PERSPECTIVES FOR NEW THERAPIES OF RARE INHERITED NEUROLOGICAL DISEASES

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Learning objectives

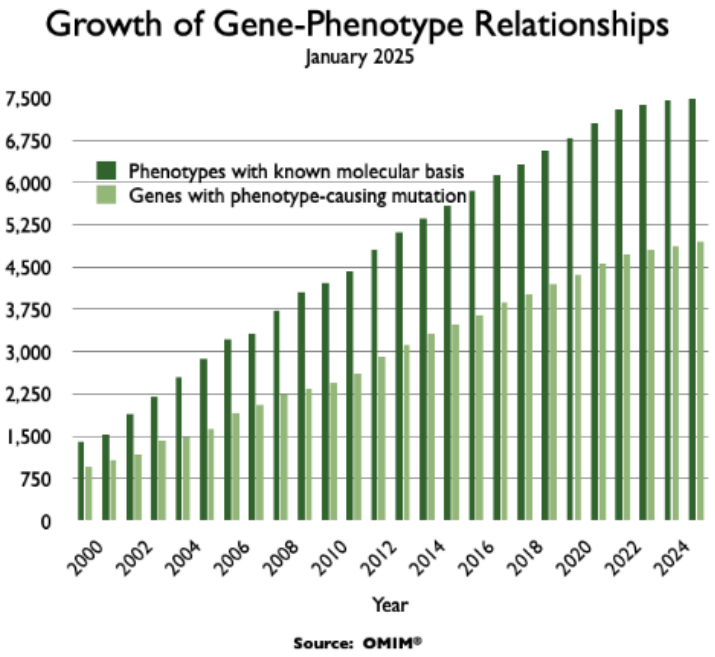
- ✓Understand the pathophysiology of rare inherited neurological disorders and their impact on therapeutic development
- ✓Identify current and emerging therapies including gene therapies, RNA targeted therapies, enzyme replacement therapies and small molecular approaches
- ✓Evaluate the challenges of the new innovative treatments

Inherited disordres

OMIM Gene Map Statistics

OMIM Morbid Map Scorecard (Updated February 28th, 2025) :

Total number of phenotypes* for which the molecular basis is known	7,601
Total number of genes with phenotype-causing mutation	4,966



Dissected OMIM Morbid Map Scorecard (Updated February 28th, 2025) :

Class of phenotype	Phenotype	Gene *
Single gene disorders and traits	6,552	4,609
Susceptibility to complex disease or infection	669	500
"Nondiseases"	151	118
Somatic cell genetic disease	236	128

Neurogenetic disorders (cc. 5000 - 6000 conditions)

Monogenic Disorders: circa. 2,000–3,000 (eg. Huntington's disease, SMA, inherited ataxias, various forms of Charcot-Marie-Tooth disease and myopathies, muscle dystrophies etc.)

Neurodevelopmental Disorders (ASD, intellectual disability, schizophrenia): high heritability, hundreds of genes are implicated, non-coding genes, structrural variants in non-coding regions, epigenetic, postranscriptional alterations)

Rare Syndromes: a large fraction (perhaps 2,000+) featuring neurological components, such as Rett syndrome or Fragile X syndrome

Diagnostics

Therapeutics

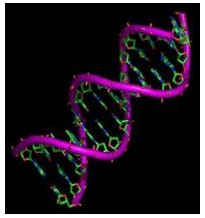
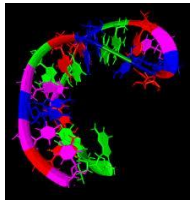
Diagnostic success

- **In general**
  - WES: ~25–40%
  - WGS: ~40–45% (captures non-coding regions and structural variants missed by WES)
- **Undiagnosed neurodevelopmental disorders** (e.g., intellectual disability, autism, or epilepsy): ~30%
- **Condition-Specific Success**:
  - **Specific phenotypes**: e.g., Huntington's disease, SMA, FSHD, myotonic dystrophy 1 and 2, SCA27B, CANVAS, NF1, Fragile X syndrome ~100%
  - **Genetic epilepsies:** e.g. Dravet syndrome ~ 60–80% with gene panels **when seizures fit known patterns**
  - **Neurodegenerative Diseases**: familial forms of Alzheimer's, Parkinson's and Creutzfeld-Jacob linked to specific genes (e.g., PSEN1, LRRK2, PRNP) are highly diagnosable (~80-90%) with gene panels, but sporadic cases often remain unresolved
  - **Muscle dystrophies**: 30-60% with gene panels

Challenges

Treating Neurogenetic Disorders

Levels of therapeutic intervention in neurogenetic diseases

MUTANT GENE[1]> MUTANT RNA[2] > DEFECTIVE PROTEIN[3] > PHENOTYPE[4]

Therapeutic targeting of either 1+2+3+4: Classical Pharmacology

Therapeutic targeting of 1 : DNA editing, Gene transfer, Gene replacement

Therapeutic targeting of 2 : Exon skipping, translational read-through,siRNA

Therapeutic targeting of 3: Protein replacement, Protein expression

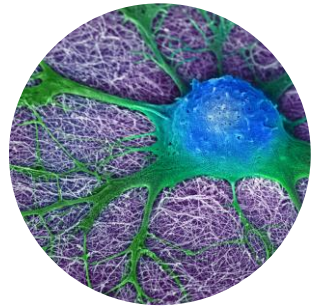
Therapeutic targeting of 4: Pharmacology, physiotherapy, surgery

Targeting miscalleneous items: enhancement of regeneration, tissue replacement by cell therapy

Therapeutic approaches of neurogenetic disorders

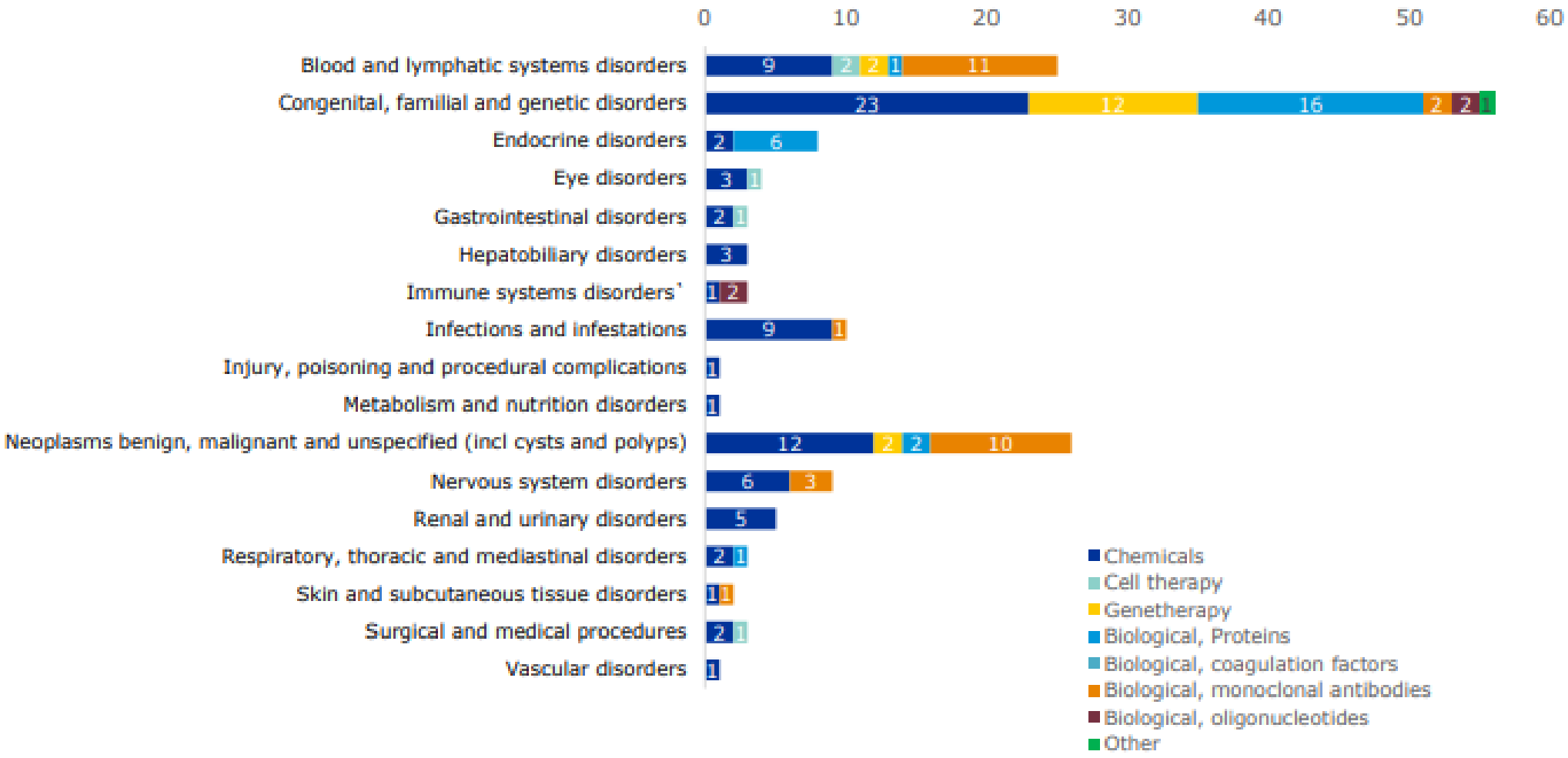
Pharmacological Therapies

Molecular Therapies

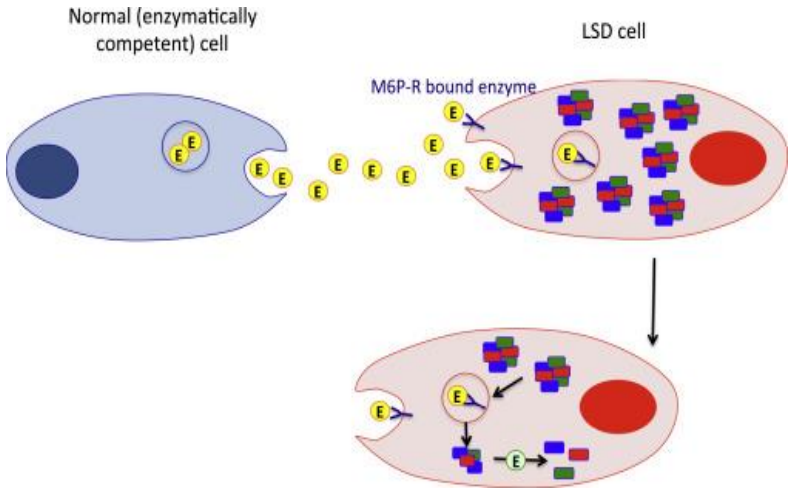


Cell Therapies

Approved drugs with orphan designation in 2024



Allogen HSCT in neurogenetic disorders



HSCT can stop the neuroinflammatory demyelinating process by replacing the affected microglia with macrophages originating from the bone marrow

HSCT normalize the TP enzyme activity avoiding the accumulation of the toxic nucleosids

Metabolic disorders

- Lysosomal disorders:
  - Mucopolysacharidosis: MPS I, II. VI, VII
  - Sphingolipidosis:
    - metachromatic leukodystrophy
    - Gaucher disease
    - Niemann Pick A, B, C
    - GM2 gangliosidosis
    - Farber
  - Glycoproteinosis
    - Alpha mannosidosis,
    - Fuccosidosis
- Peroxisomal disorders: Adrenoleukodystrophy
- Mitochondrial disorders
  - MNGIE (Mitochondrial-Neuro-Gastrointestinal Encephalopathy)

doi.org/10.1016/j.ymthe.2017.03.025

Biologics for the treatment of genetic disorders

DNA targeted therapies

- Gene replacement – replace the defective gene
- Gene transfer – upregulate therapeutic proteins
- Gene editing – correct the altered gene (CRISPR/CAS9)
- Gene addition – introduce a new gene

RNA targeted therapies

- Exon skipping with antisense oligonucleotids (ASOs)
- Translation correction - increase protein production with ASOs
- The inactivation of the mRNA with miRNA/ASO

Protein replacement therapies

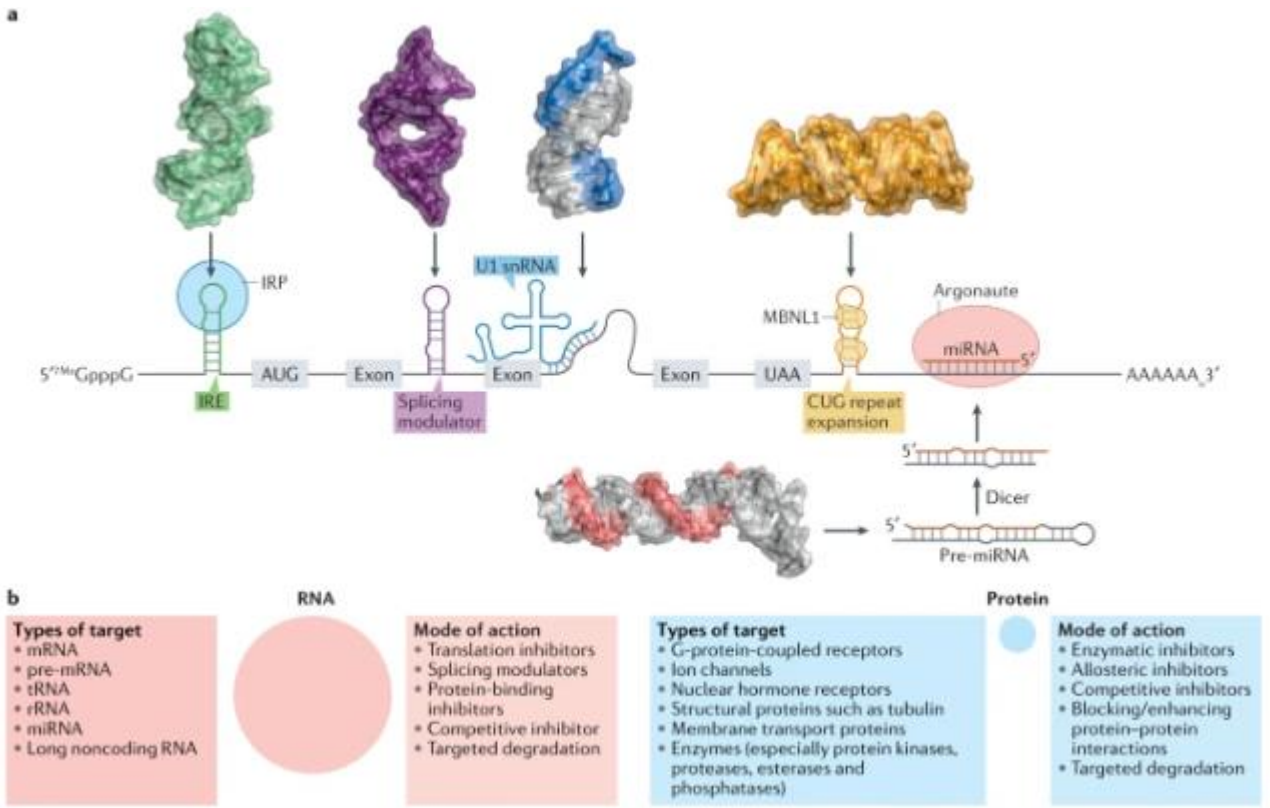
- Replace the missing protein

Pharmacological approaches acting on RNA level

RNA modifying therapies

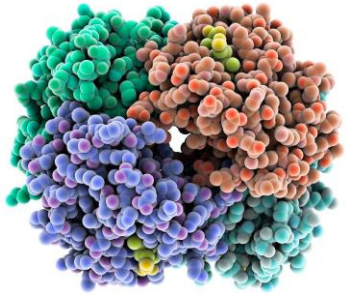
- Translation correction
  - increase protein production - with with small molecule
    Risdiplam (SMA)

- Translation manipulation
  - Read-through with small molecule
    Translarna (Duchenne MD)



doi.org/10.1038/s41573-022-00521-4

Pharmacological approaches acting on protein level/1



Substrate Reduction Therapy (SRT)

MoA: Inhibits synthesis of toxic substrates that accumulate due to enzyme deficiency.

- Examples: Miglustat and Eliglusta (Niemann-Pick C, Gaucher)

Read-Through Therapy (Nonsense Suppression)

MoA: Induces ribosomes to bypass premature stop codons, allowing full-length protein production.

- Examples: Ataluren (Duchenne muscular dystrophy)

Pharmacological Chaperones

MoA: Small molecules stabilize misfolded proteins, enhancing correct folding and trafficking to functional locations (e.g. lysosomes)

- Examples: Migalastat (Fabry disease), Tezacaftor/Lumacaftor/ Elexacaftor (Cystic fibrosis) – called CFTR corrector as well

Stabilizers / Allosteric Modulators

MoA: Bind to and stabilize the native conformation or enhance residual activity of the defective protein.

