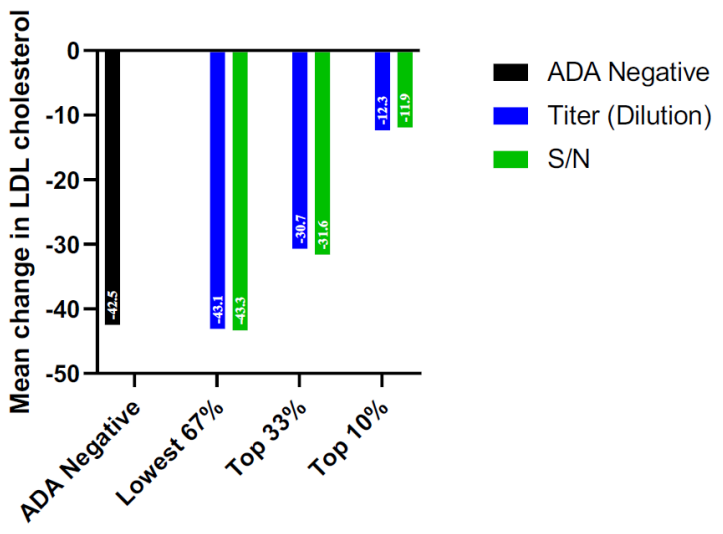
Published Data to Support the Use of S/N

S/N May Be Used Instead of Titer to Assess Effect on PK/PD Endpoints

Impact of ADA Magnitude on Dose-Normalized Drug Mean Trough Concentrations



Similar Effect of Bococizumab ADA on the Durability of LDL Cholesterol Lowering Using Titer or S/N



Conclusions: Use of S/N does not change the interpretation of the impact of ADA on PK/PD endpoints.

Manning, MS. *et al.* AAPS J (2022), 24(81).

McCush, F. *et al.* AAPS J (2023), 25(85).

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Discussion and forward-looking proposal…

1. **Innate immune response - primary trigger**
   - Flu-like symptom – cytokine release
   - Local inflammation (e.g. injection site reaction)

2. **Adaptive immune response - low clinical impact**
   - GalNAc-siRNA have shown a low incidence of ADA and no discernible impact on PK, PD or safety
   - ASO have shown a relatively high ADA incidence but minimal impact on efficacy and safety in clinical studies.

3. **The risk-based immunogenicity assessment strategy**
   - For low-risk molecule (e.g. GalNAc siRNA, ASO), “collect and hold” strategy can potentially be justified throughout the entire clinical development process.
   - Molecules that utilized novel and complex delivery systems, such as siRNA-peptide or siRNA-protein conjugates may pose a higher immunogenic risk.

4. **Streamline sample analysis by using 1-tiered ADA testing and no Nab assessment strategy**

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- BAS colleagues

Alnylam®

- Sarah Bond
- Valerie Clausen

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Questions?

Key takeaway: Minimizing immunogenicity assessment: a risk-based proposal for event driving testing