Understanding the genetic code and approaches to gene directed therapies

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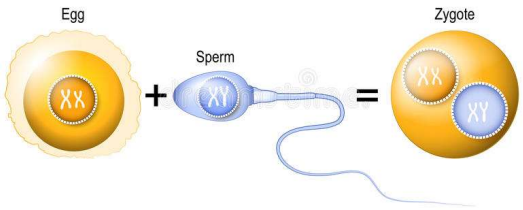
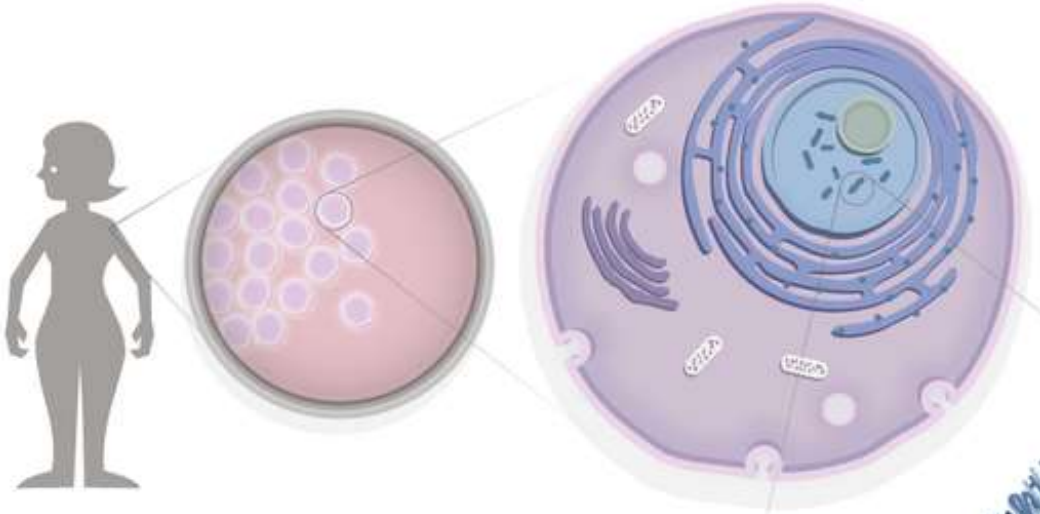
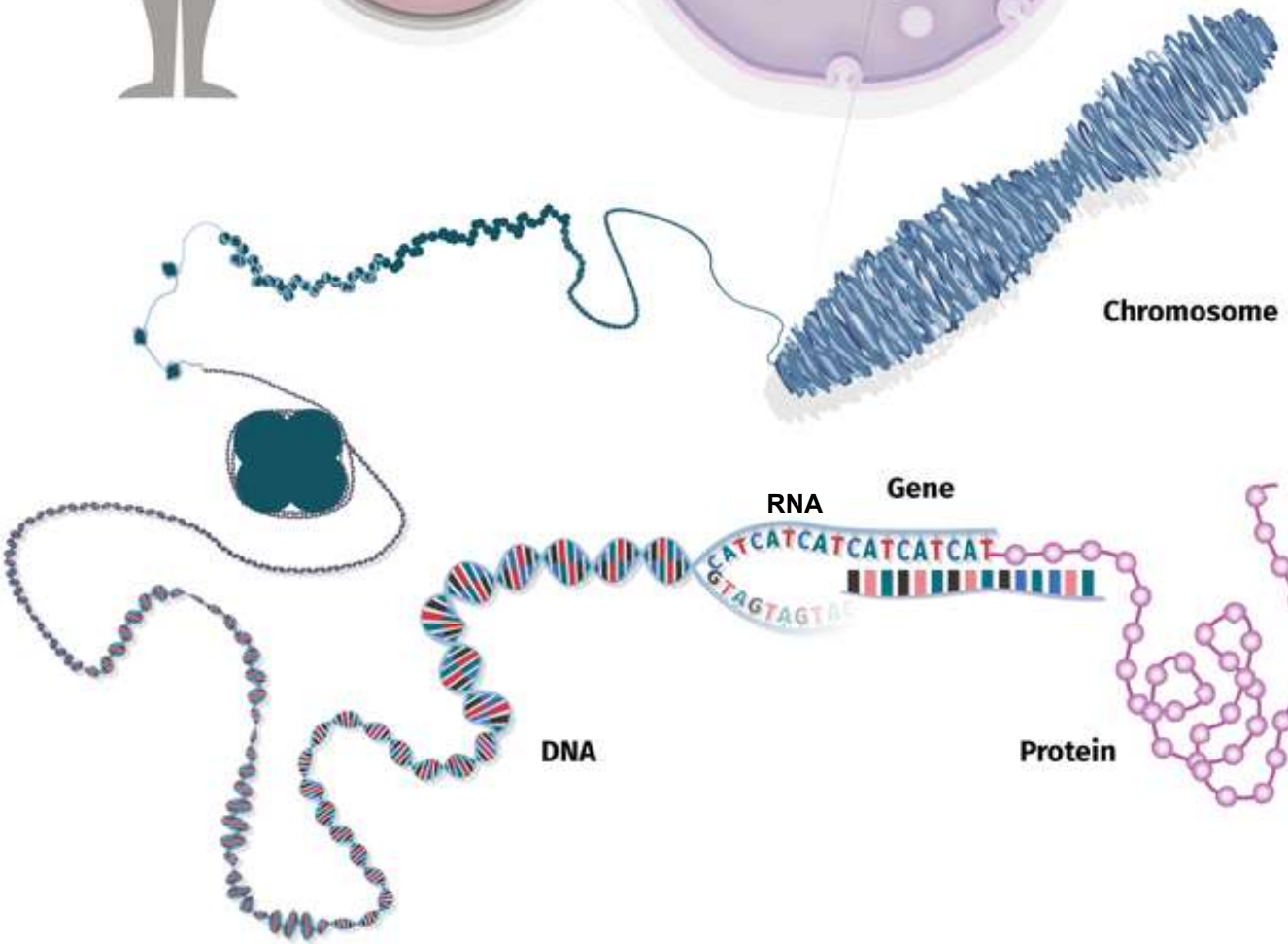
University of Alabama at Birmingham

