Immunological challenges associated with nucleic acid-based therapeutics

Fiscal Year (FY) 2025 Generic Drug Science and Research Initiatives Public Workshop
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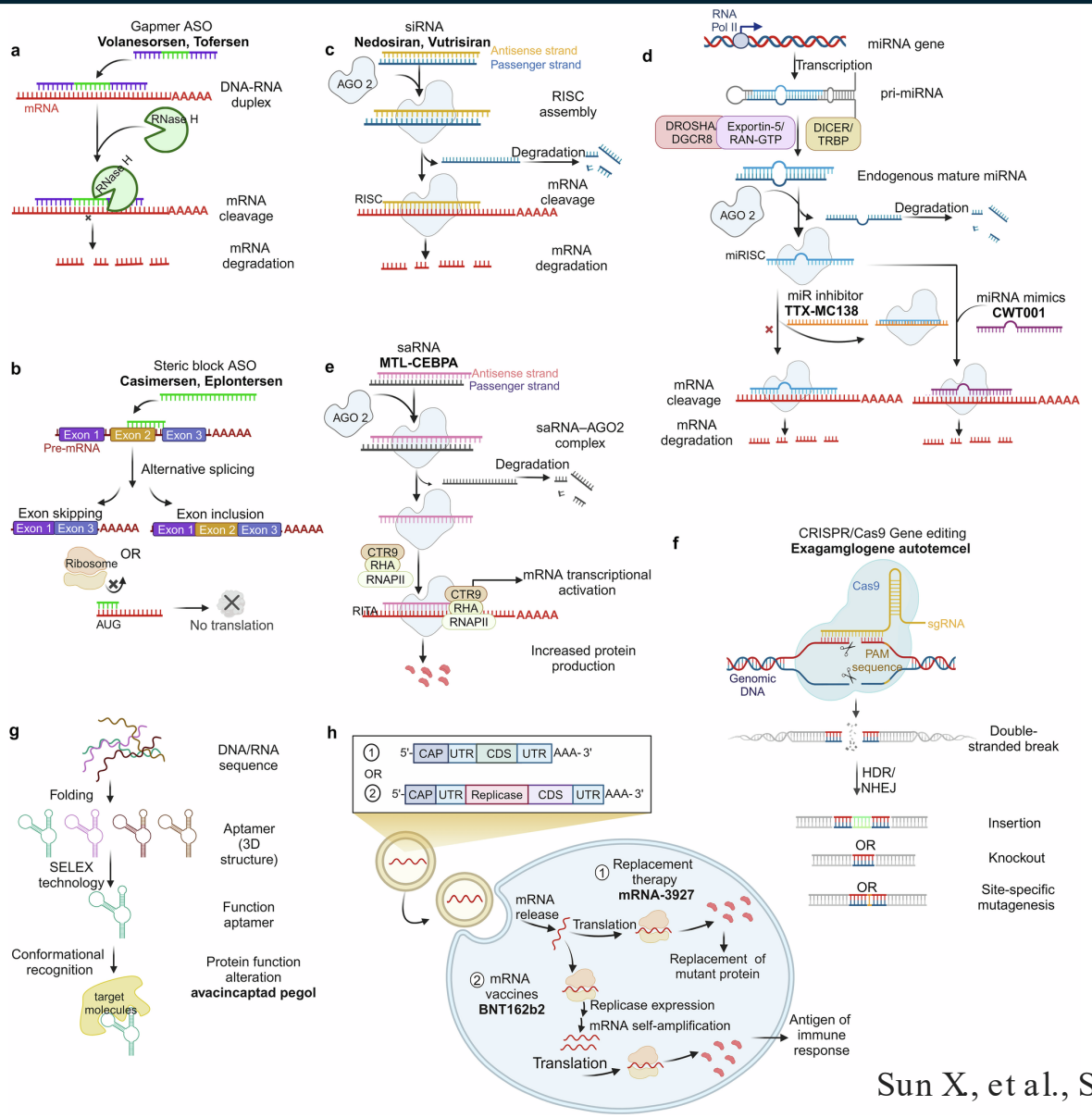
Nucleic acid-based drugs and their mechanism of action