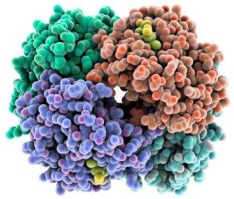
- Examples: Tafamidis (transthyretin amyloidosis), Ivacaftor (CFTR modulator in Cystic Fibrosis)

Proteostasis Regulators

MoA: Modulate the protein quality control system (e.g. proteasome, autophagy, unfolded protein response).

- Examples: Experimental; potential use in cystic fibrosis, ambroxol GBA1 associated PD

Pharmacological approaches acting on protein level/2



Transcriptional modulators

MoA: binds transcription factors or nuclear receptors to influence gene expression. Mechanistically they act via proteins (e.g. receptors, transcription factor), functionally they influence mRNA production, which indirectly affects downstream protein levels

- 1. Example: vamorolone - acts via the glucocorticoid receptor to modulate inflammation-related gene expression without the full side effect profile of traditional steroids (DMD)
- 2. Example: omaveloxolone -activates transcription factor Nrf2, Nrf2 regulates the expression of genes involved in antioxidant response, mitochondrial function, anti-inflammatory signaling (Friedreich ataxia)

Post-translational modifyers

MoA: Modify expression of genes through chromatin remodeling or methylation changes.

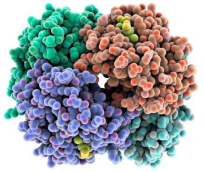
- Example: givinostat - histon deacetylase inhibitors (HDAC inhibitor) leads to increased histone acetylation, promoting a more open chromatin structure and enhanced transcription of genes involved in muscle regeneration and anti-inflammatory pathways (DMD)

Signaling pathway regulators

MoA: regulate dysregulated intracellular signaling cascades (e.g. MAPK, mTOR, GR pathways), supress protein synthesis, influence cell growth

- 1. Example: everolimus - mTOR inhibitors suppress protein synthesis (tuberous sclerosis, lymphangioleiomyomatosis)
- 2. Example: selumetinib, trametinib - MEK inhibitors **reducing ERK activation** and downstream transcription of growth-promoting genes (NF1)

Pharmacological approaches acting on protein level/3



Ion channel blockers

Drug	Target	Indication	Type
Mexiletine	Voltage-gated Na^+ channels	Non-dystrophic myotonias (e.g., paramyotonia congenita, SCN4A mutations)	**Na^+ channel blocker**
Acetazolamide	Indirect effect on Cl^-/K^+ channels	Periodic paralysis (CACNA1S, SCN4A mutations)	**Carbonic anhydrase inhibitor**
Quinidine	KCNQ1 (K^+ channel)	Short QT syndrome, some types of long QT syndrome	**K^+ channel blocker**
4-aminopyridine	Kv channels	Lambert-Eaton myasthenic syndrome	**K^+ channel blocker**
Zilebesiran (investigational)	ENaC (epithelial Na^+ channel)	Liddle syndrome or salt-sensitive hypertension	**Na^+ channel modulator**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for
details

General applications of therapeutic gene transfer

1. Molecular therapy for genetic diseases

2. Establishment of a stable gene reservoir as a source of therapeutic proteins in non – genetic diseases

3. To increase the tumor cells sensitivity to drugs

4. Destruction of malignant cells in neoplasias

5. DNA vaccines

Biologics for the treatment of genetic disorders

DNA targeted therapies

- Gene replacement – replace the defective gene
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RNA modifying therapies

- Exon skipping with antisense oligonucleotids (ASOs)
- Translation correction - increase protein production with ASOs
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Protein replacement therapies

- To replace the missing protein

Gene delivery

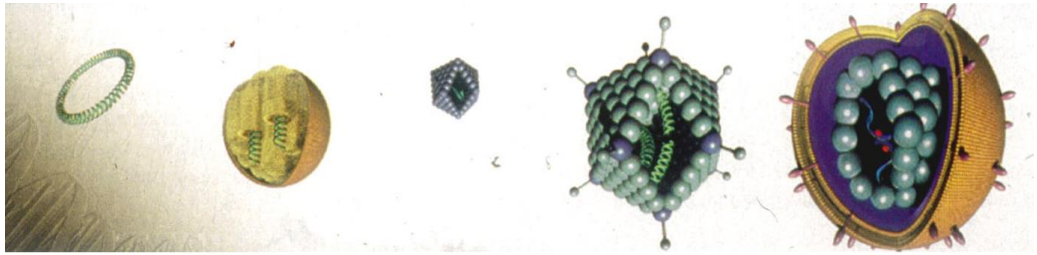
Vectors

Nonviral

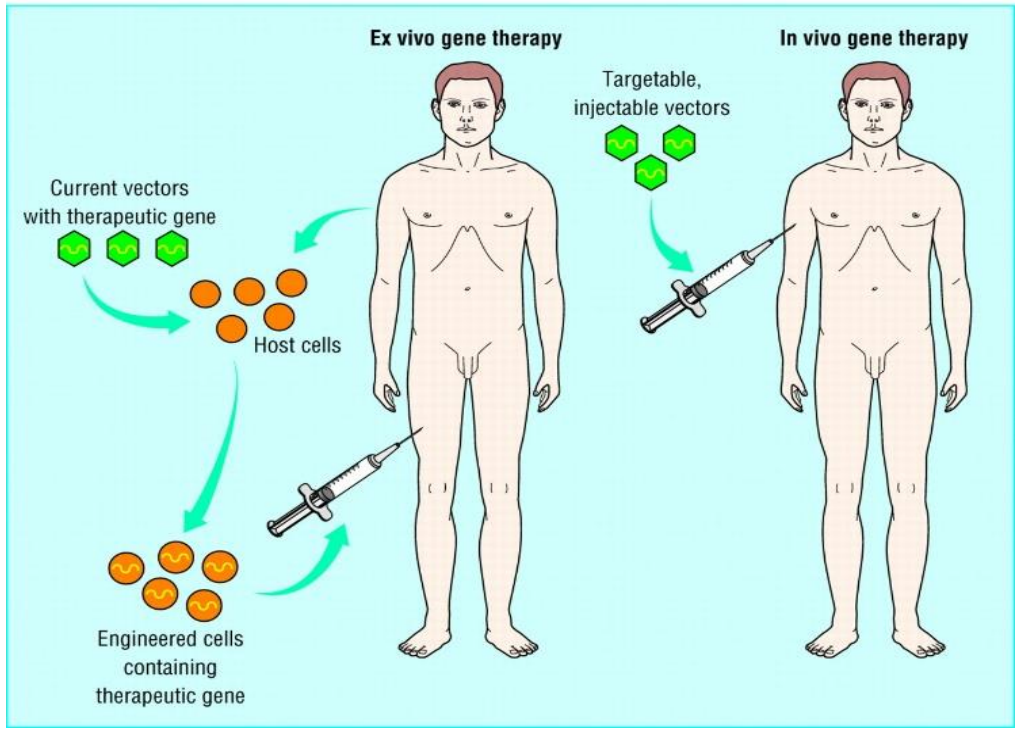
Plasmid
Arteficial Chr
Liposomes
DNAsomes
Nanoparticlees

Viral

Retro
Herpes
Adeno
AAV
Lenti



Strategies



5 main classes

- Retrovirus
- Lentivirus
- Herpes Simplex Virus type 1 (HSV-1)
- **Adeno-Associated Virus (AAV)**
- Adenovirus

Retrovirus, Lentivirus → Integrating → Genomes integrate into the host genome

Herpes Simplex Virus type 1 (HSV-1), **Adeno-Associated Virus (AAV)**, Adenovirus → **Generally non-integrating** → **Persist in the cell nucleus predominantly as extrachromosomal episomes**

Thomas CE, et al. *Nat Rev Genet* 2003;4(5):346–358

Ex vivo gene therapies

#	Hatóanyag (INN)	Kereskedelmi név	Típus	Célpont / célgén	Indikáció
1	Tisagenlecleucel	Kymriah®	CAR-T, autológ	CD19	ALL, DLBCL
2	Axicabtagene ciloleucel	Yescarta®	CAR-T, autológ	CD19	LBCL, PMBCL, FL, MZL
3	Brexucabtagene autoleucel	Tecartus®	CAR-T, autológ	CD19	MCL, ALL
4	Lisocabtagene maraleucel	Breyanzi®	CAR-T, autológ	CD19	LBCL, CLL/SLL
5	Idecabtagene vicleucel	Abecma®	CAR-T, autológ	BCMA	Multiplex myeloma
6	Ciltacabtagene autoleucel	Carvykti®	CAR-T, autológ	BCMA	Multiplex myeloma
7	Afamitresgene autoleucel	Tecelra®	TCR-T, autológ	MAGE-A4	Szinoviális szarkóma
8	Elivaldogene autotemcel	Skysona®	ex vivo lentivírus	ABCD1 gén	Cerebrális ALD
9	Atidarsagene autotemcel	Libmeldy®	ex vivo lentivírus	ARSA gén	Metachromatikus leukodystrophia
10	Betibeglogene autotemcel	Zynteglo®	ex vivo lentivírus	HBB gén (β-globin)	Transzfúziós thalassaemia
11	Lovotibeglogene autotemcel	Lyfgeni[illegible]	ex vivo lentivírus	HBB gén (HbA^T87Q)	Sarlósejtes anaemia

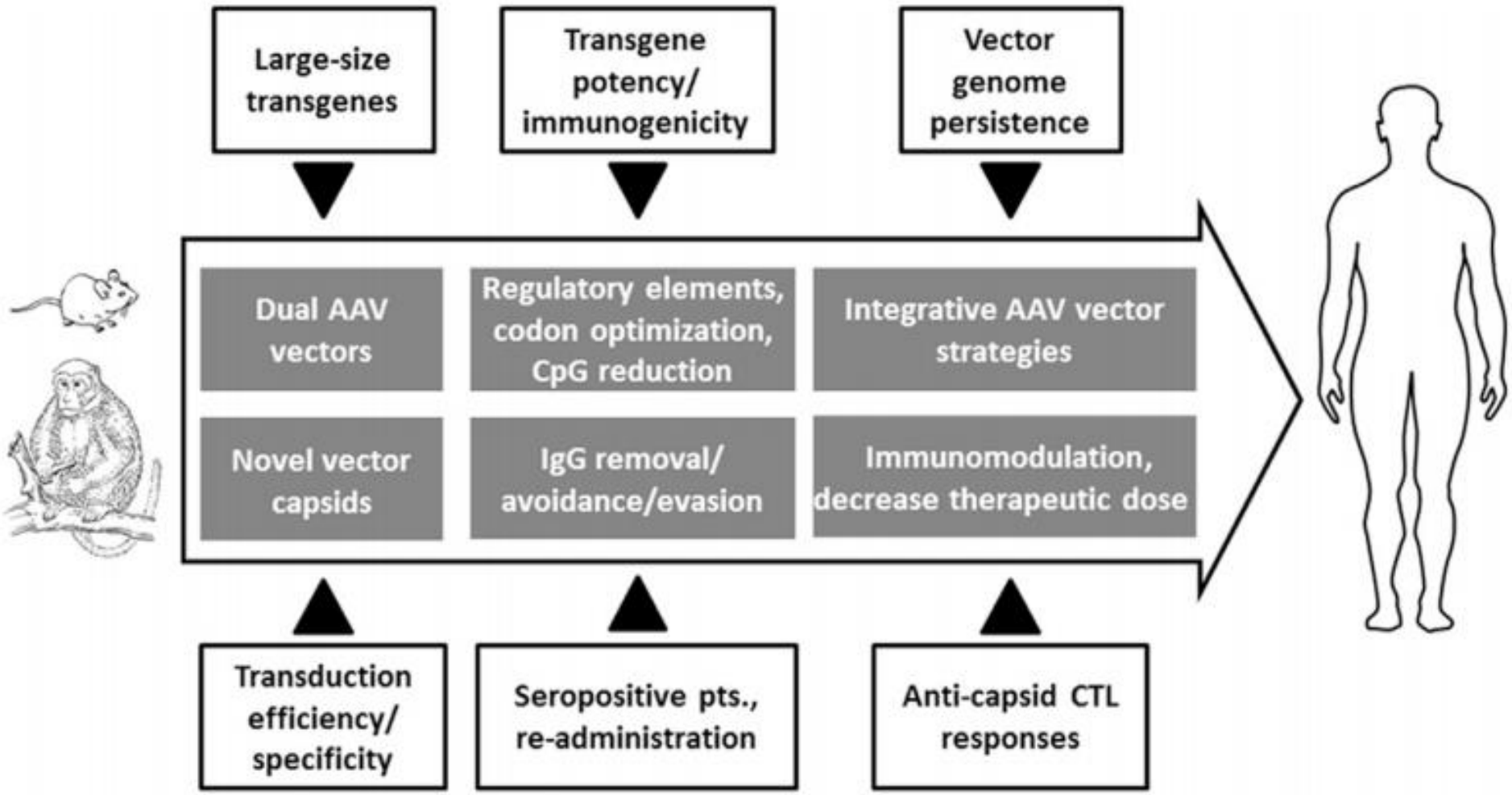
In vivo gene therapies

#	Hatóanyag (INN)	Kereskedelmi név	Vektor/Platform	Indikáció
1	Onasemnogene abeparvovec	Zolgensma®	AAV9	SMA (0–2 év)
2	Voretigene neparvovec	Luxturna®	AAV2	RPE65 retinal dystrophy
3	Valoctocogene roxaparvovec	Roctavian®	AAV5	Hemofília A
4	Etranacogene dezaparvovec	Hemgenix®	AAV5	Hemofília B
5	Fidanacogene elaparvovec	Beqvez®	AAV	Hemofília B
6	Delandistrogene moxeparvovec	Elevidys®	AAVrh74	Duchenne-féle izomdisztrófia (DMD)
7	Lenadogene nolparvovec	Lumevoq®	AAV2	LHON optic neuropathy
8	Beremagene geperpavec	Vyjuvek®	HSV-1	Dystrophic epidermolysis bullosa
9	Eladocagene exuparvovec	Kebilidi / Upstaza	AAV	AADC deficiency

Gene therapies of neurological disorders

Disease	Medication	Type of gene th.	Pt eligibility	Approved in
SMA	Onasemnogene abeparvovec	AAV9 vector based in vivo DNA	Older than 2 years, bw. below 21 kg	USA, EU, UK, Japan, Asutralia, Canada, Brazil, Israel, Taiwan, South Korea
AADC	Eladocagene exuparvovec	AAV2-based in vivo DNA	Older than 18 months with severe phenotype	EU, UK
C-ALD	Elivaldogene autotemcel	Lentiviral based ex vivo autologous DNA	4-17y of age with early, active C-ALD	USA
MLD	Atidarsagene autotemcel	Lentiviral based ex vivo autologous DNA	Presymptomatic late infantile, presymptomatic early juvenile,-early symptomatic early juvenile	USA,EU,UK, Switzerland
DMD	Delandistrogene moxeparvovec	AAVrh74 in vivo DNA	Older than 4 years, for ambulatory and non-ambulatory patients	USA

The challenges of AAV based gene therapies



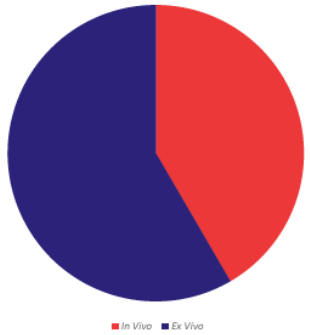
Molecular Therapy: Methods & Clinical Development Vol. 8 March 2018

What does the near future bring in the field of gene therapy?