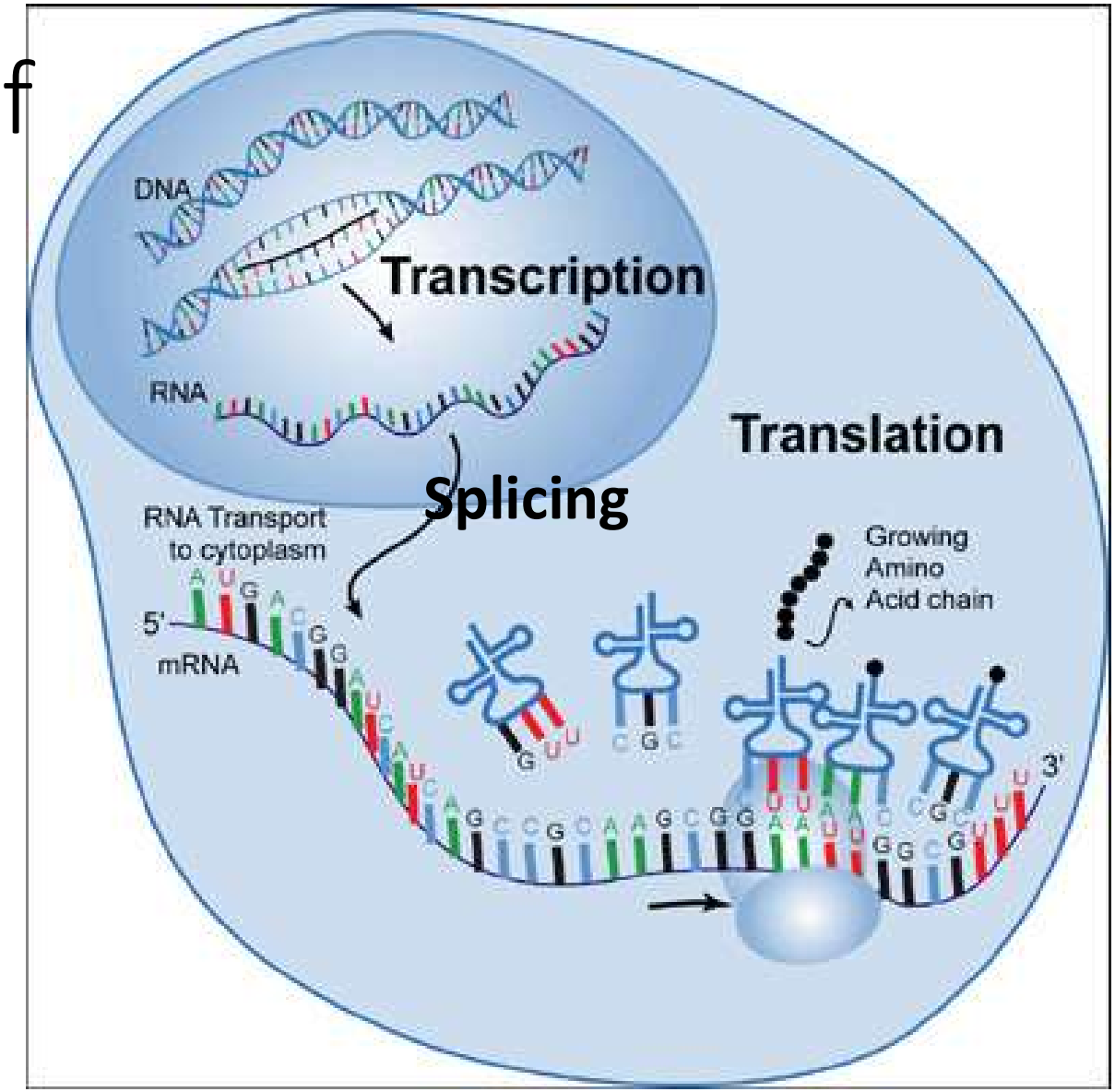
Response Evaluation In Neurofibromatosis Schwannomatosis
INTERNATIONAL COLLABORATION