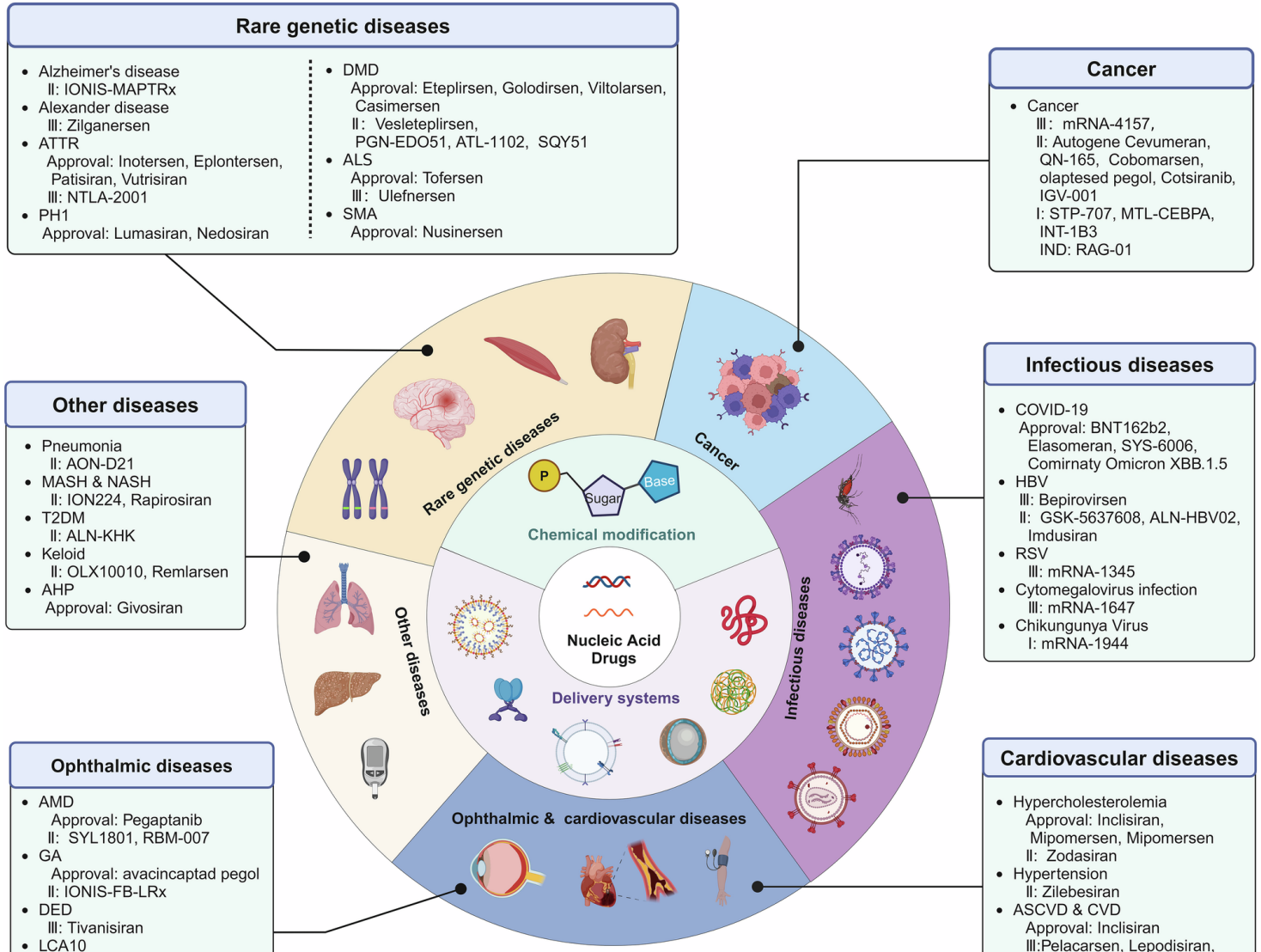


Clinically approved therapy

- ASOs
- siRNA
- mRNA vaccines
- Aptamer
- CRISPR

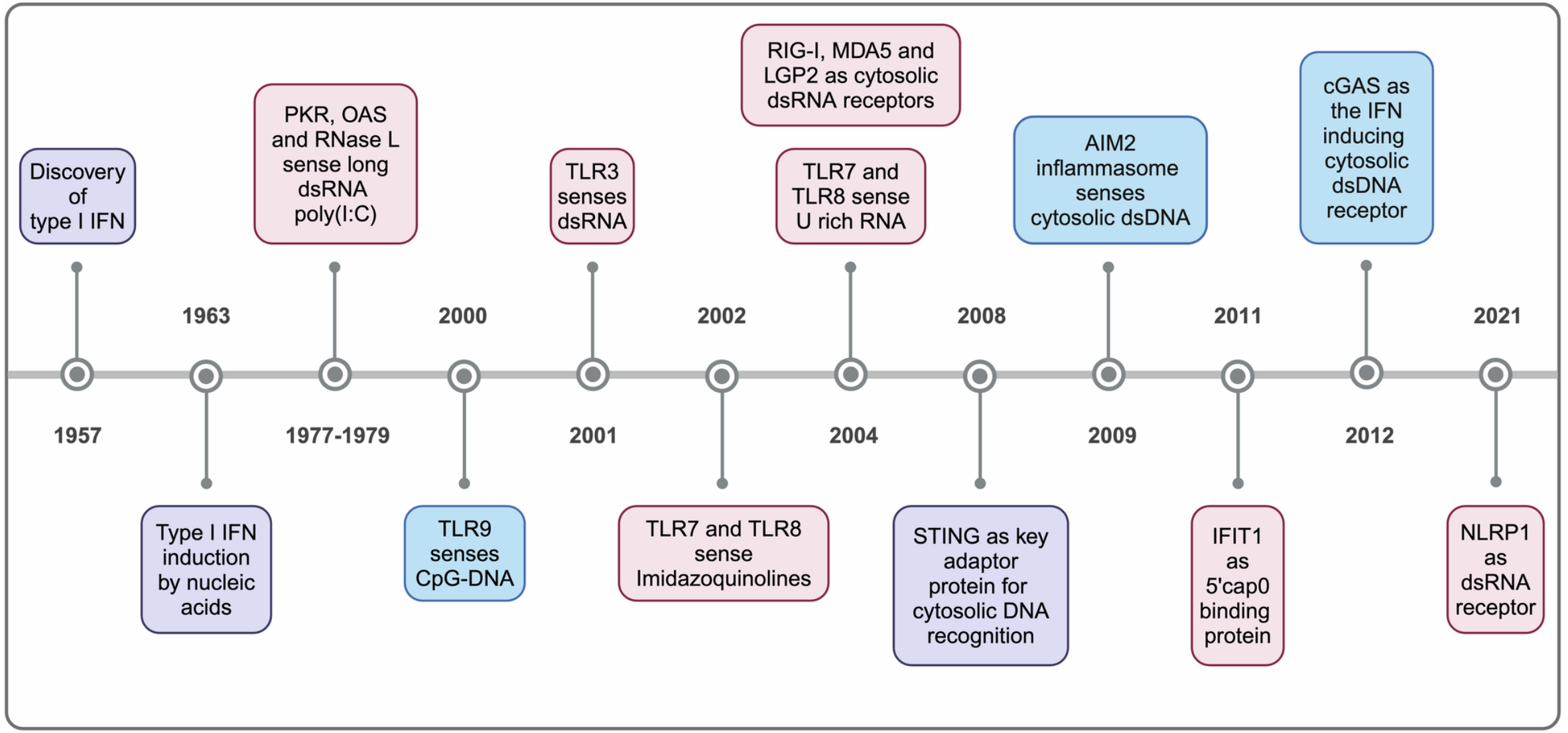
Sun X., et al., Sig Transduct Target Ther, 2024.

Therapeutic applications of nucleic acid drugs



Sun X, et al., Sig Transduct Target Ther, 2024.

Discovery of nucleic acid sensing mechanisms



Significant discoveries: fundamental DNA sensors (blue), RNA sensors (red) and key signaling proteins (purple)

Chen Z., et al., Sig Transduct Target Ther, 2025.

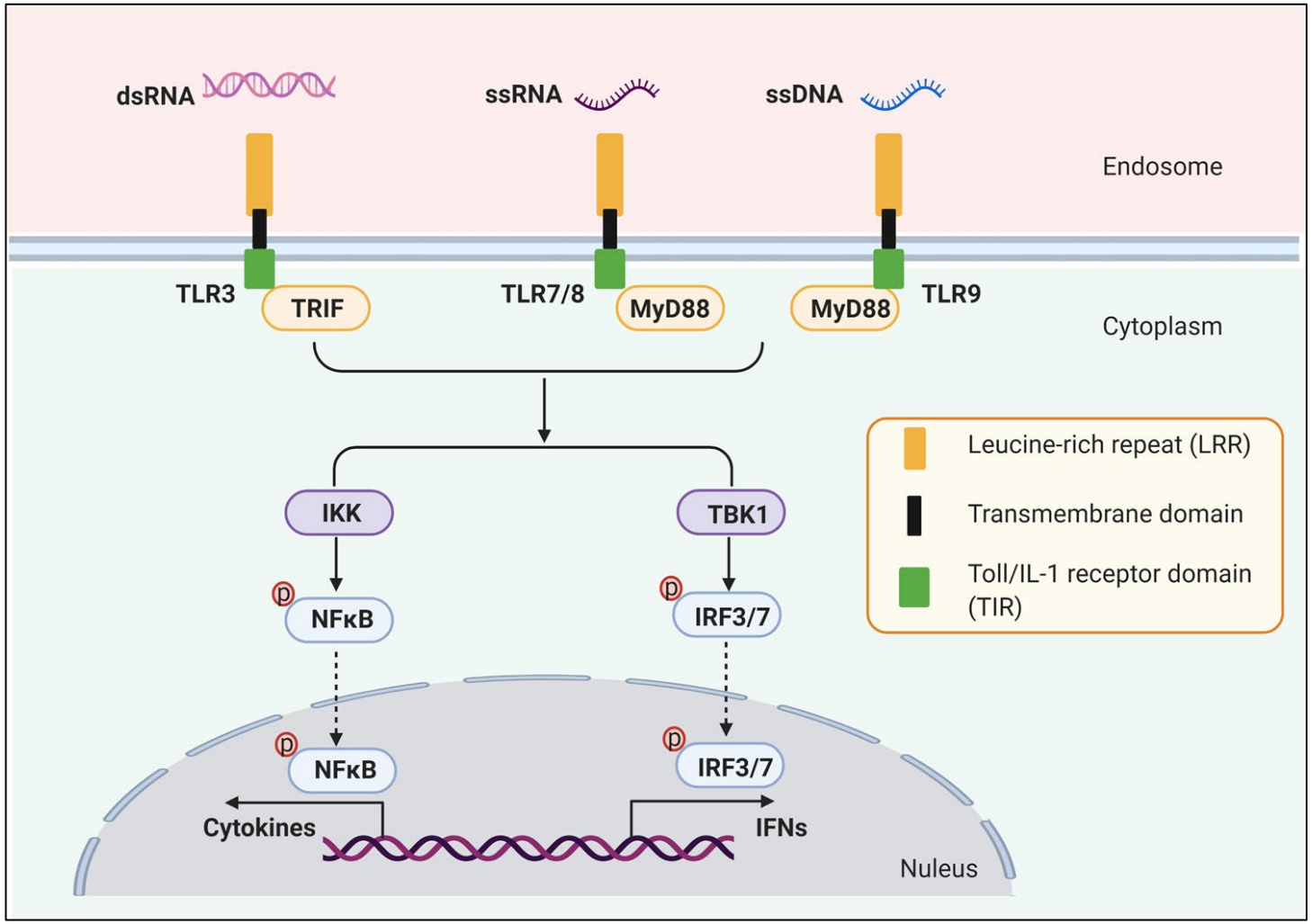
Mechanism of nucleic acids-mediated immune response

Receptor mediated recognition

Toll like receptors

- TLR3:dsRNA
- TLR7/8:ssRNA
- TLR9:ssDNA

Immunological cascade of events



Chen M., et al., Sig Transduct Target Ther, 2020.