Gene therapy pipeline: quarterly comparison

- Q1 2024 saw an increase in the number of therapies at each pipeline stage of development except pre-registration
- The number of gene therapies at pre-registration is the lowest it has been since prior to Q4 2022, while Phase I development has seen the largest percentage increase in over a year (11%) between Q4 2023 and Q1 2024
- Therapies currently in pre-registration:
  - In the US
    - RP-L201 (Rocket Pharmaceuticals)
    - EB-101 (Abeona Therapeutics)
    - afami-cel (Adaptimmune Therapeutics)
    - obe-cel (Autolus Therapeutics)

In vivo vs. *Ex vivo* genetic modification



Global Status	Q1 2023	Q2 2023	Q3 2023	Q4 2023	Q1 2024
Preclinical	1,493	1,539	1,522	1,528	1,471
Phase I	245	240	256	270	301
Phase II	247	260	267	274	282
Phase III	30	30	30	33	35
Pre-registration	7	6	7	6	4
Total	**2,022**	**2,075**	**2,082**	**2,111**	**2,093**

Source: Pharmaprojects | Citeline, April 2024

Biologics for the treatment of genetic disorders

DNA targeted therapies

- Gene replacement – replace the defective gene
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RNA targeted therapies

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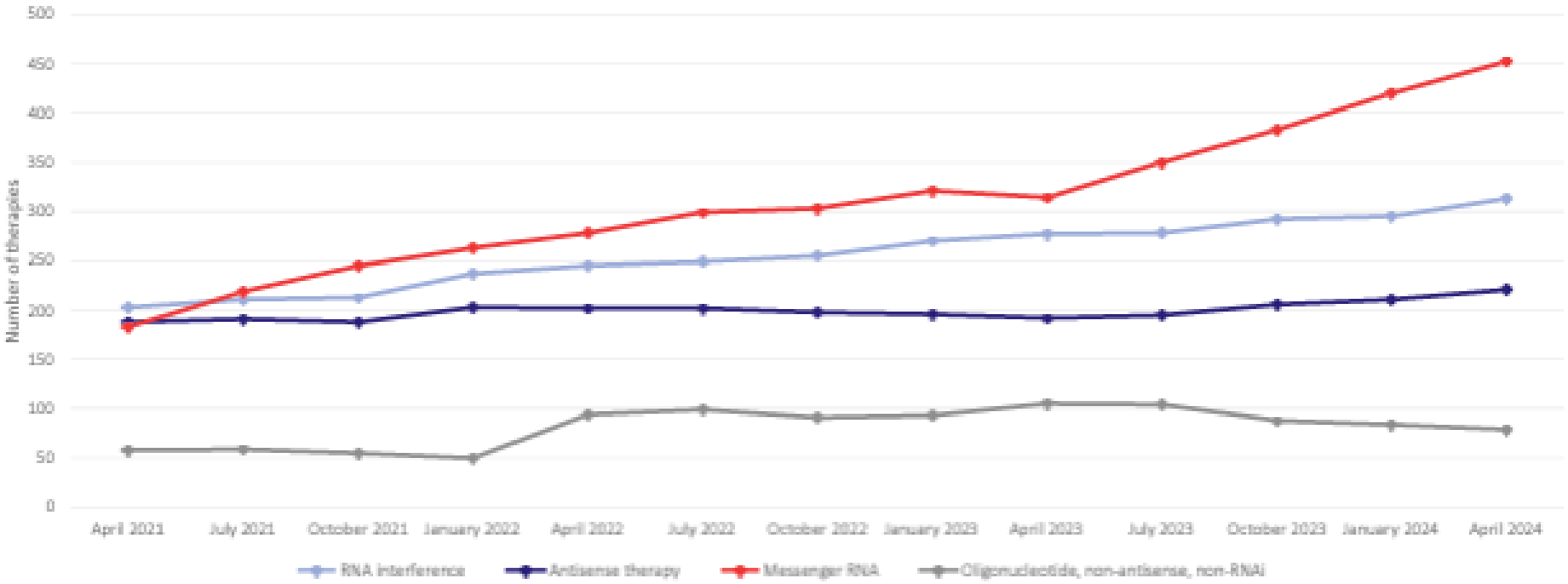
Protein replacement therapies

- To replace the missing protein

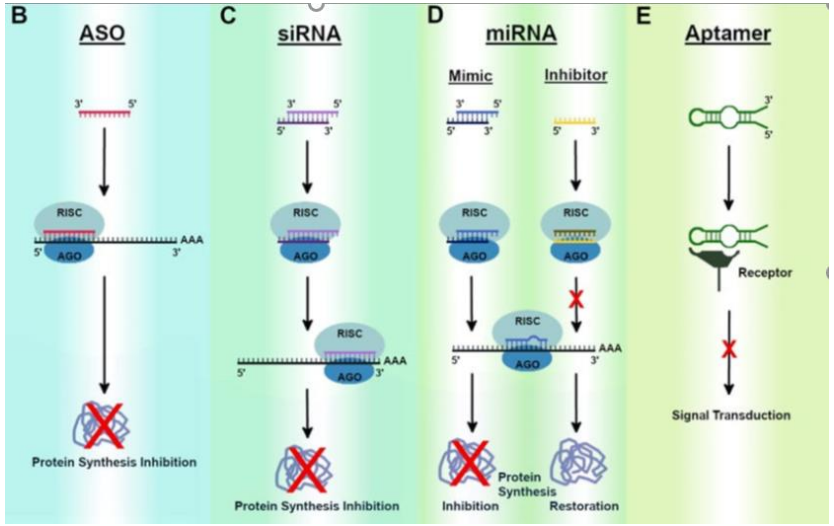
RNA therapy pipeline: most common modalities

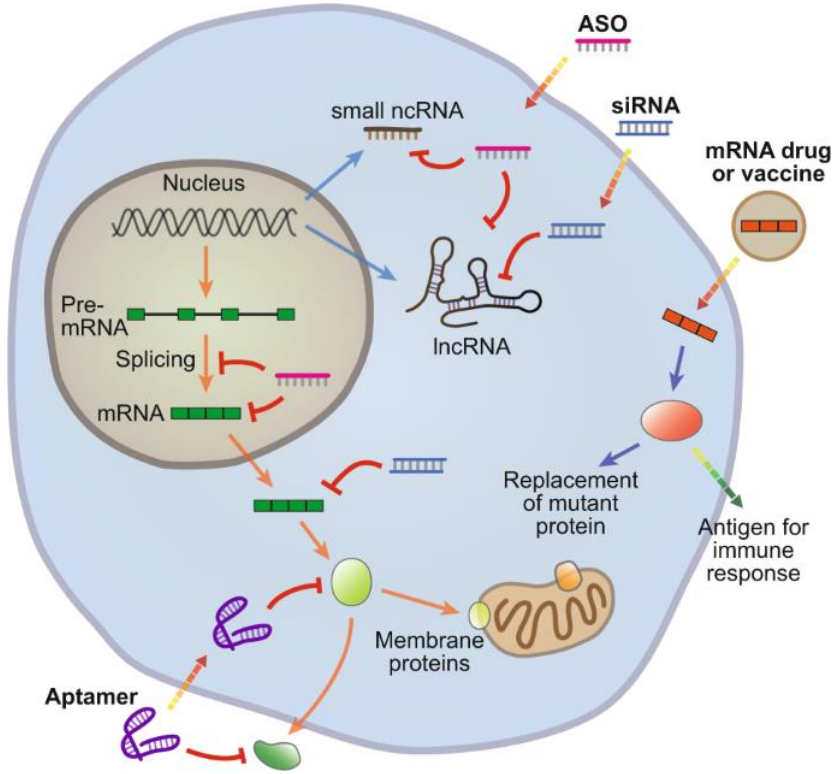
- Of RNA therapies in the pipeline, messenger RNA (mRNA) and RNA interference (RNAi) continued to be the preferred RNA modalities for research



Source: Pharmaprojects | Citeline, April 2024

The types of RNA targeted therapies





ASOs: small single stranded RNA

siRNA: small double stranded RNA translational repression of their target protein

miRNA: small RNAs, either inhibit protein synthesis when they bind to an mRNA, or free up mRNA by binding to the miRNA that represses the translation

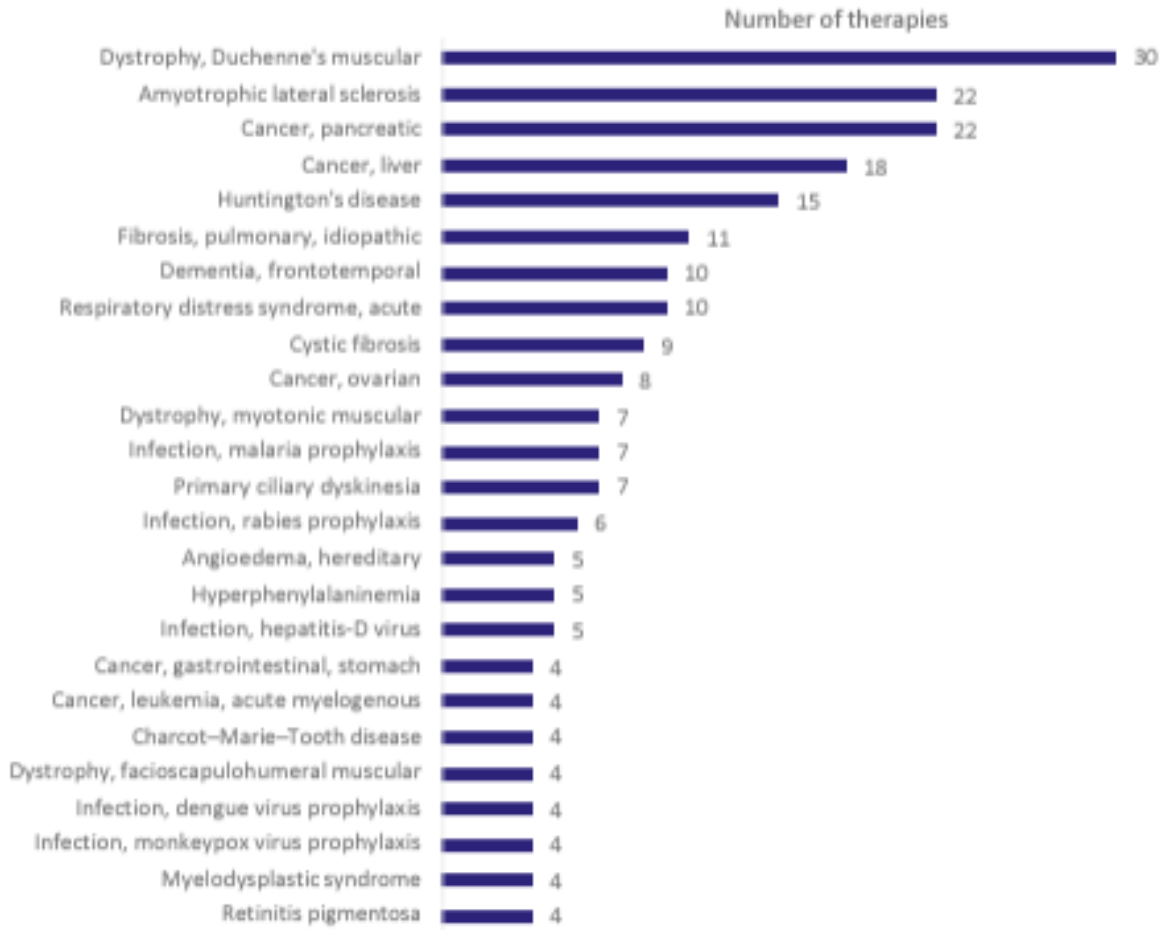
mRNA: encoding proteins – SARS-CoV-2 vaccines, MRT5005- cystic fibrosis, mRNA-3704 methyl malonic aciduria

Aptamer: short single-stranded nucleic acids that form secondary and tertiary structures and interact with a specific enzyme or molecule and therefore can promote or inhibit many different molecular pathway

RNA therapies: most common rare diseases targeted

Of the RNA therapies currently in the pipeline (from preclinical through pre-registration):

- Top specified rare oncology indications were pancreatic, liver, and ovarian cancer
- For non-oncology rare diseases, Duchenne muscular dystrophy, amyotrophic lateral sclerosis, and Huntington's disease were the most targeted indications



Source: Pharmaprojects | Citeline, April 2024

RNA targeted therapies 2024/1

Approved RNA therapies 2024 3/1

Product name	Generic name	Year first approved	Disease(s)	Locations approved*	Originator company
Kynamro	mipomersen sodium	2013	Homozygous familial hypercholesterolemia	US, Mexico, Argentina, South Korea	Ionis Pharmaceuticals
Exondys 51	eteplirsen	2016	Dystrophy, Duchenne muscular	US	Sarepta Therapeutics
Spinraza	nusinersen	2016	Muscular atrophy, spinal	US, EU, UK, Canada, Japan, Brazil, Switzerland, Australia, South Korea, China, Argentina, Colombia, Taiwan, Turkey, Hong Kong, Israel	Ionis Pharmaceuticals
Ampligen	rintatolimod	2016	Chronic fatigue syndrome	Argentina	AIM ImmunoTech
Tegsedi	inotersen	2018	Amyloidosis, transthyretin-related hereditary	EU, UK, Canada, US, Brazil	Ionis Pharmaceuticals
Onpattro	patisiran	2018	Amyloidosis, transthyretin-related hereditary	US, EU, UK, Japan, Canada, Switzerland, Brazil, Taiwan, Israel, Turkey, Australia	Alnylam
Vyondys 53	golodirsen	2019	Dystrophy, Duchenne muscular	US	Sarepta Therapeutics
Waylivra	volanesorsen	2019	Hypertriglyceridemia; lipoprotein lipase deficiency	EU, UK, Brazil, Canada	Ionis Pharmaceuticals
Comirnaty	tozinameran	2020	Infection, coronavirus, novel coronavirus prophylaxis	UK, Bahrain, Israel, Canada, US, Rwanda, Serbia, United Arab Emirates, Macao, Taiwan, Mexico, Kuwait, Singapore, Saudi Arabia, Chile, Switzerland, EU, Ghana, Colombia, Philippines, Indonesia, Australia, Hong Kong, Peru, South Korea, New Zealand, Japan, Brazil, Sri Lanka, Vietnam, South Africa, Thailand, Oman, Egypt, Malaysia	BioNTech
Moderna COVID-19 vaccine	COVID-19 vaccine, Moderna	2020	Infection, coronavirus, novel coronavirus prophylaxis	US, Canada, Israel, EU, Switzerland, Singapore, Qatar, Vietnam, UK, Philippines, Thailand, Japan, South Korea, Brunei, Paraguay, Taiwan, Botswana, India, Indonesia, Saudi Arabia, Mexico, Australia, Nigeria, Colombia	Moderna Therapeutics

RNA targeted therapies 2024/2

Approved RNA therapies as of Q1 2024 (2/3)