Egg
Sperm
Zygote
XX
XY
XX
XY

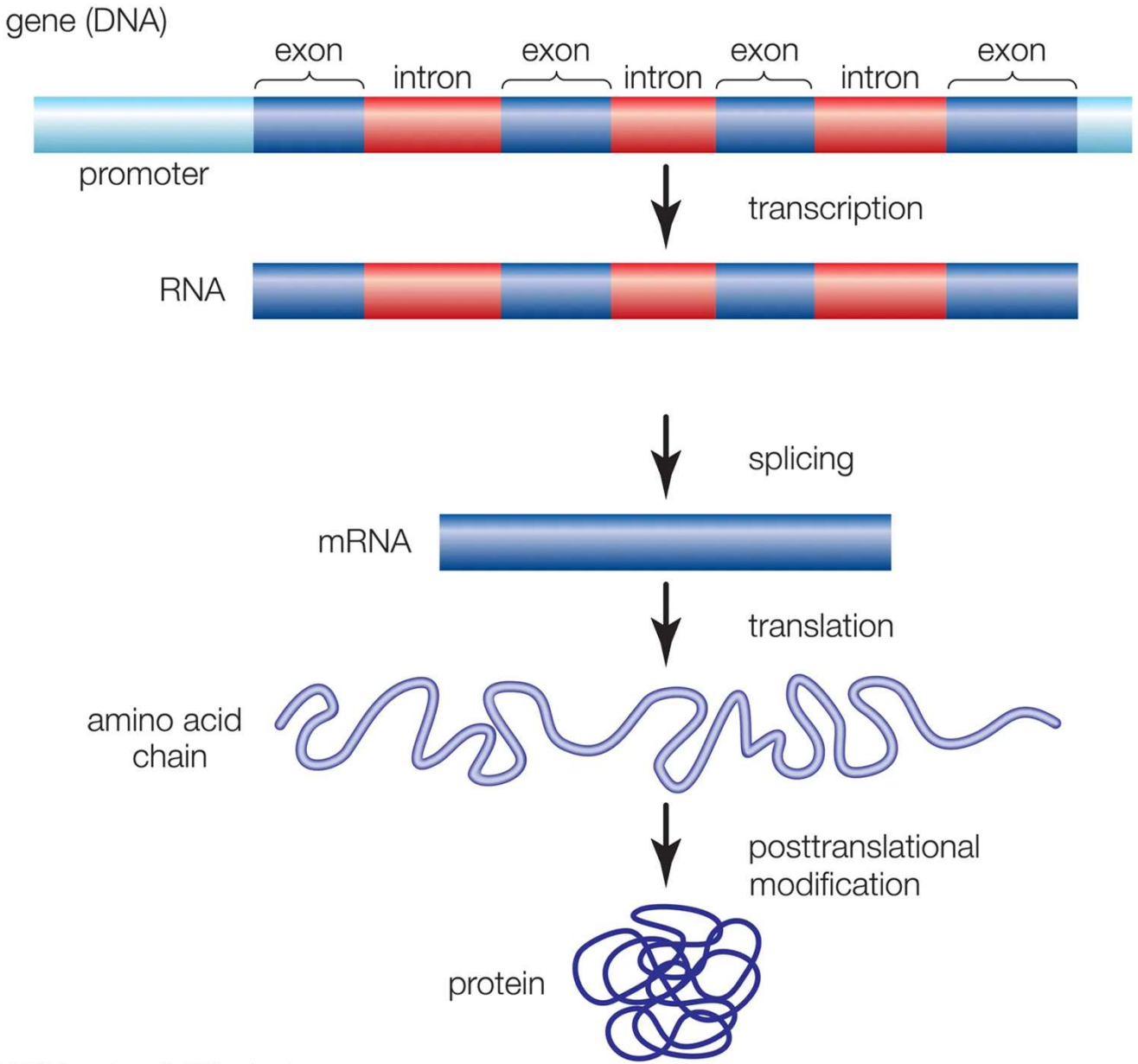

Cell


Chromosome
Gene