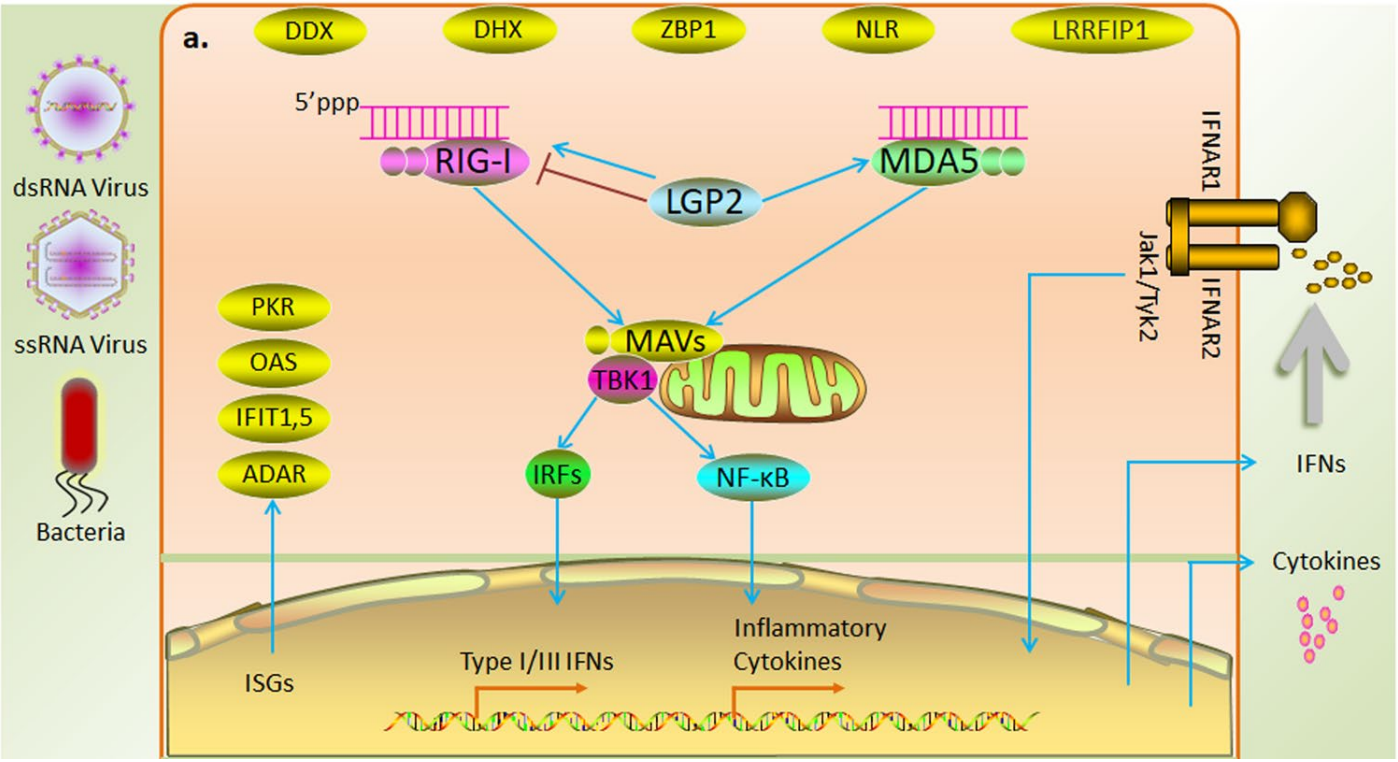
Cytosolic sensing of nucleic acids

In addition to immune cells (Dendritic cells, Macrophages, Neutrophils, NK cells), epithelial cells and fibroblasts on the mucosal surface can stimulate an effective innate immune response.

Cytoplasmic sensors

- RLS family (RIG-1, MDA-5, LGP2): RNA
- cGAS/IFI16-STING: DNA
- AIM2: dsDNA
- Cyclic GMP-AMP: DNA

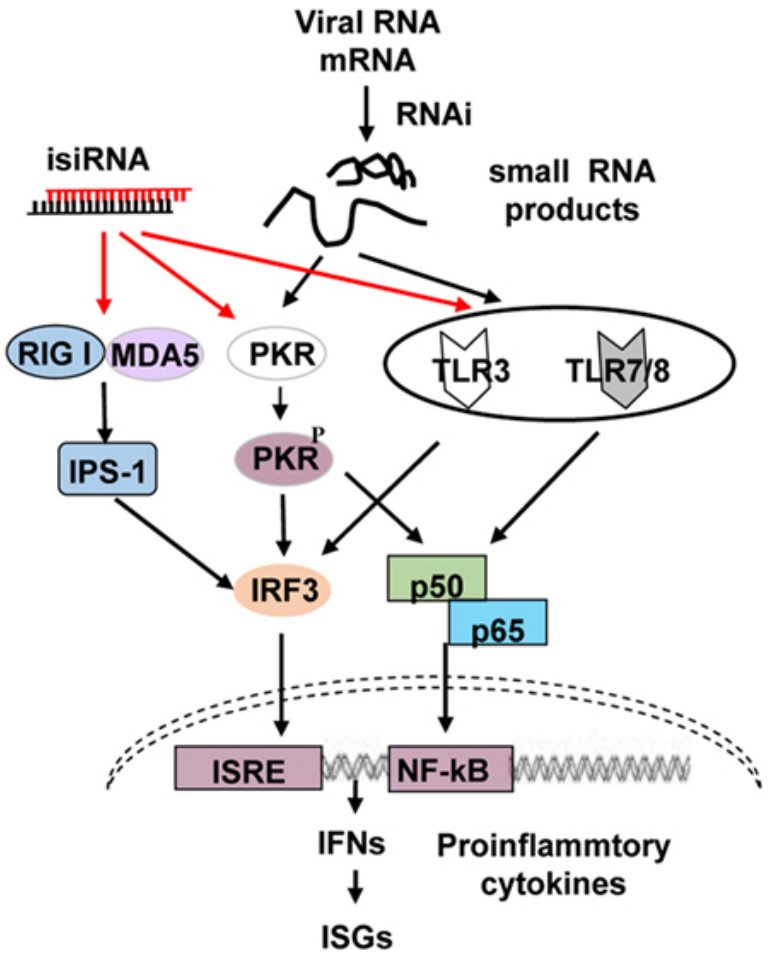
Cytosolic RNA sensing and regulation mechanisms



Kong LZ., et al., Exp Mol Med, 2023; Chen Z., et al., Sig Transduct Target Ther, 2025.