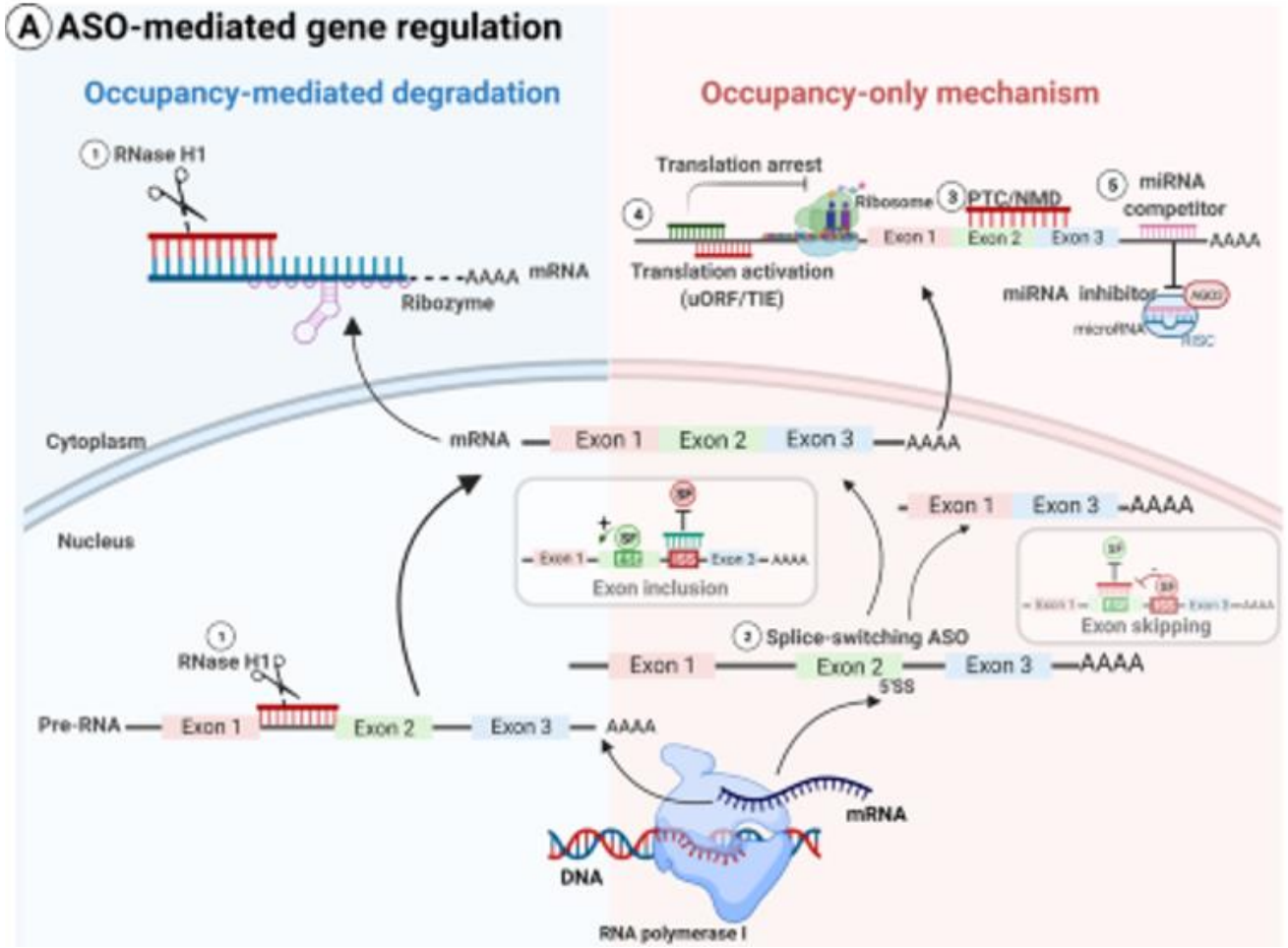
Product name	Generic name	Year first approved	Disease(s)	Locations approved*	Originator company
Givlaari	givosiran	2020	Porphyria	US, EU, UK, Canada, Switzerland, Brazil, Israel, Japan	Alnylam
Oxlumo	lumasiran	2020	Hyperoxaluria	EU, UK, US, Brazil	Alnylam
Viltepso	viltolarsen	2020	Dystrophy, Duchenne muscular	US, Japan	NS Pharma
Leqvio	inclisiran	2020	Atherosclerosis; heterozygous familial hypercholesterolemia; hypercholesterolemia	EU, UK, Australia, Canada, Israel, US, Saudi Arabia, Japan, China	Alnylam
Amondys 45	casimersen	2021	Dystrophy, Duchenne muscular	US	Sarepta Therapeutics
Nulibry	fosdenopterin	2021	Molybdenum cofactor deficiency	US, EU, UK, Israel	Orphatec
Gennova COVID-19 vaccine	COVID-19 vaccine, Gennova Biopharmaceuticals	2022	Infection, coronavirus, novel coronavirus prophylaxis	India	Gennova Biopharmaceuticals
Amvuttra	vutrisiran	2022	Amyloidosis, transthyretin-related hereditary	US, EU, UK	Alnylam
Moderna Spikevax Bivalent Original/Omicron vaccine	COVID-19 bivalent original/Omicron vaccine, Moderna	2022	Infection, coronavirus, novel coronavirus prophylaxis	UK, Canada, Taiwan, Switzerland, Japan, EU, Australia, South Korea, Singapore, US	Moderna Therapeutics
ARCoV	COVID-19 vaccine, Suzhou Abogen Biosciences	2022	Infection, coronavirus, novel coronavirus prophylaxis	Indonesia	Suzhou Abogen Biosciences
Pfizer & BioNTech's Omicron BA.4/BA.5-adapted bivalent booster vaccine	Omicron BA.4/BA.5-adapted bivalent booster vaccine	2022	Infection, coronavirus, novel coronavirus prophylaxis	US, UK	BioNTech
CSPC Pharmaceutical COVID-19 vaccine	COVID-19 vaccine, CSPC Pharmaceutical	2023	Infection, coronavirus, novel coronavirus prophylaxis	China	CSPC Pharmaceutical
Sinocelltech COVID-19 vaccine	COVID-19 alpha/beta/delta/Omicron variants S-trimer quadrivalent recombinant protein vaccine	2023	Infection, coronavirus, novel coronavirus prophylaxis	China, UAE, US	SinoCellTech

RNA targeted therapies 2024/3

Approved RNA therapies as of Q1 2024 (3/3)

Product name	Generic name	Year first approved	Disease(s)	Locations approved*	Originator company
Qalsody	tofersen	2023	Amyotrophic lateral sclerosis	US	Ionis Pharmaceuticals
ARCT-154	COVID-19 mRNA vaccine, Arcturus	2023	Infection, coronavirus, novel coronavirus prophylaxis	Japan	Arcturus Therapeutics
Daichirona	COVID-19 vaccine, Daiichi Sankyo	2023	Infection, coronavirus, novel coronavirus prophylaxis	Japan	Daiichi Sankyo
Wainua	eplontersen	2023	Transthyretin-related hereditary amyloidosis	US	Ionis Pharmaceuticals
Rivfloza	nedosiran	2023	Hyperoxaluria	US	Dicerna Pharmaceuticals
SYS-6006.32	Bivalent COVID-19 mRNA vaccine, CSPC Pharmaceutical	2023	Infection, coronavirus, novel coronavirus prophylaxis	China	CSPC Pharmaceutical

The mode of action of ASOs



I. **"Occupancy mediated"** (enzymatic RNS degradation - RNase H activity): ASOs bind to target RNS molecules with the help of endogenous enzymes, resulting in a downregulation effect

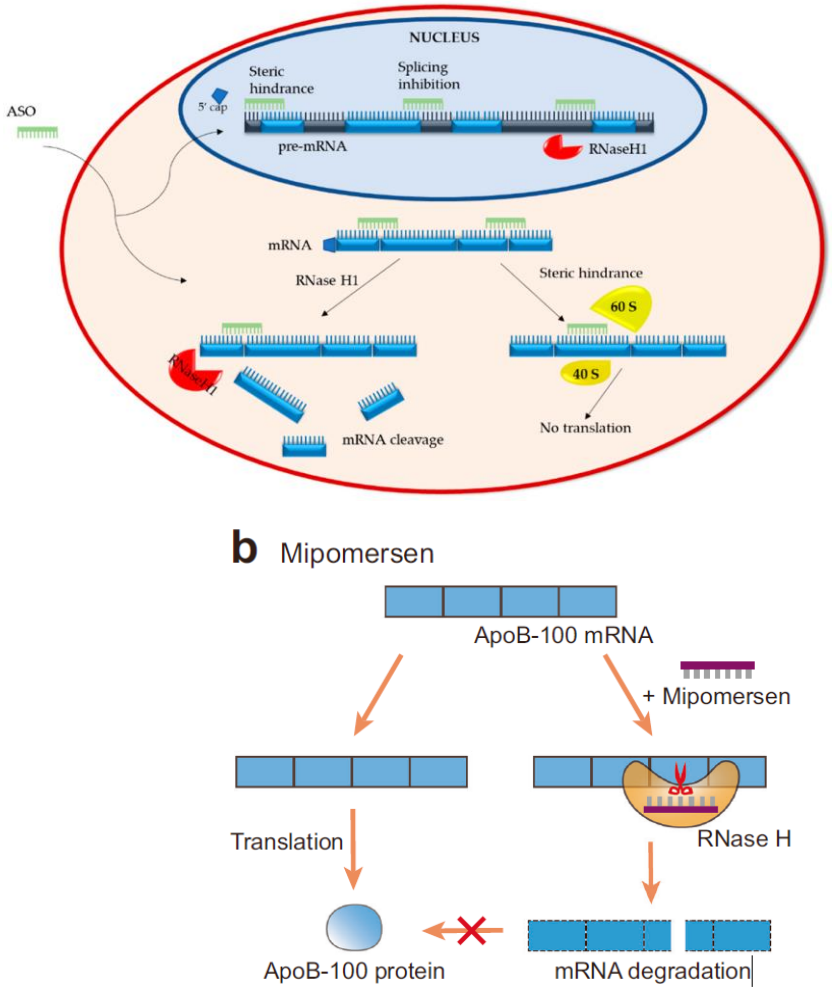
II. "**Occupancy only mode**" (RNase H independent): Specific enzymes are not involved, and the binding of the target molecule does not result in down- or up-regulation effects.

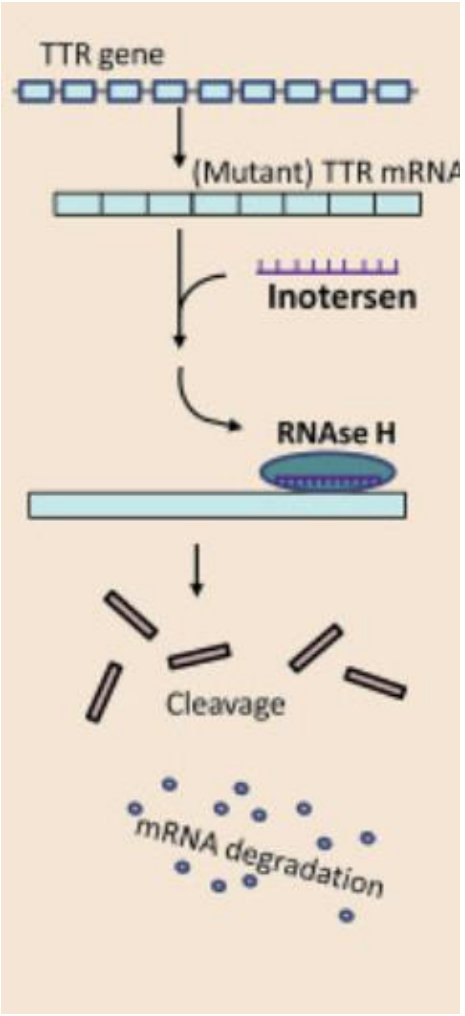
doi.org/10.1038/s41419-022-05075-2

I. “Occupancy mediated” (enzymatic RNA degradation)

Mipomersen binds to ApoB100 mRNA, leading to the cleavage of the mRNA. ApoB100 is an important component of LDL and VLDL, and a lipid-lowering effect can be achieved

Inotersen and **eplontersen** induce RNase H1-mediated degradation of hepatic transthyretin (TTR) mRNAs, thereby reducing TTR protein synthesis and serum TTR levels.





Young-Kook. Exp & Mol Med 2022: 54:455 – 465

Milasen from a N-of-1 trial in Batten's disease (neuronal ceroid lipofuscinosis 7)

- The disease is caused: insertion of SVA (SINE-VNTR-Alu) retrotransposon in CLN7 (MFSD8) that alters the splicing of transcripts
- A series of ASOs were tested in patient fibroblasts to target cryptic splice sites in the MFSD8 pre-mRNA and identified one which restored exon 6–7 splicing

II. Occupancy only mode of action of the ASO regulate the splicing of pre-mRNAs via a steric hindrance-based mechanisms

1. Control the down-/up-regulation of target transcripts by splicing switching ASOs

2. Remove abnormal mRNAs by activating endogenous surveillance programs
   via nonsense-mediated mRNA decay when ASOs act on pre-mRNAs to create mRNAs with premature termination codons

3. Inhibit or activate translation
   Can downregulate the target RNA via translational arrest, 5'cap inhibition, or polyadenylation changes

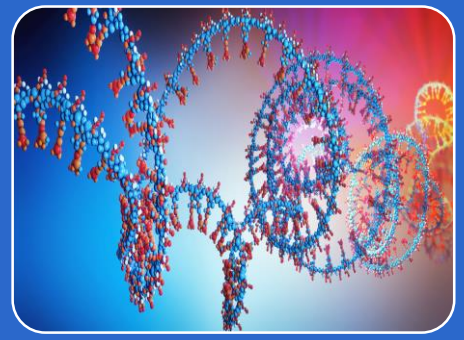
4. Upregulate target RNAs by binding to inhibitory elements, such as upstream open reading frames (uORFs) or other translation inhibitory components

5. Inhibit miRNA-mediated downregulation by directly binding to miRNAs

Steric hinderance = sterikus akadály

Control the down-/up-regulation of target transcripts by splicing switching ASOs

Can change the splicing pattern by targeting splicing regulatory cis-elements.
Cis-acting elements activate or inhibit adjacent splice sites through recruiting trans-splicing factors. They consist of splicing enhancers and silencers.



Targeting a splicing enhancer sequence blocks the binidng of the stimulatory splicing factor to its cognate enhancer-binding site

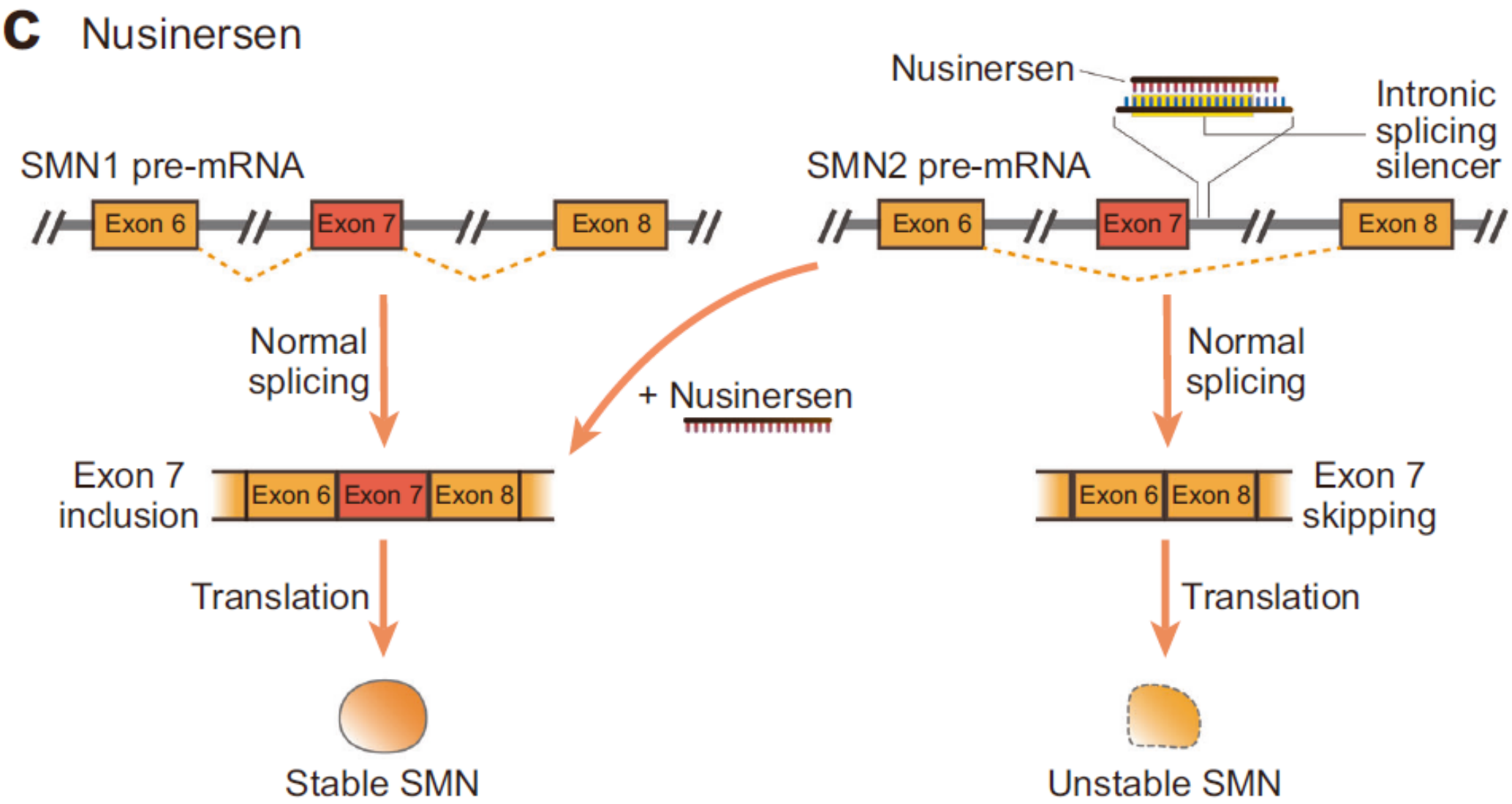
↓

Inhibiting splicing - causing exon skipping (e.g. eteplirsen)

Targeting a splicing silencer sequence blocks the binding of the inhibitory splicing factor

↓

Negatively regulates splicing activation - resulting in exon inclusion (e.g. nusinersen)



Young-Kook. Exp & Mol Med 2022: 54:455 – 465

RNA targeted therapies in DMD

Exon skipping:

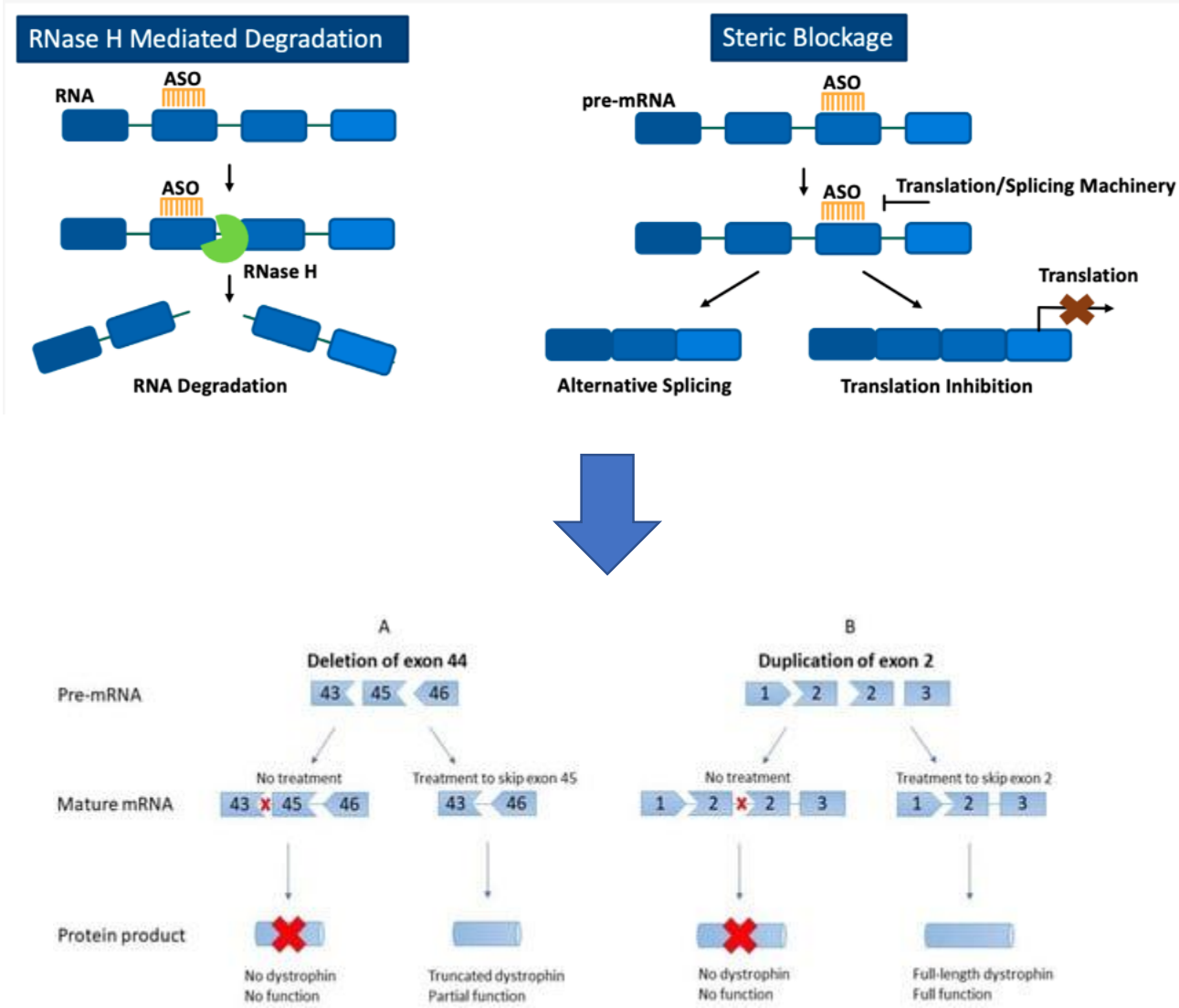
Splicing modulation with ASOs to restore the reading frame

ASO: a short, single-stranded nucleic acid polymer that affects splicing by masking specific splicing signals (e.g., exon splicing enhancers and recognition sequences).

Correctable dystrophin defects: deletions, duplications, certain nonsense mutations occurring in exons whose skipping does not disrupt the translational reading frame (approximately 55% of mutations, e.g., exon 23).

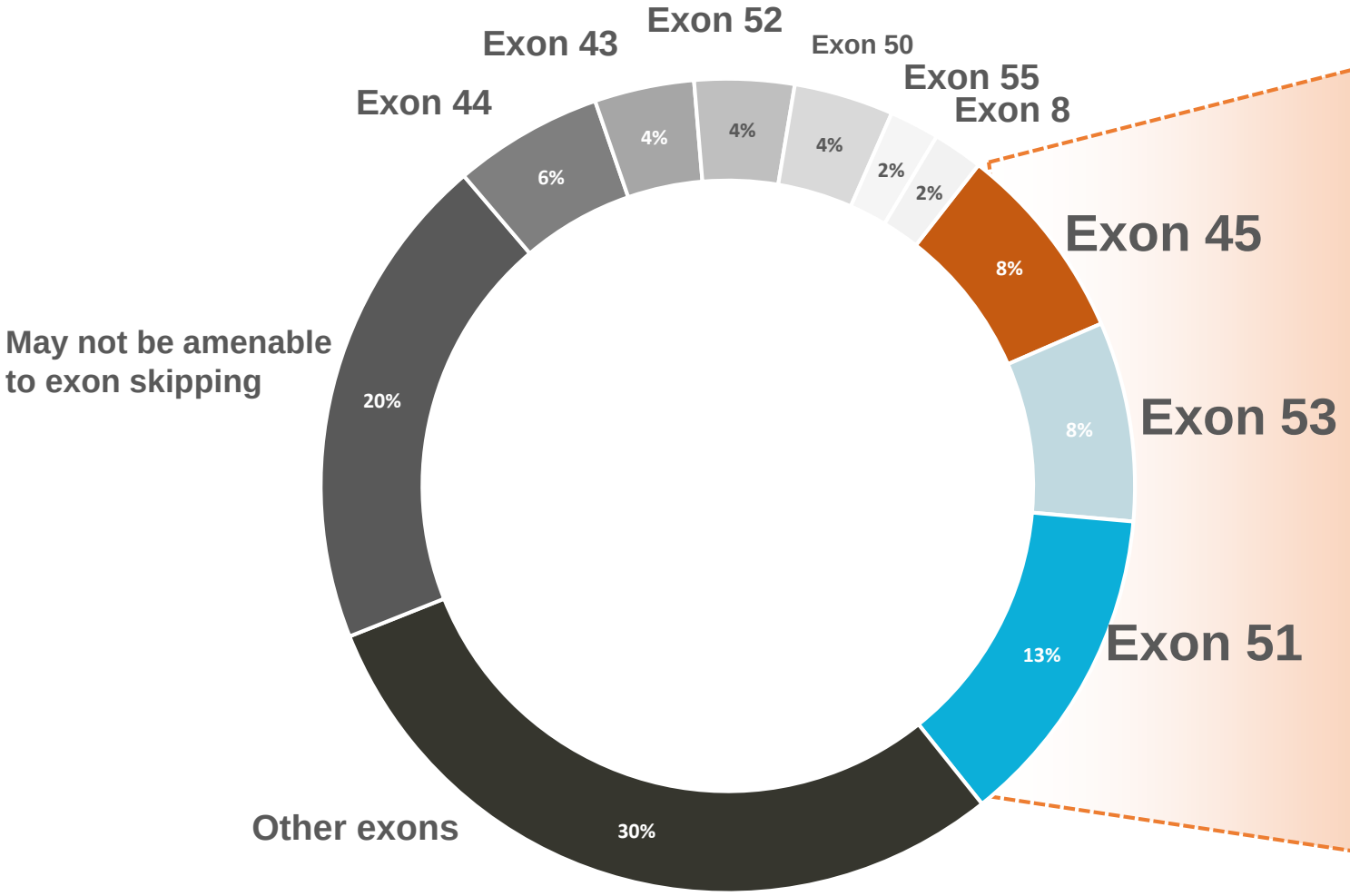
Stop codon readthrough

Translation does not stop at an early stop codon in the mRNA; it continues to the next STOP codon within the same reading frame (e.g., Ataluren).



Leckie and Yokota *Molecules* **2024**, *29*(11), 2658, Wilton-Clrak and Yokota. *Genes* **2022**, *13*(2), 257

The most common targets of exon skipping in DMD



Skipping exons 45, 53, and 51 together will address approximately

30%

of *DMD* mutations

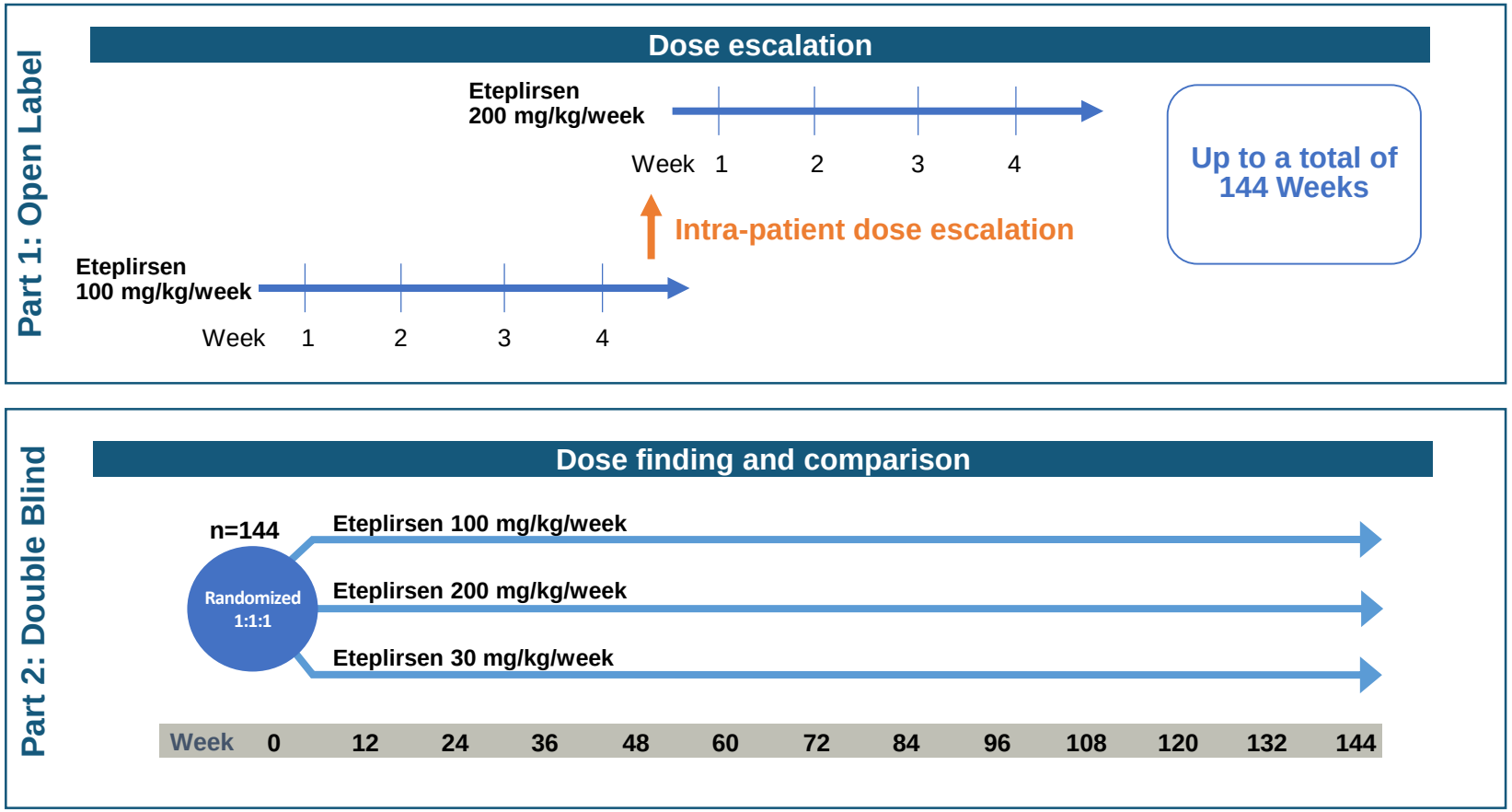
- DMD=Duchenne muscular dystrophy .
- Aartsma-Rus A, et al. *Hum Mutat.* 2009;30:293-9. Note: The mutation data highlighted above is derived from the Leiden Duchenne Muscular Dystrophy Mutation Database, which includes a limited data set; numbers are approximate and therefore may not add up to 100%.

MIS51ON (Study 402): A randomized, double-blind, dose finding and comparison study of a high dose of eteplirsen, preceded by an open-label dose escalation

- **Study population**
- 160 patients in total
- Age 4–13 years
- Ambulatory, able to perform TTR ≤ 10s
- On a stable dose or dose equivalent of oral corticosteroids for at least 12 weeks prior to randomization

Primary endpoints

- **Part 1 and 2**
  - Safety and tolerability
- **Part 2**
  - Change from baseline at Week 144 in NSAA total score



NSAA=North Star Ambulatory Assessment; TTR=time to rise.
ClinicalTrials.gov Identifier: NCT03992430.

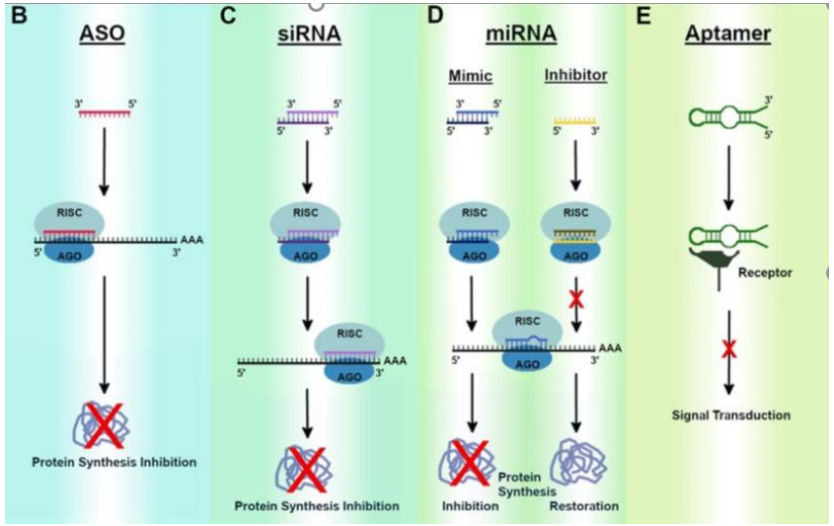
Exon skipping developments in DMD

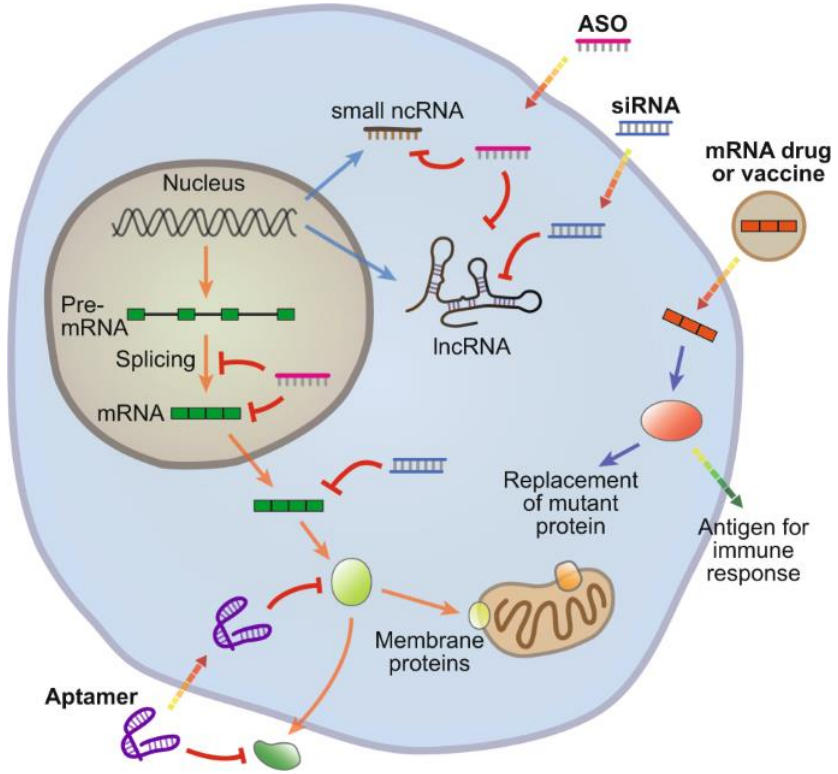
Vesleteplirsen	Sarepta Therapeutics	PPMO (R_6Gly)	51	Phase II
WVE-N531	Wave Life Sciences	PS/PN stereoselective	53	Phase Ib/II
Renadirsen	Daiichi Sankyo	2'OMe/ENA mixmer	45	Phase II
AOC 1044	Avidity Biosciences	PMO–antibody conjugate	44	Phase I/II
DYNE-251	Dyne Therapeutics	PMO–Fab fragment conjugate	51	Phase I/II
ENTR-601-44	Entrada Therapeutics	PPMO (EEV)	44	Preclinical
PGN-EDO51	PepGen	PPMO (EDO)	51	Phase I
SQY51	SQY Therapeutics	Tricylco-DNA	51	Phase I/II (in 2023)

EDO, enhanced delivery oligonucleotide; EEV, enhanced endosomal escape vehicle; ENA, ethylene-bridged nucleic acid; PMO, phosphorodiamidate morpholino oligonucleotide; PPMO, peptide–PMO conjugate; PS/PN, phosphorothioate and phosphoryl guanidine linkages.