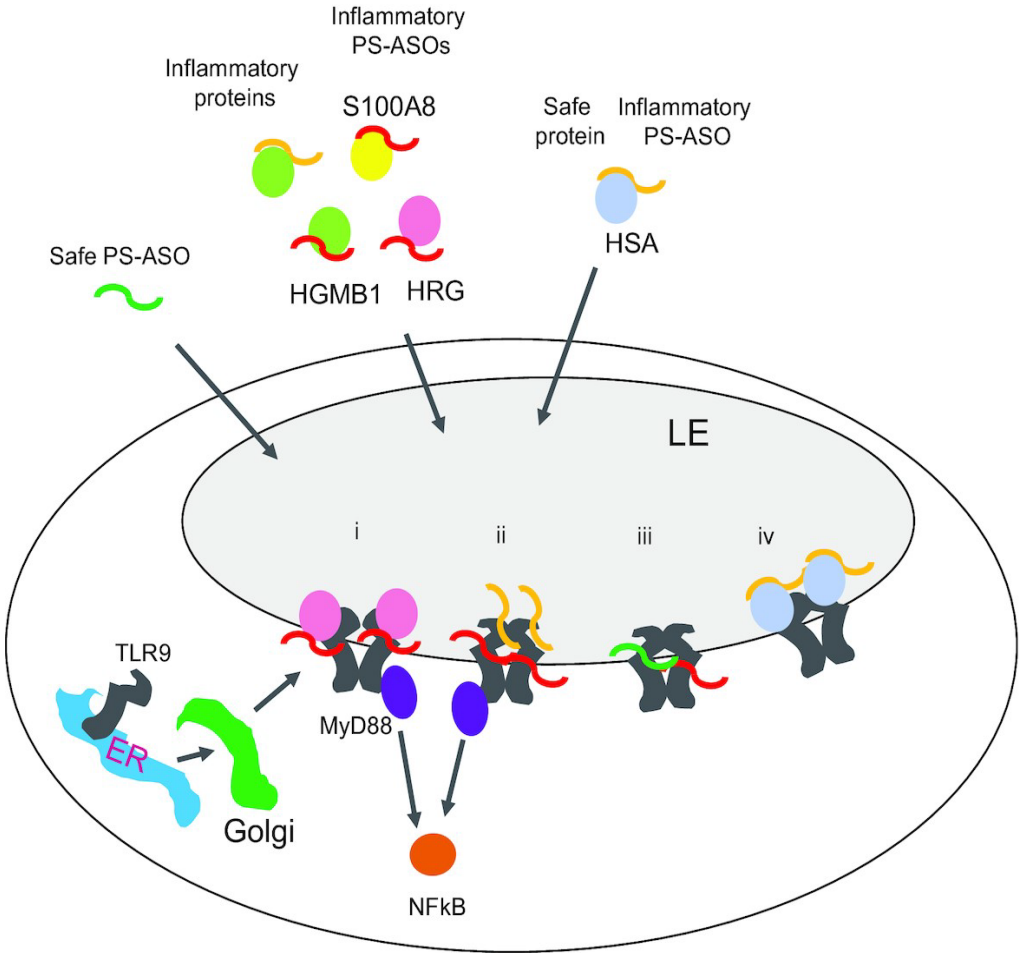
Oligonucleotides mediated innate immune response

siRNA can activate RIG-I/MDA5 pathway or TLR3



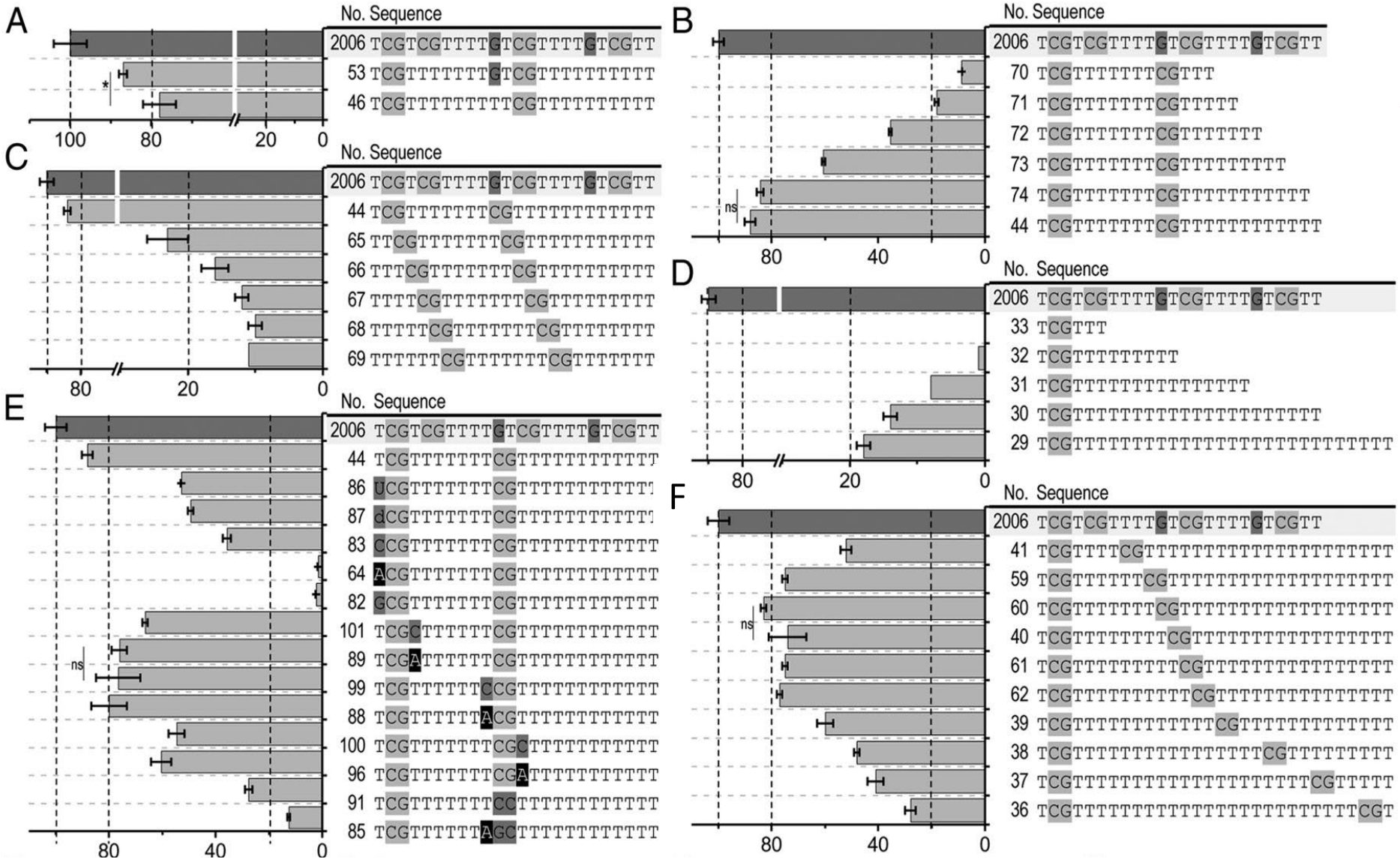
Meng Z., & Lu M., Front. Immunol., 2017

PS-ASOs can directly act on TLR9 or interact with inflammatory proteins



Pollak AJ., et al., Nucleic Acids Res, 2022

Oligonucleotides' features affecting immune response

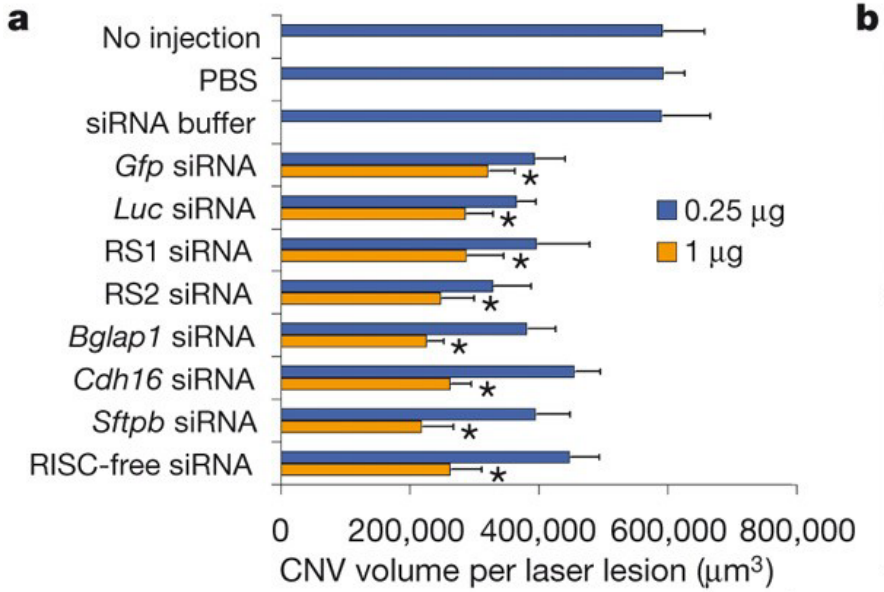


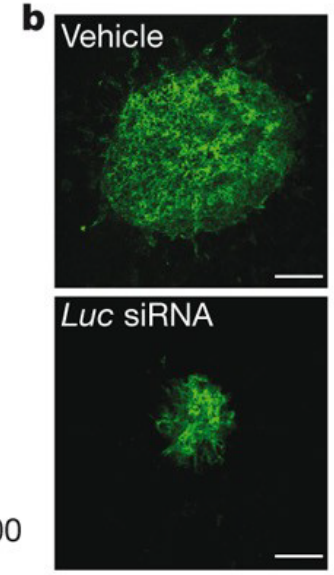
5' CpG motif and an adjacent thymidine group leads to TLR9 stimulation

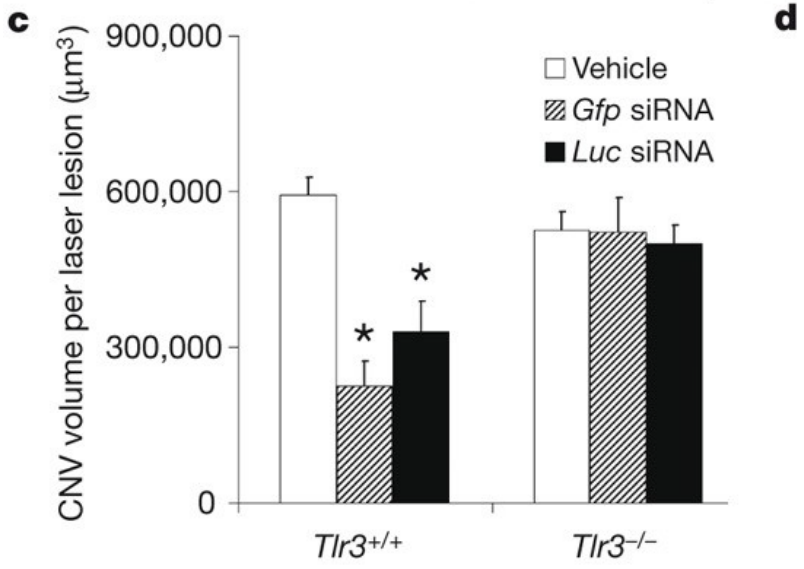
A. CpG motifs. B. 3' T-tail length. C. 5' T-tail length. D. Oligo length with one CpG motif. E. Nucleotides adjacent to CpG. F. Position of CpG motifs.

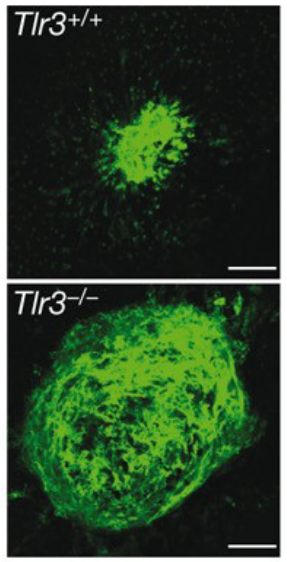
Pohar J., et al., J Immunol, 2015

Sequence independent immune response









Sequence- and target-independent angiogenesis suppression by siRNA via TLR3

siRNA's sequence-independent immune response is mediated through TLR3.

Guanine- and uridine-rich siRNAs are more prone to binding to TLR7/8, making them sequence-specific.

Kleinman M., et al., Nature, 2008

Chemical modifications to overcome immune response

1. Nucleosides modifications

Pseudouridine (Ψ)

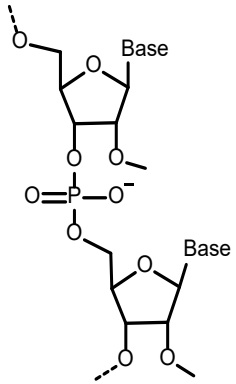
N1-Methyl-pseudouridine (m1Ψ)

2'-Thiouridine (s2U)

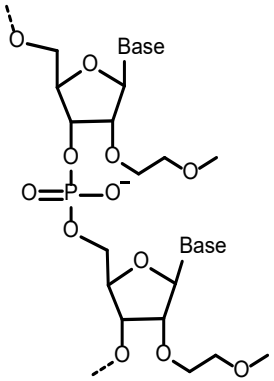
5-Methyl-cytidine (m^5C)

2. 5' Cap in mRNA

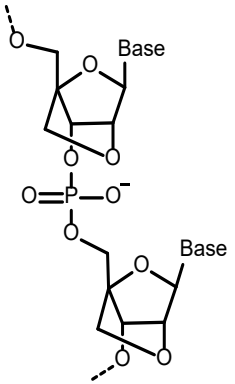
3. Sugar modifications



2'-O-Methyl (2'-OMe)

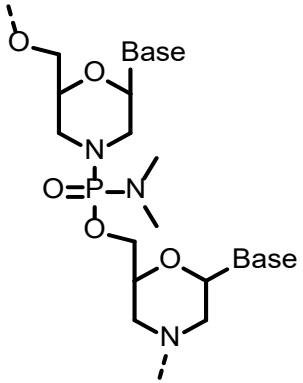


2'-O-Methoxyethyl (2'-MOE)

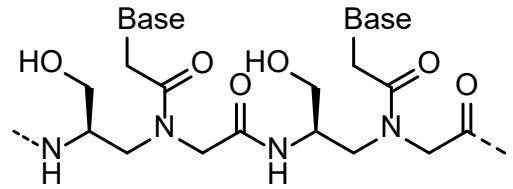


Locked nucleic acid (LNA)

4. Alternate backbone chemistry



Phosphorodiamidate morpholino oligomers (PMO)