Roberts, T.C. et al *Nat Rev Drug Discov 2023;* **22**, 917–934

The types of RNA targeted therapies





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miRNA: small RNAs, either inhibit protein synthesis when they bind to an mRNA, or free up mRNA by binding to the miRNA that represses the translation

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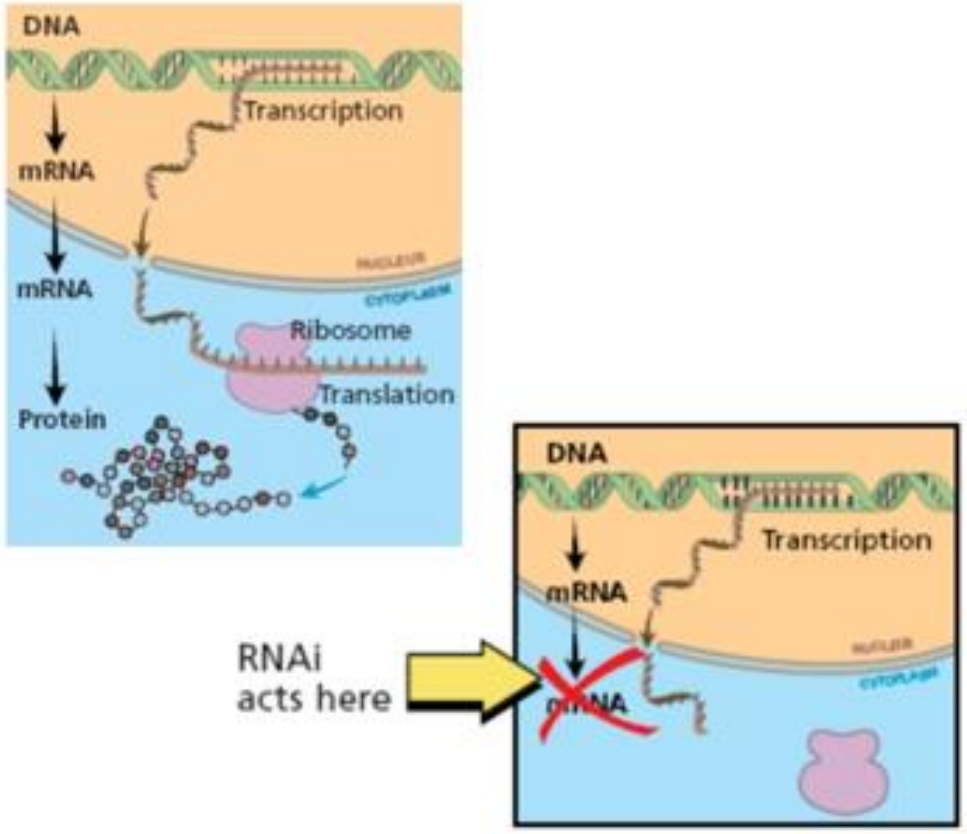
RNA interference



In RNA interference, RNA in double-stranded form breaks down the mRNA for a specific gene, thus stopping production of protein.

Role in the cell:

Defense mechanism:
Defense against infections
Defense against transposons and other insertional elements

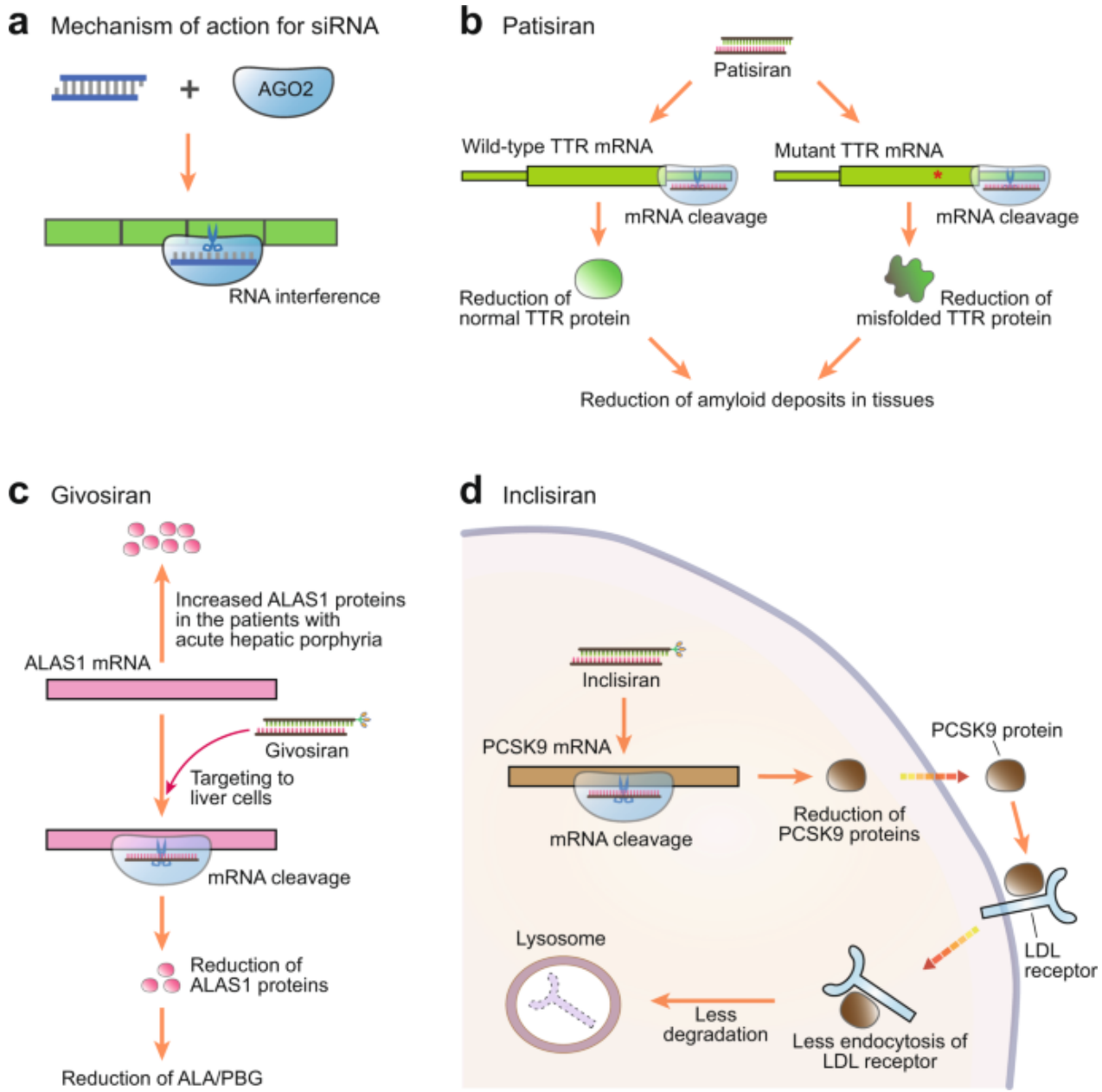
Genome wide regulation
RNA i plays in regulating development and genome maintenance

The mode of action of siRNAs

.

Four siRNA drugs (patisiran, givosiran, lumasiran and inclisiran) have been approved by FDA

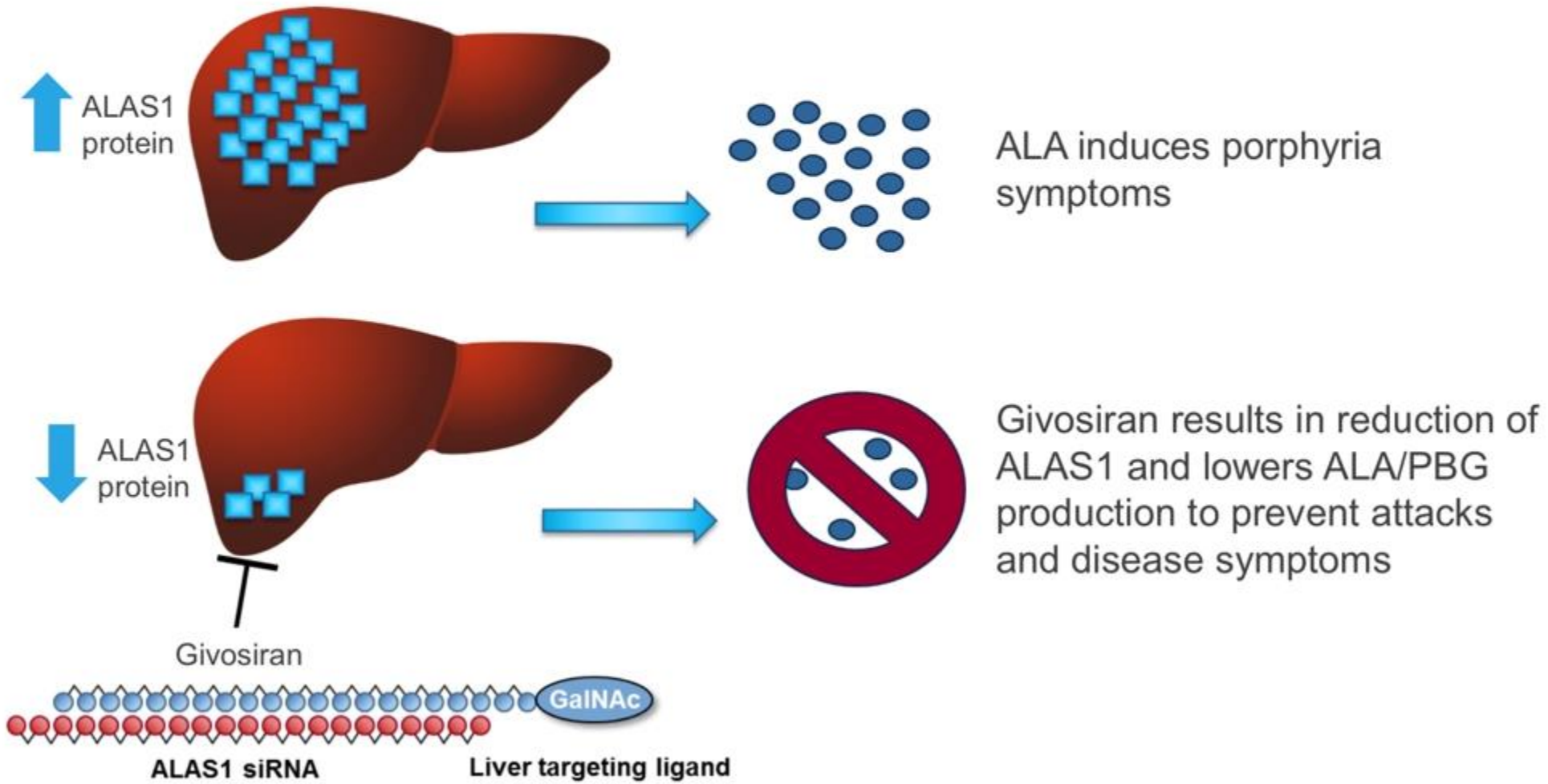
Six siRNA candidates (vutrisiran, fitusiran, cosdosiran, nedosiran, tivanisiran and teprasiran) are undergoing Phase III clinical trials



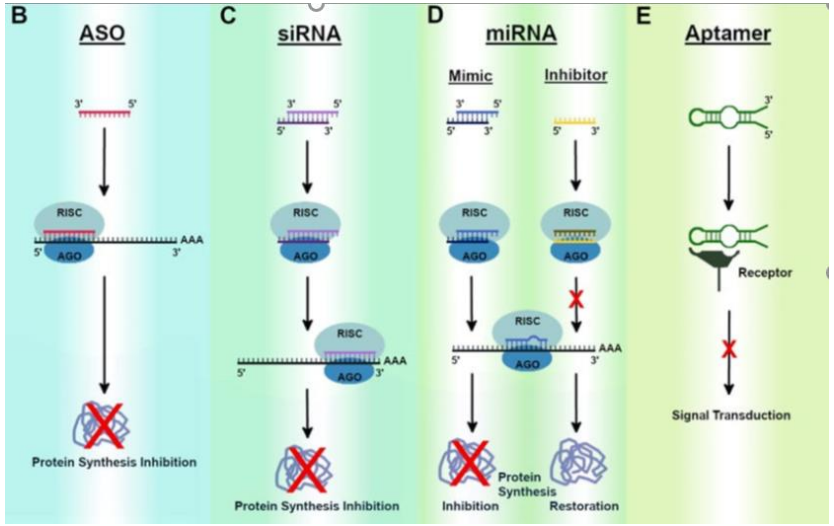
Givosiran: Investigational RNAi Therapeutic for AHP

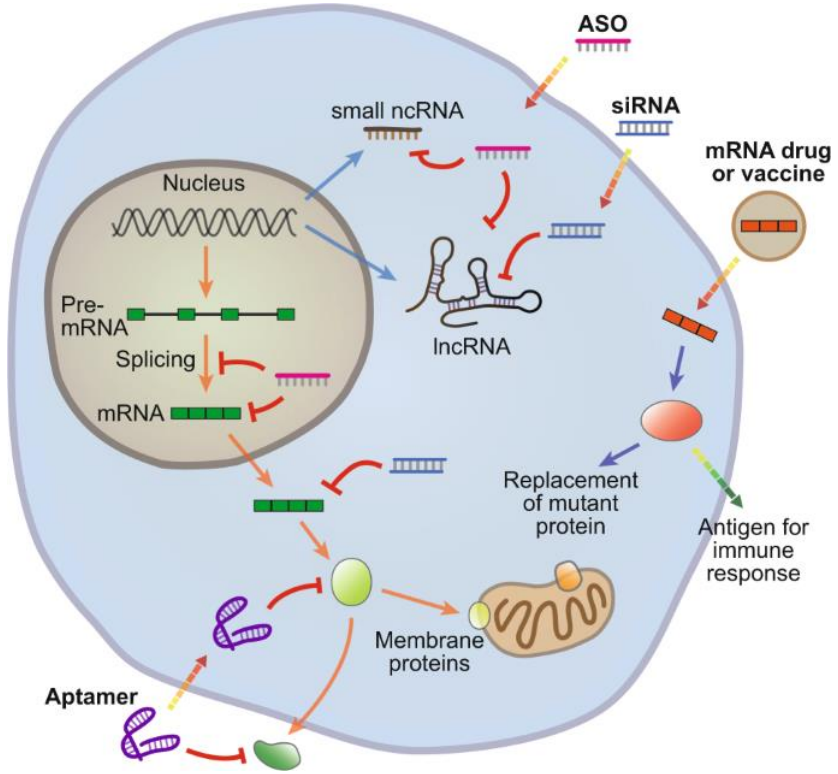
Therapeutic Hypothesis

- Reduction of Liver ALAS1 Protein to Lower ALA and PBG



The types of RNA targeted therapies





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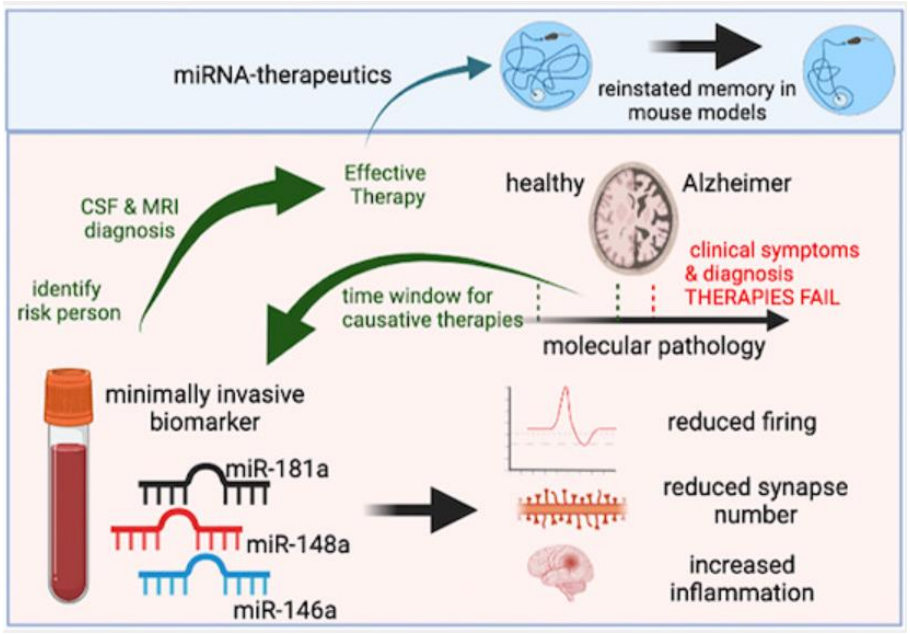
miRNA targeted therapies

miRNA inhibitors (Anti-miRs) and miRNA mimics can be used to down- or upregulate miRNAs.