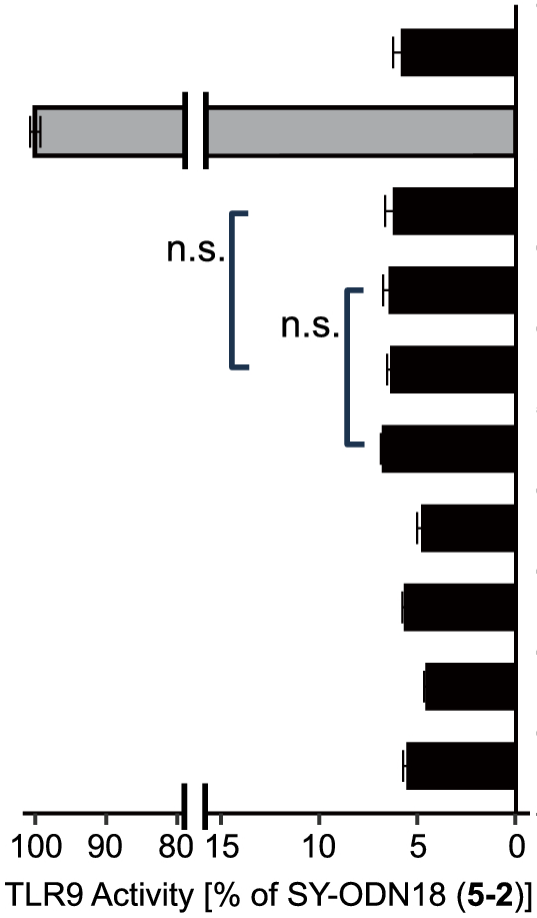


Peptide nucleic acid (PNA)

Shi Y., et al., Chem Rev, 2024

Modified oligonucleotides confer immune resistance

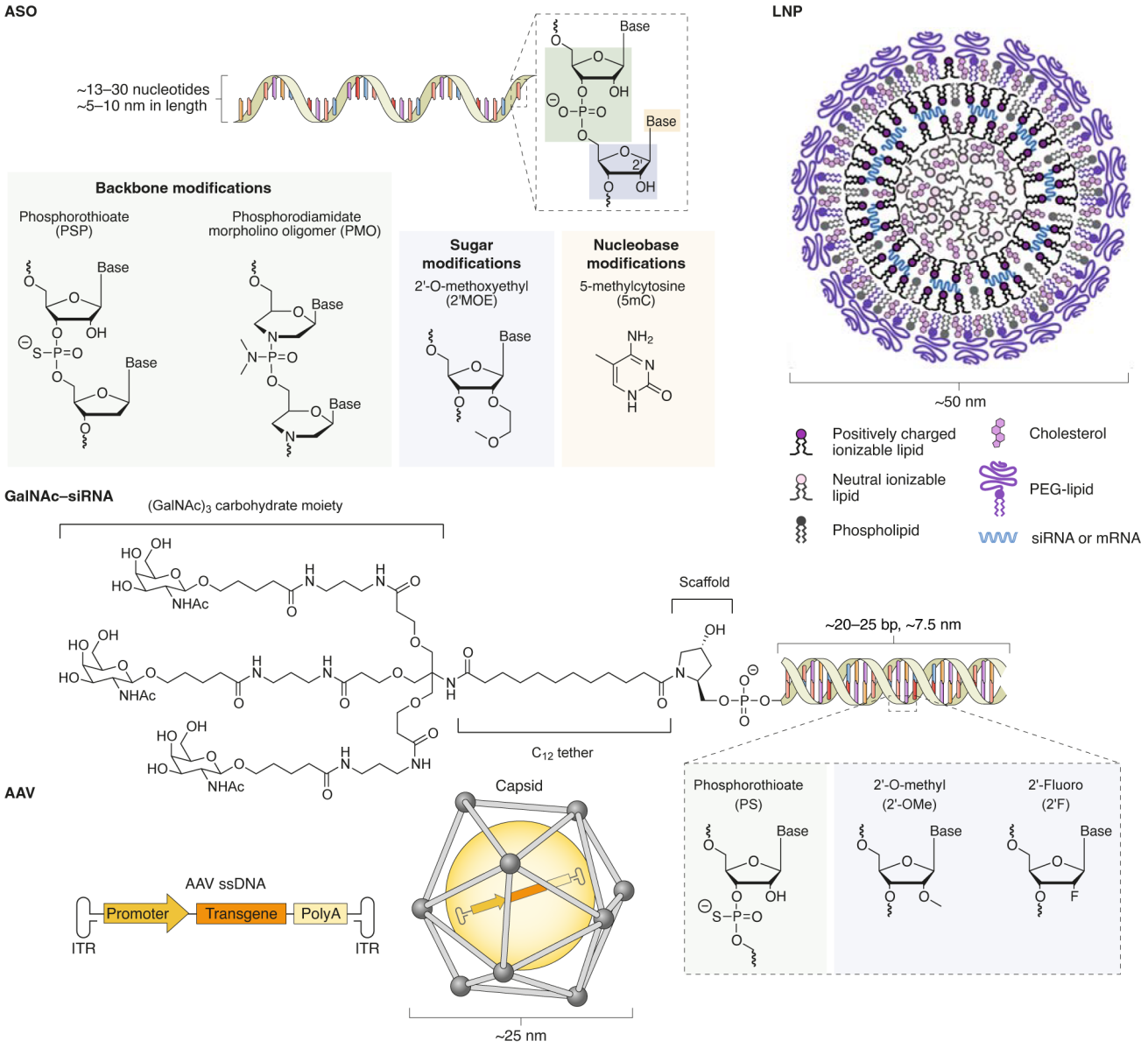
Sugar modifications in CpG-containing oligonucleotides result in reduced TLR9 activation.



No	CpG motif	Type	Sugar-modified nucleotides	Sequences (5′→3′)
5-1	–	–	–	–
5-2	1CG-2CG	–	–	TCGTTTTGTCGTTTTGTC
5-3	1CG-2CG	Fully modified	2′-OMe	TCGTTTTGTCGTTTTGTC
5-4	1CG-2CG	Fully modified	2′-MCE	
5-5	1CG-2CG	Mixmer	2′-OMe	
5-6	1CG-2CG	Mixmer	2′-MCE	
5-7	1CG-2CG	Mixmer	2′-MOE	TCGTTTTGTCGTTTTGTC
5-8	1CG-2CG	Mixmer	LNA	
5-9	1CG-2CG	Mixmer	ENA	
5-10	1CG-2CG	Mixmer	BNA^{NC} (N-Me)	

Yoshida T., et al., Sci Rep, 2024

Nucleic acid delivery methods



ASOs
Local delivery | Nuclease susceptibility | Immune activation

LNPs
Nuclease stability | Successful mRNA delivery| Immune activation via carrier components | Limited tissue-specific targeting