1. Miravirsen (SPC3649) and RG-101 are anti-miRs targeting miR-122 for treating hepatitis C virus infection
2. miRNAs targeting neurodegeneration



doi.org/10.15252/emmm.202013659

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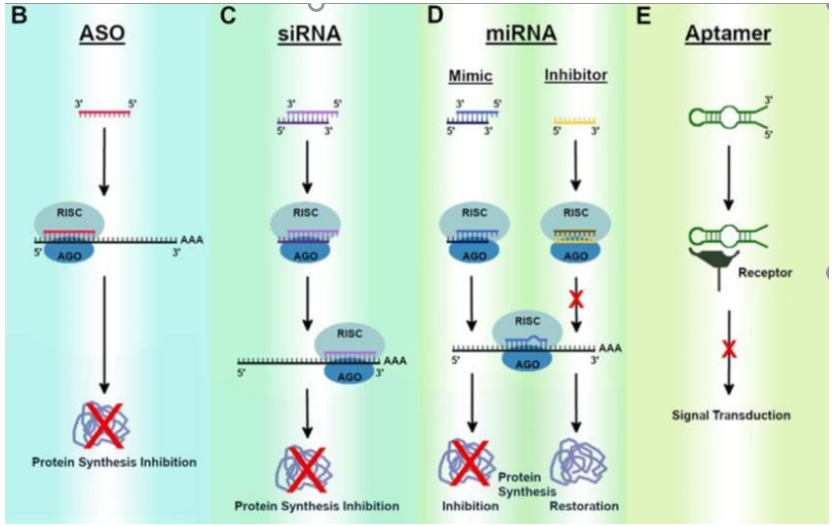

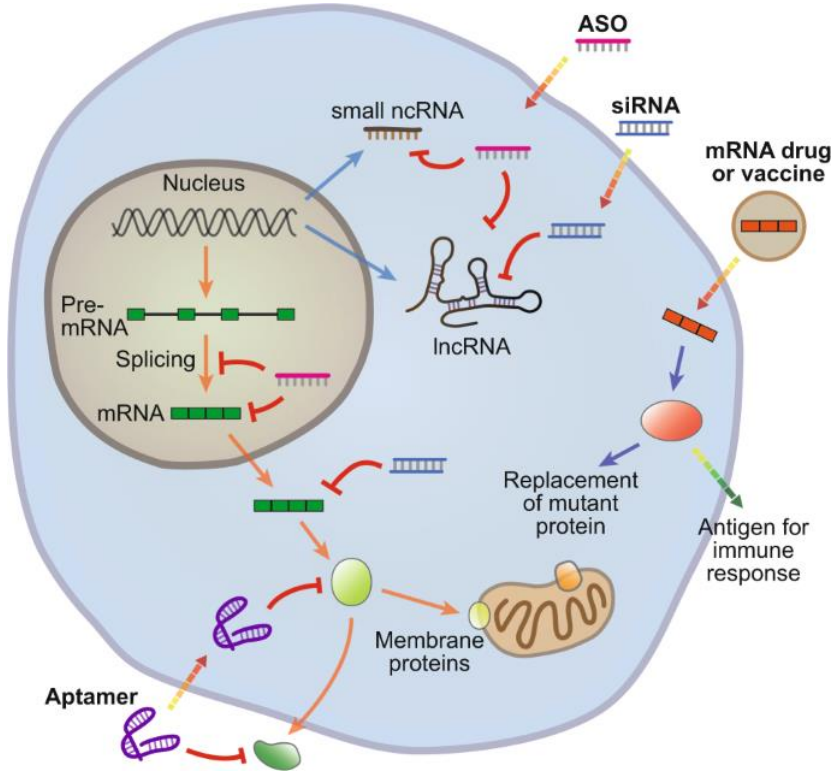

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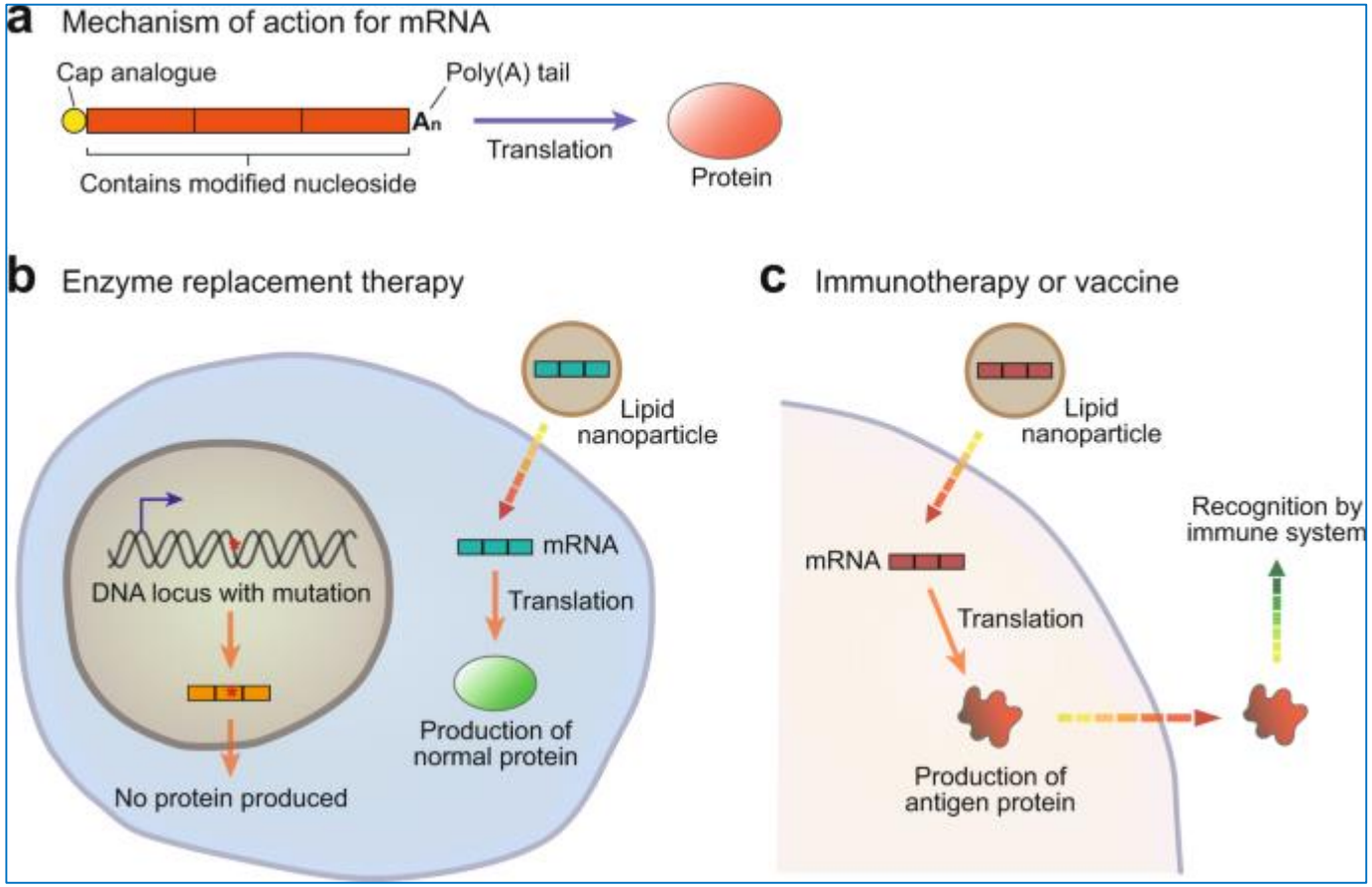
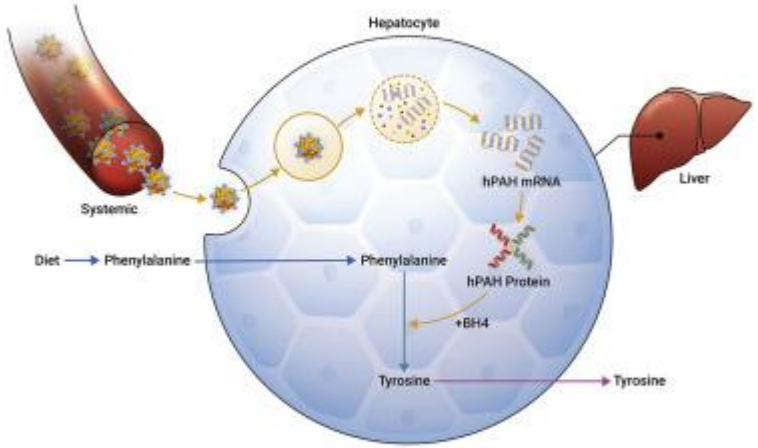
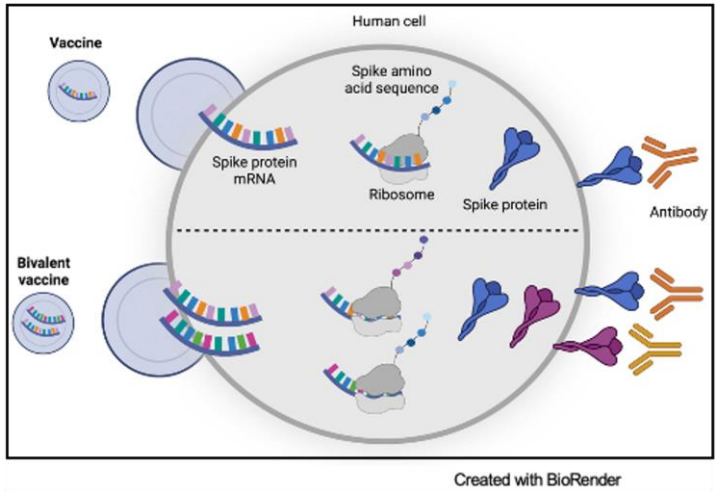
The mode of action of mRNA targeted drugs



PKU

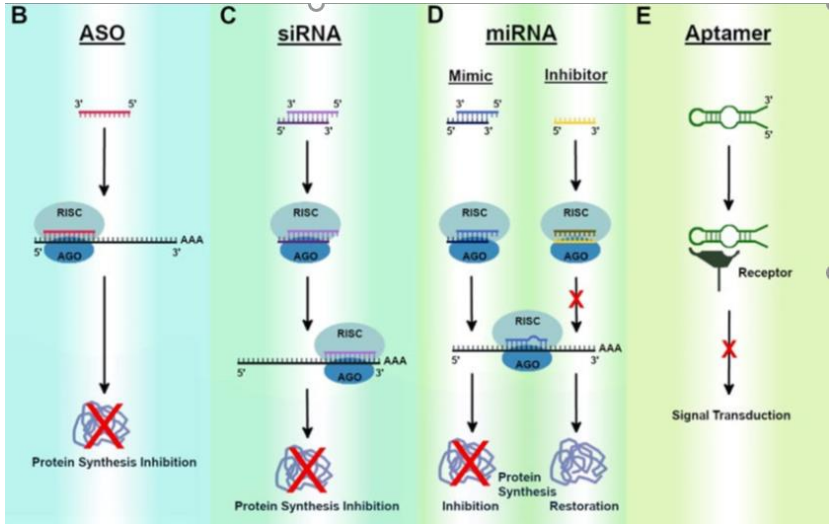


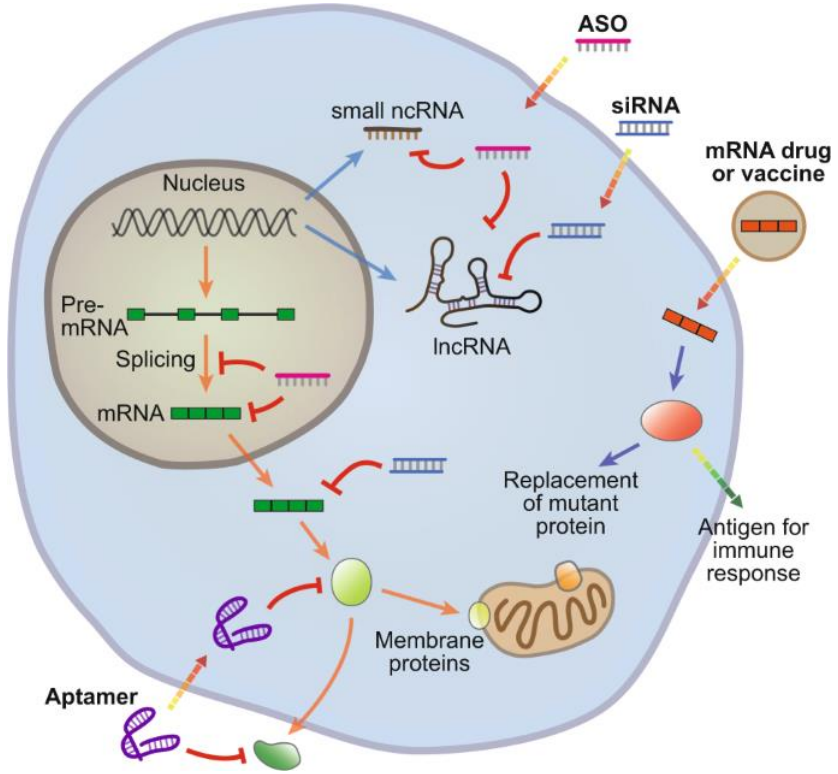
Vaccines



Comirnaty, Spikevax

The types of RNA targeted therapies





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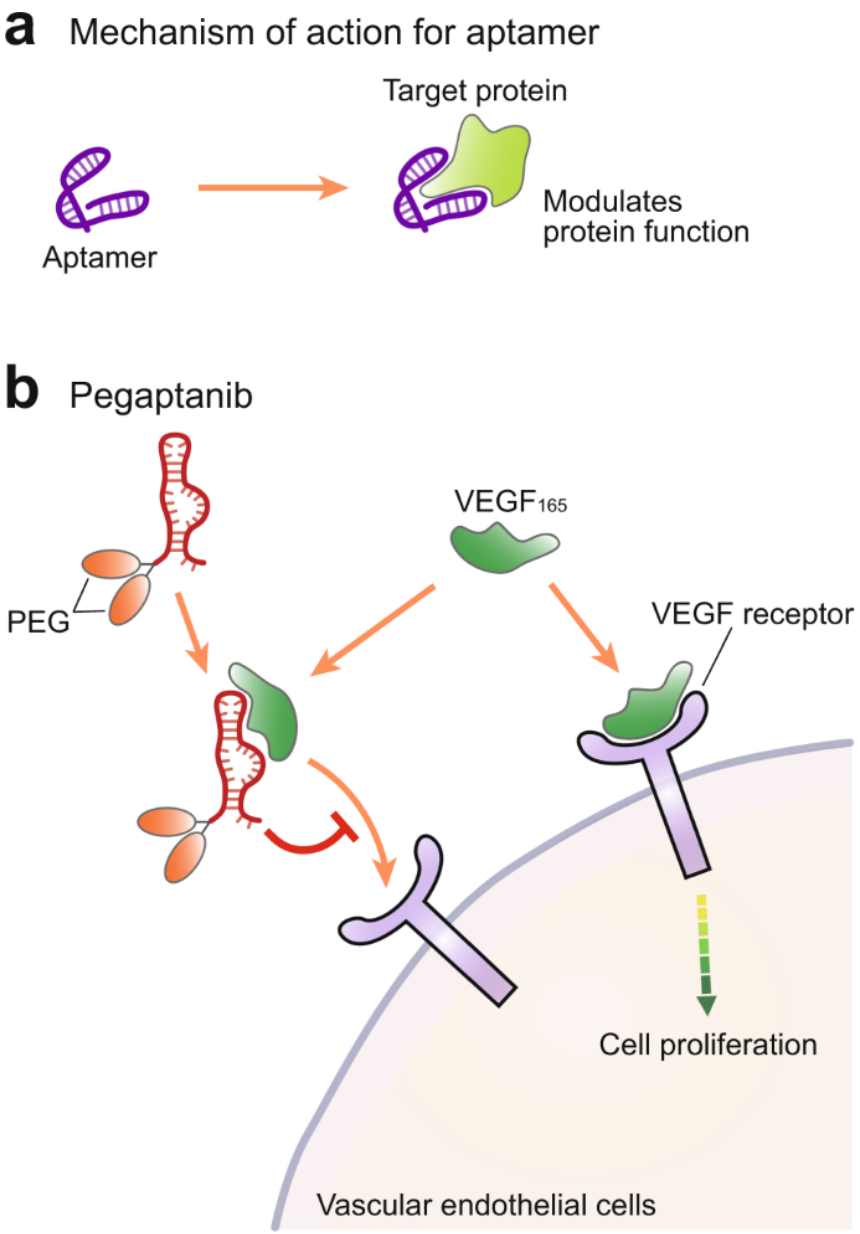
The mode of action of aptamer drugs

1. Antagonist aptamers disrupt the interaction between disease-associated targets, such as protein-protein or receptor-ligand interactions

2. Cell type-specific aptamers serve as carriers to deliver other therapeutic agents to the target cells or tissues

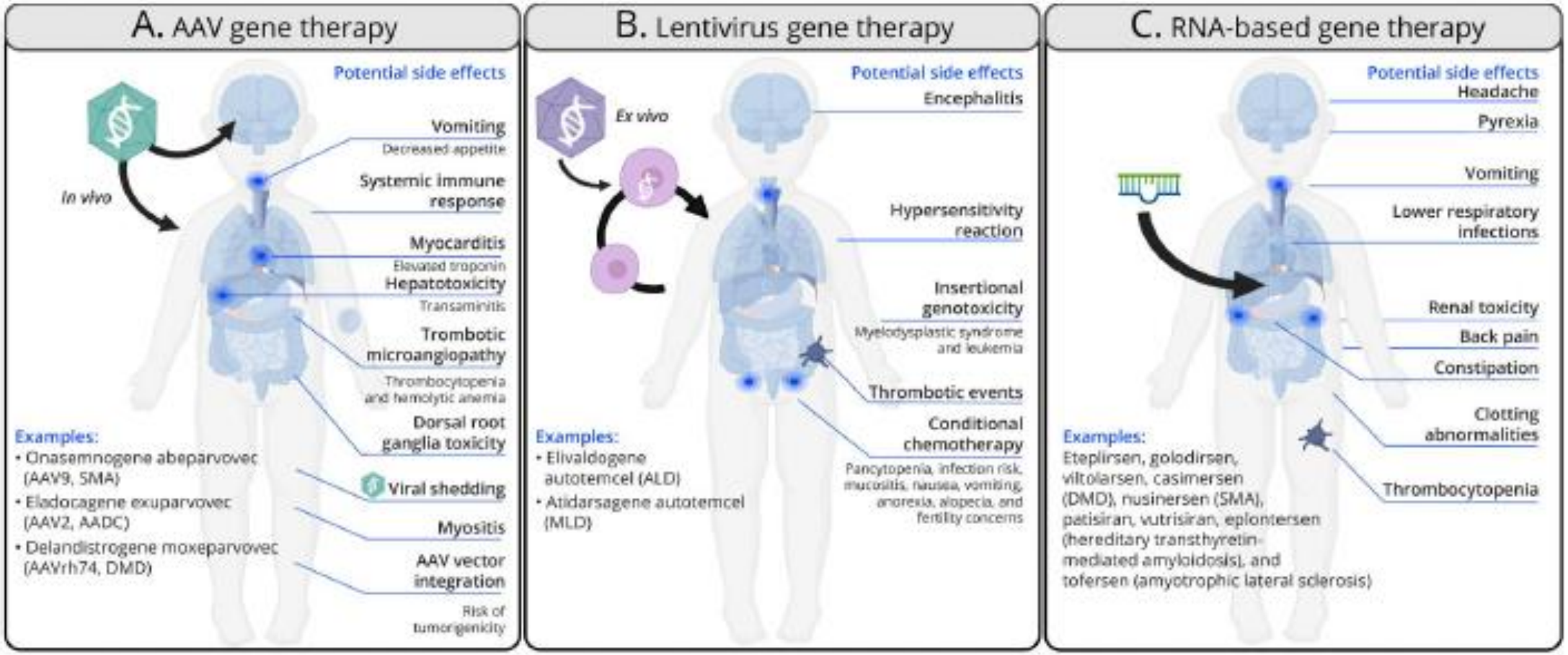
Pegaptanib (Macugen) was the first FDA-approval aptamer drug targeting VEGF to treat age-related macular degeneration.
Numerous other aptamers are in the preclinical or clinical development pipeline for possible treatment of diseases, such as visual disorders, oncology, neuroinflammation



RNA targeted therapies for neurogenetic disorders

Disease	Medication	Type of gene th.	Pt eligibility	Approved in
SMA	nusinersen	ASO- RNA	All SMA pts	USA, EU, UK, Canada, Japan, Brazil, Switzerland, Australia, South Korea, China, Argentina, Colombia, Taiwan, Turkey, Hong Kong, Israel
DMD	Eteplirsen	ASO-RNA	Exon 51 skipping	USA
	Golodirsen	ASO-RNA	Exon 53 skipping	USA
	Vitolarsen	ASO-RNA	Exon 53 skipping	USA, Japan
	Casimersen	ASO-RNA	Exon 45 skipping	USA
hTTR amyloidosis	Patisiran	siRNA	Polyneuropathy adults	USA, EU, Switzerland, Brazil, Japan
	Vutisiran	siRNA	Polyneuropathy adult	USA, EU, Switzerland, Brazil, Japan
	Eplontersen	ASO-RNA	Polyneuropathy adult	USA
ALS	Tofersen	ASO-RNA	SOD1 associated ALS	USA, EU

The side effects of the DNA and RNA targeted therapies



doi:10.1212/NXG.0000000000200229

Advantages

- Easy to generate high-purity RNA
- The production is much faster and cheaoer than the production of either traditional small molecule drugs or recombinant proteins
- The manufacturing process is independent of the RNA sequence.
- Compared to DNA-based gene therapy, RNA has a superior safety profile and the regulatory requirements are easy to follow
- RNA doesn’t integrate into the host genome

.

Limitations

- Specificity: off-target and on-target effects due to similarity or an overdose of the RNA drugs
- Delivery: the instability of RNA, the inefficient intracellular delivery, and the efficiency of crossing the BBB
- Tolerability: immune reaction that is caused by the activation of pathogen-associated molecular pattern receptors, such as the Toll-like receptors.

The evasion of innate immune activation, increase RNA stability, and development of targeted delivery can facilitate the growth of RNA therapeutics.

Biologics for the treatment of genetic disorders

DNA targeted therapies

- Gene replacement – replace the defective gene
- Gene transfer – upregulate therapeutic proteins
- Gene editing – correct the altered gene (CRISPR/CAS9)
- Gene addition – introduce a new gene

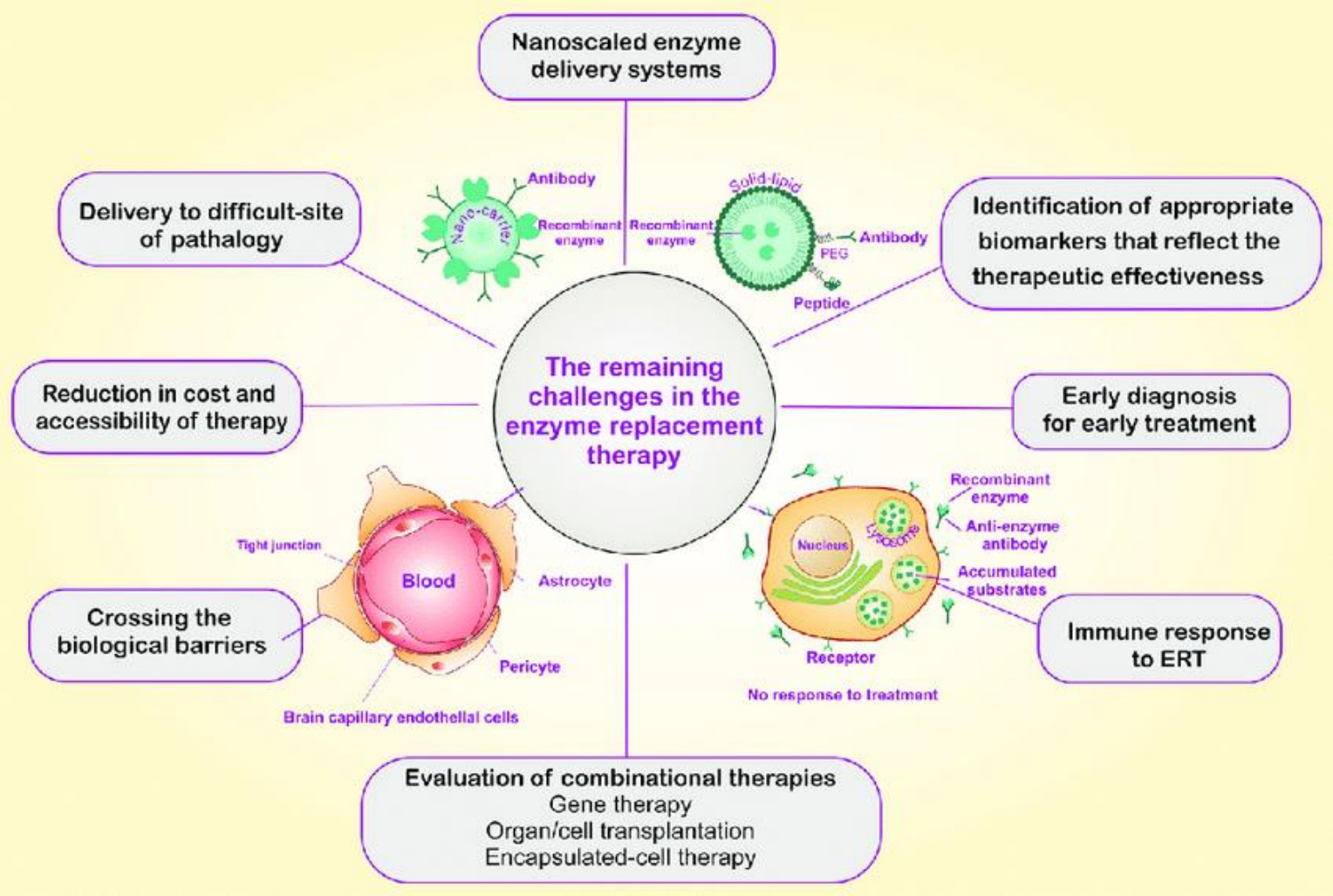
RNA targeted therapies

- Exon skipping with antisense oligonucleotids (ASOs)
- Translation correction - increase protein production with ASOs
- The inactivation of the mRNA with miRNA/ASO

Protein replacement therapies

- To replace the missing protein

Challenges of the ERTs



Enzyme replacement therapies in LSDs

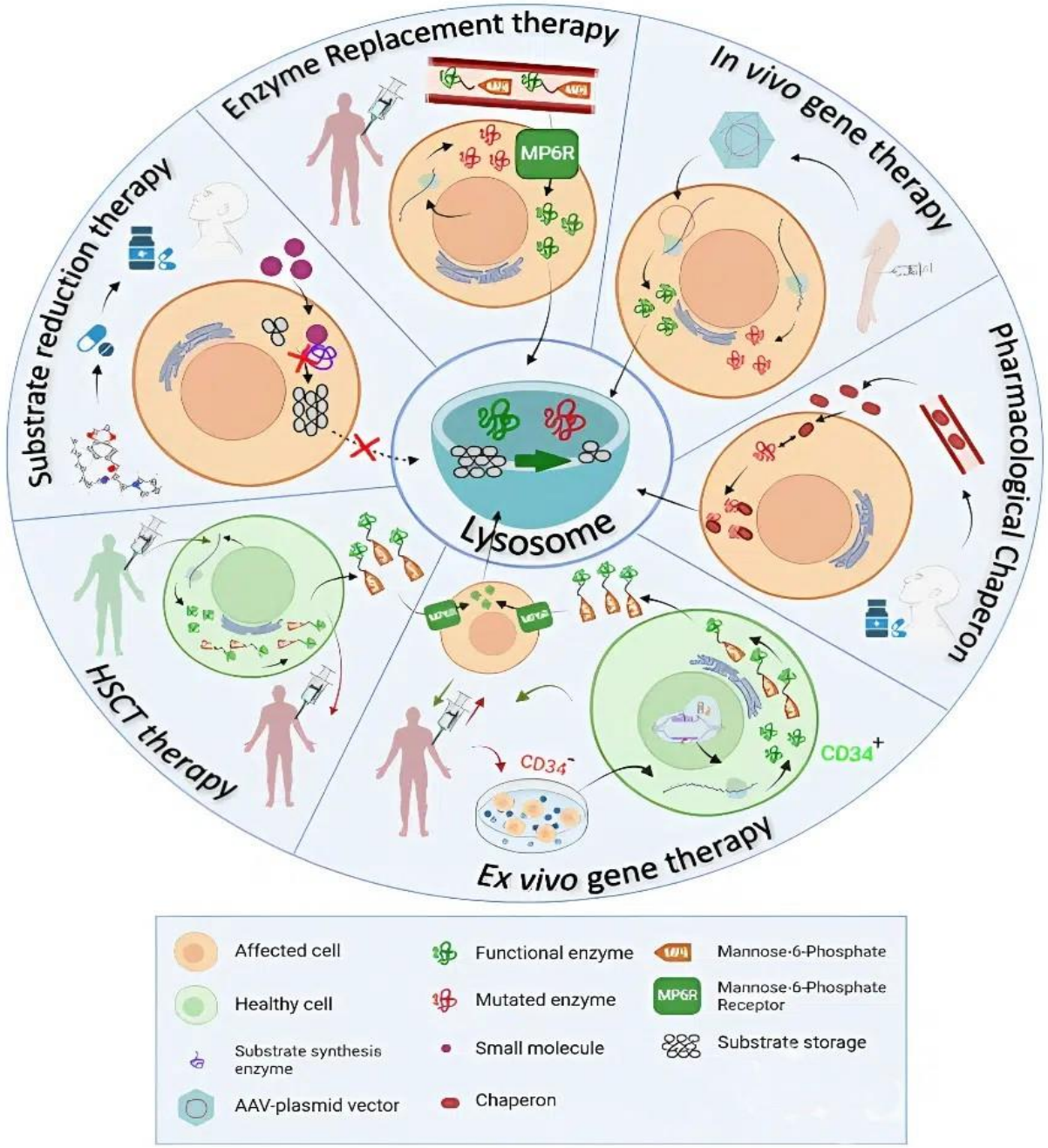
- Gaucher: Glucocerebrosidase deficiency - Imiglucerase (Cerezyme), Velaglucerase alfa (VPRIV), Taliglucerase alfa (Elelyso)
- **Fabry:** α-Galactosidase A deficiency-Agalsidase alfa (Replagal), Agalsidase beta (Fabrazyme), Pegunigalsidase alfa (Elfabrio)
- **Pompe:** Acid α-glucosidase deficiency - Alglucosidase alfa (Myozyme, Lumizyme), Avalglucosidase alfa (Nexviazyme)
- **Lysosomal Acid Lipase Deficiency**: Lysosomal acid lipase deficiency - Sebelipase alfa (Kanuma)
- **Alpha-Mannosidosis:** α-Mannosidase deficiency - Velmanase alfa (Lamzede)
- **Neuronal Ceroid Lipofuscinosis**: Tripeptidyl peptidase 1 deficiency - Cerliponase alfa (Brineura)
- **MPS I**: α-L-Iduronidase deficiency- Laronidase (Aldurazyme)
- **MPSII:** Iduronate-2-sulfatase deficiency- Idursulfase (Elaprase), Idursulfase beta (Hunterase, in some regions)
- **MPSIVA**: N-acetylgalactosamine-6-sulfatas deficeincy - Elosulfase alfa (Vimizim)
- **MPSVI**: Arylsulfatase B deficeincy - Galsulfase (Naglazyme)
- **MPSVII**: β-Glucuronidase deficiency - Vestronidase alfa (Mepsevii)

Enzyme replacement therapies in non-LSDs

Hypophosphatasia: Tissue-nonspecific alkaline phosphatase (TNSALP) deficiency - Asfotase alfa (Strensiq)

Adenosine Deaminase Deficiency (ADA-SCID): Adenosine deaminase (ADA) deficiency - Pegademase bovine (Adagen), Elapegademase-lvlr (Revcovi)

Therepautic options of LSDs



Key messages

- There are several disease-modifying causal treatments available for many hereditary diseases.
- Biologics are important tools of targeted therapy and have become part of the standard of care today.
- To evaluate the effectiveness of new molecular therapies, collecting real-world data is essential, thus both patients and clinicians play a key role in health technology assessments.
- Many more new therapies are expected in the future.
- Successful clinical trials require genetically stratified, committed patients and well-prepared clinicians.


Thank you for your attention!
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