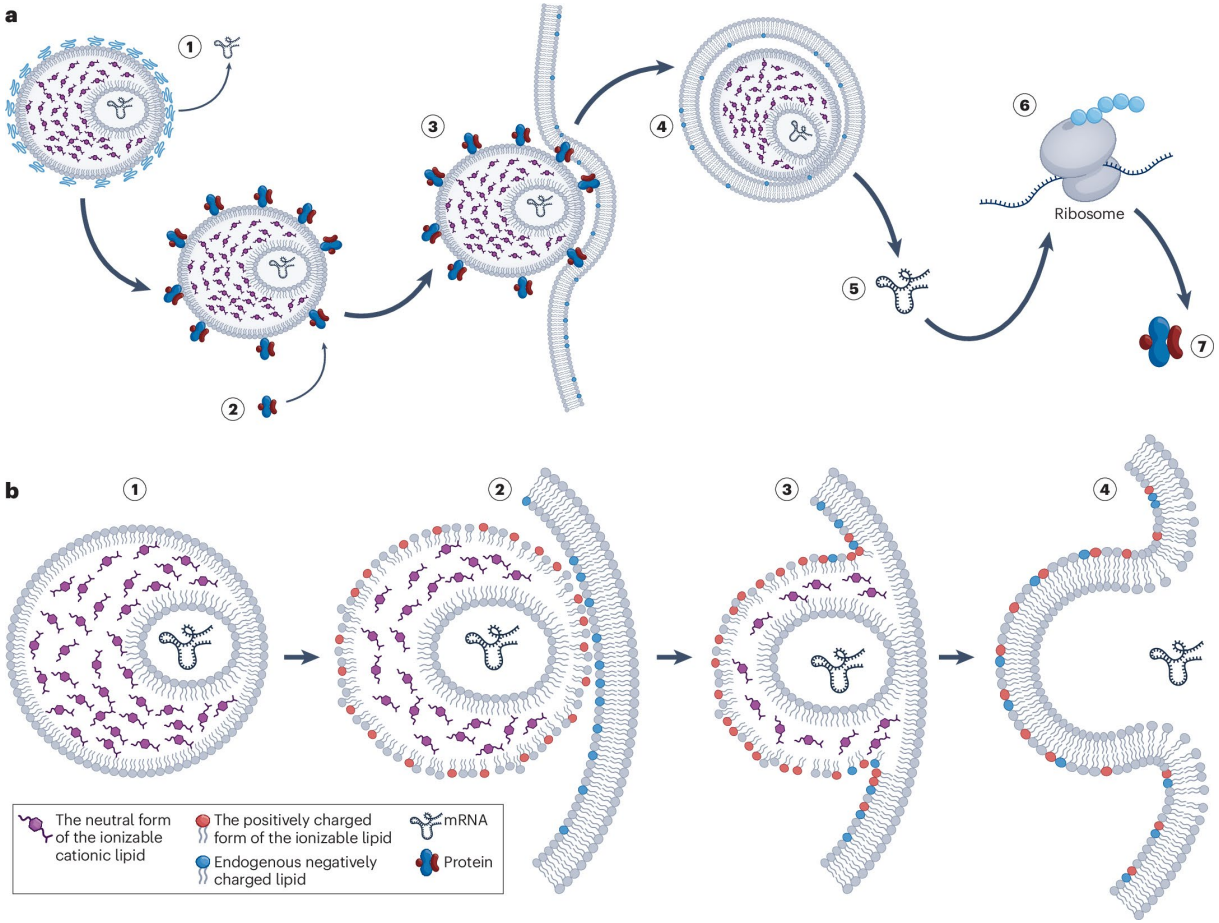
Bioconjugates
Enables cell/tissue-specific delivery | Allows synthetic and formulation flexibility | May trigger immune activation | Demonstrated clinical success

AAV vectors
Gene delivery platform | High transduction rates and broad tropism | Limited DNA loading (<5kb) | May trigger immune response

Kulkarni JA., et al., Nat Nanotechnol, 2021

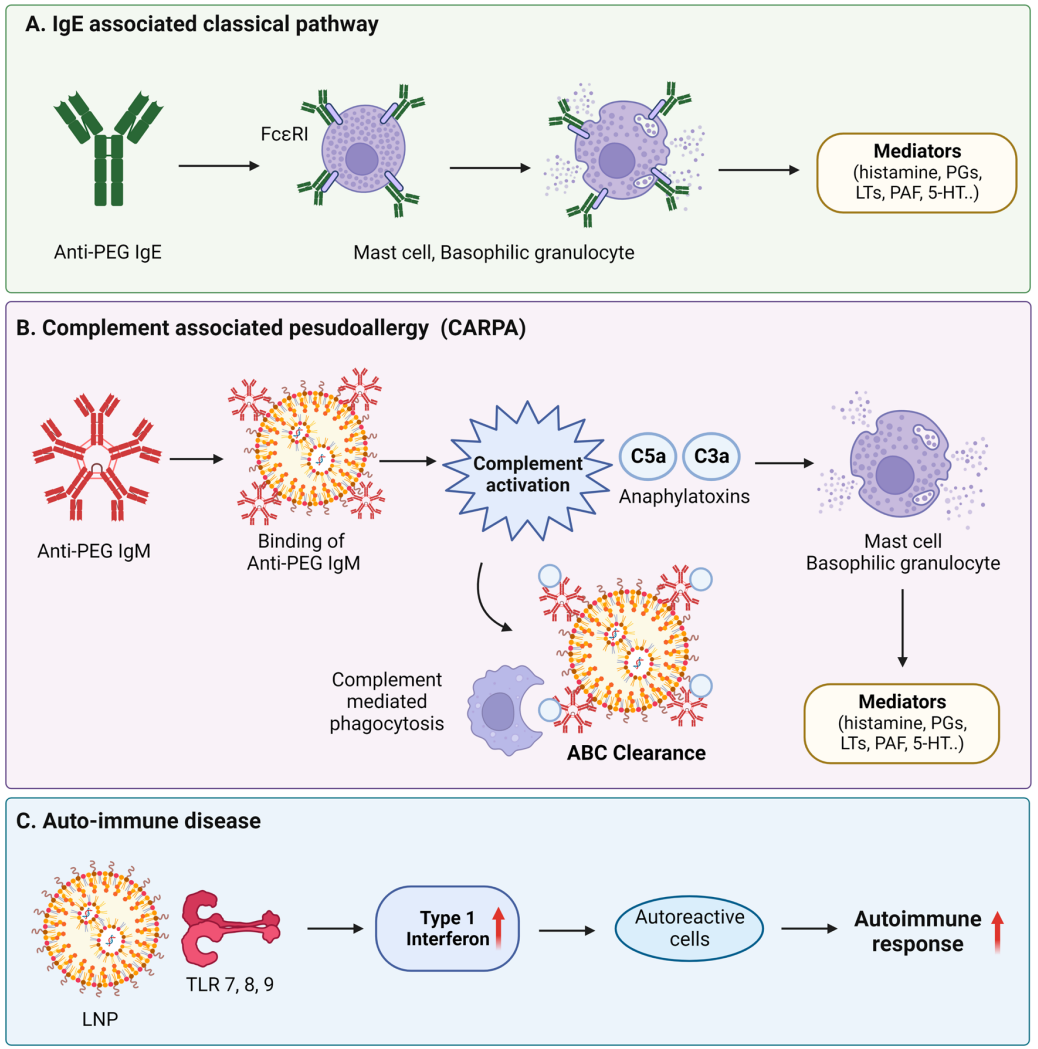
Lipid nanoparticles (LNP) as nucleic acid delivery carriers

LNP-mediated mRNA delivery



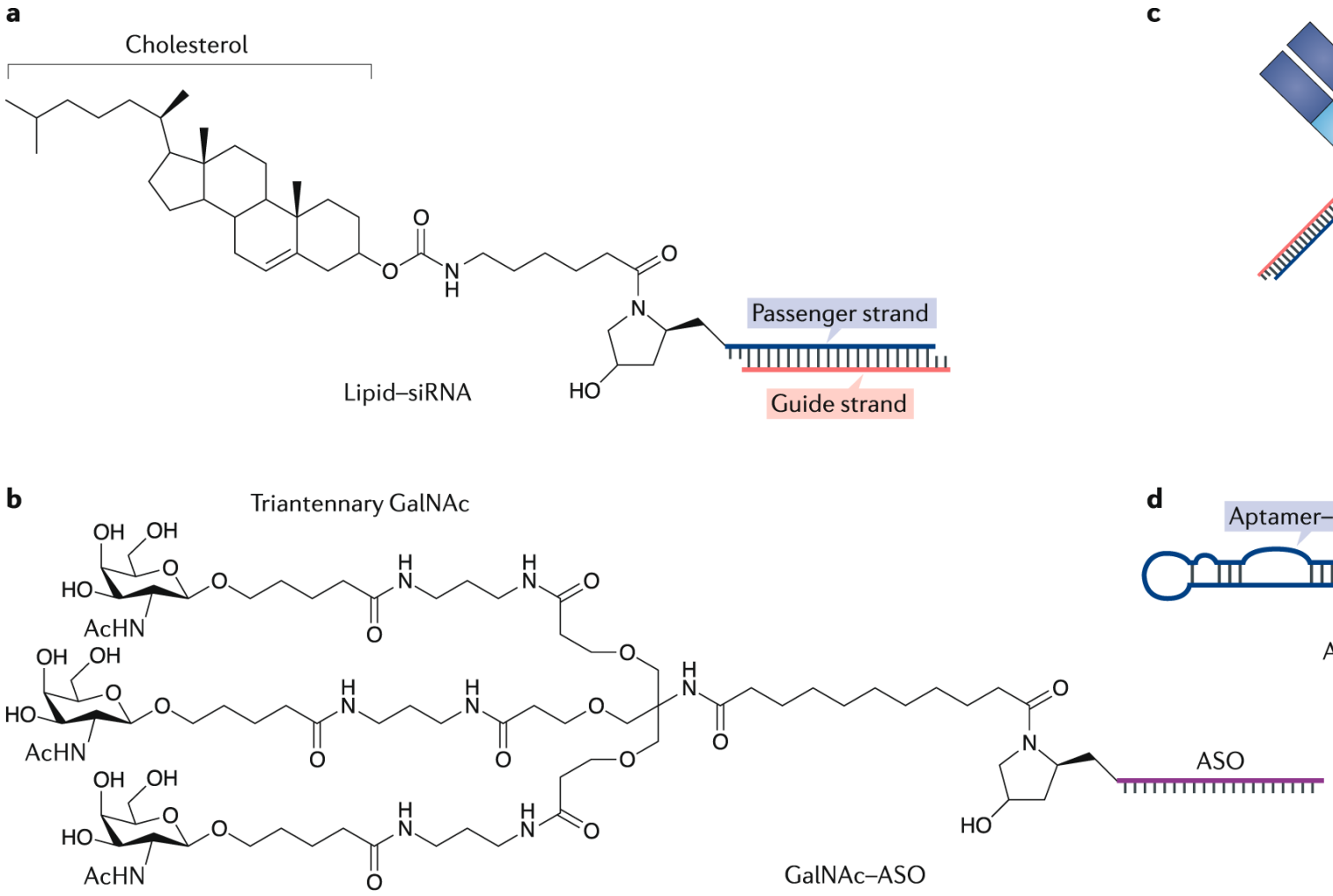
Cullis PR., and Felgner PL., Nat Rev Drug Discov, 2024.

LNP-mediated immune response



Lee Y., et al. Exp Mol Med, 2023.

Bioconjugates mediated nucleic acid delivery

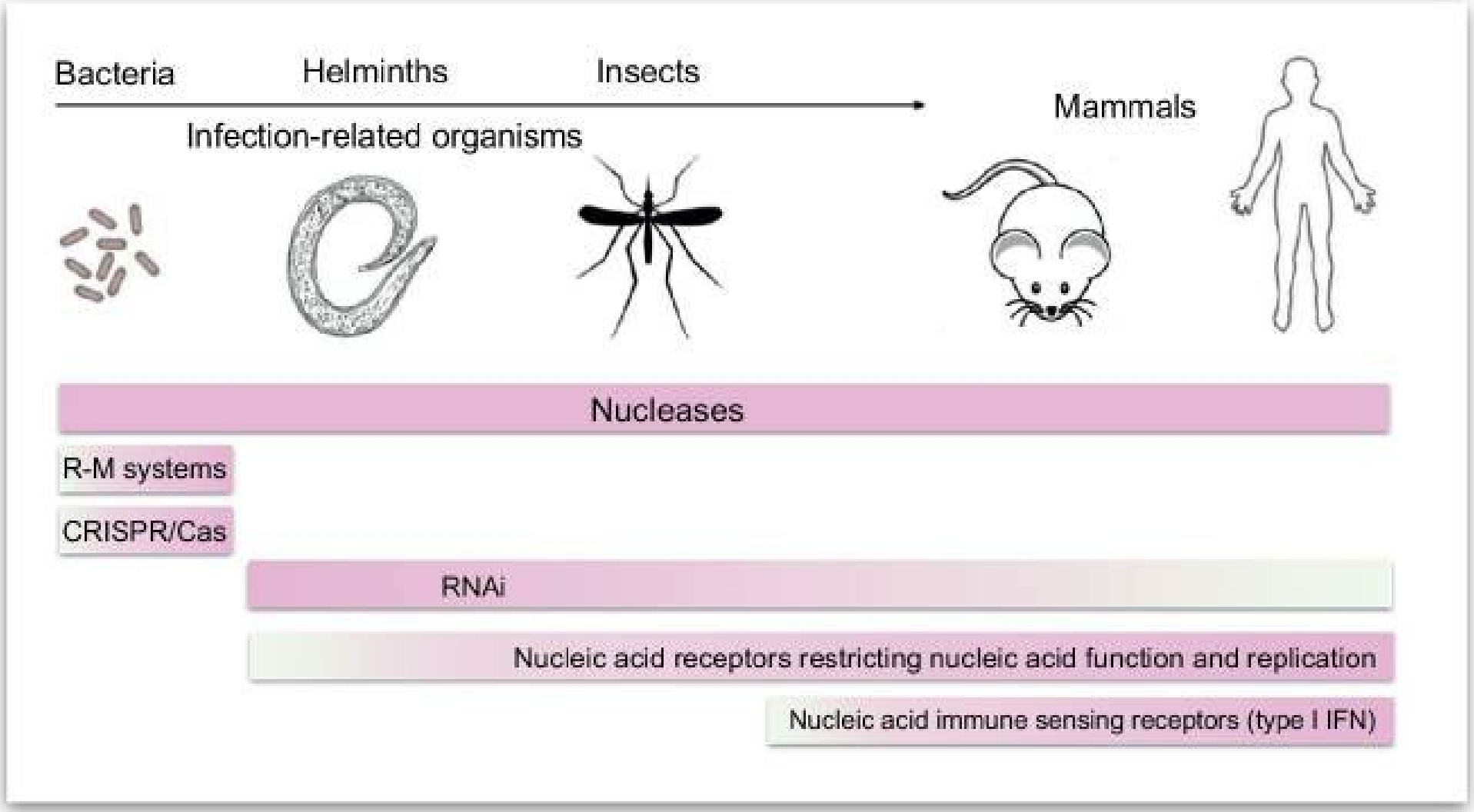


Advantages: Targeted delivery, lesser off target effects, but can stimulate immune response

Roberts TC., et al., Nat Rev Drug Discov, 2020

Considerations of nucleic acid-mediated immune activation

Immune responses are unique to each species.



Hartmann G., Adv Immunol, 2016.

Methods to detect nucleic acid mediated immune activation

Innate immune system activation:

TLR engagement: TLR3, TLR7/8, TLR9
Inflammasome activation

Complement activation

Immunostimulatory effects:

Panel of cytokines by ELISA or western blot analysis in PBMC cells or plasma following in vivo treatment.

mRNA levels of cytokines by qPCR.

Activation of Immune cells:

Proliferation / activation-based assays

Lymphocyte proportion by flow cytometry

Detection timelines:

Innate immune response develops in minutes to hours.

Adaptive immune response develops from days to weeks.

Andersson P., Antisense RNA Design, Delivery, and Analysis, 2022; Goyenvalle A., et al., Nucleic Acid Ther., 2023; https://www.fda.gov/media/169117/download.

Summary

- The immune system outcompetes us! A comprehensive evaluation is needed for a specific oligonucleotide-based drug product.
- Delivery systems associated with specific oligonucleotides introduce an additional layer of complexity for assessing the immune response.
- Challenges exist in transitioning rodent studies to predict human results. However, there is room for early observation at both the cell culture and preclinical levels to assess the immune response to both oligonucleotides alone and their delivery system.