RNA
DNA
Protein

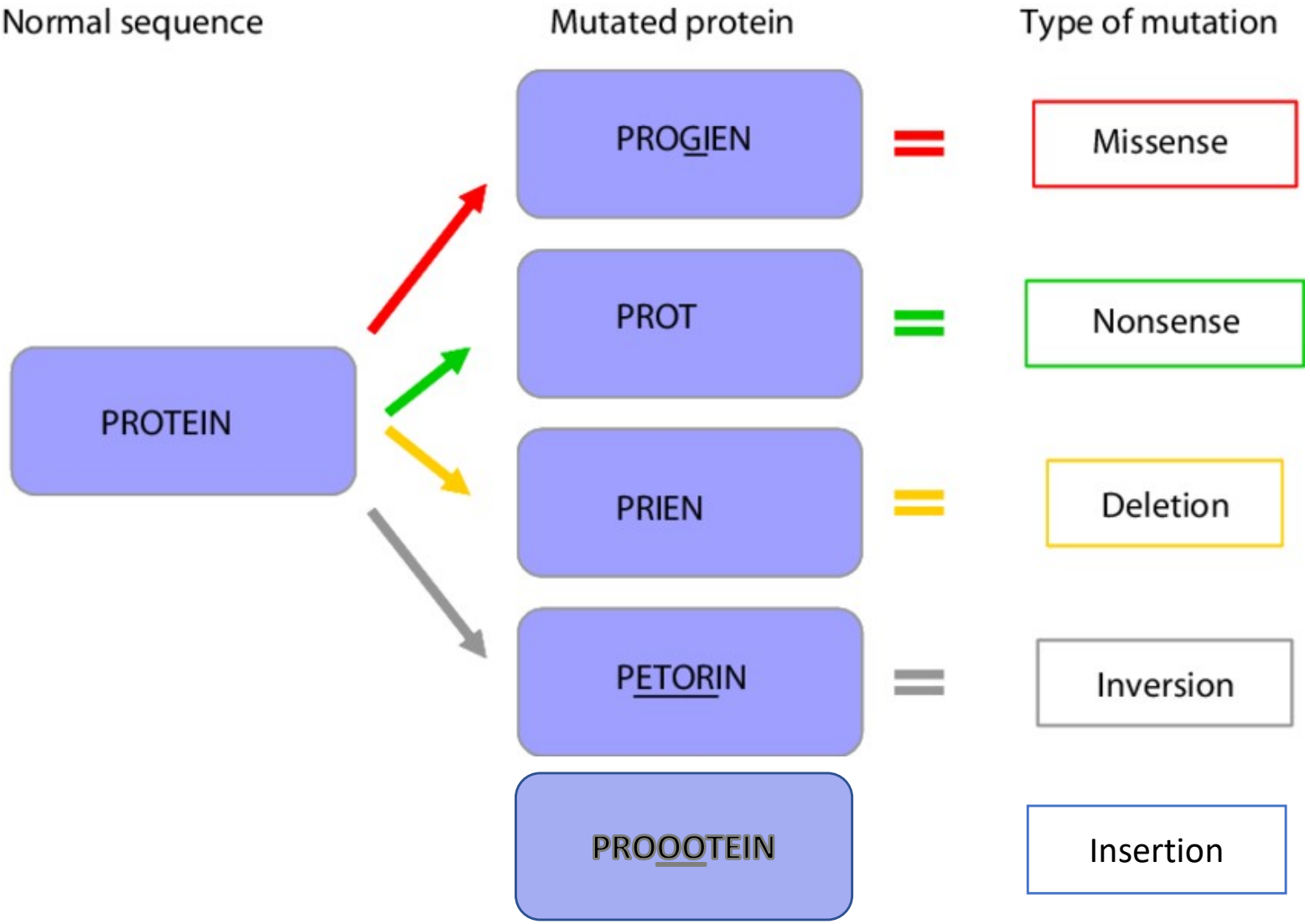
Central Dogma of Molecular Biology

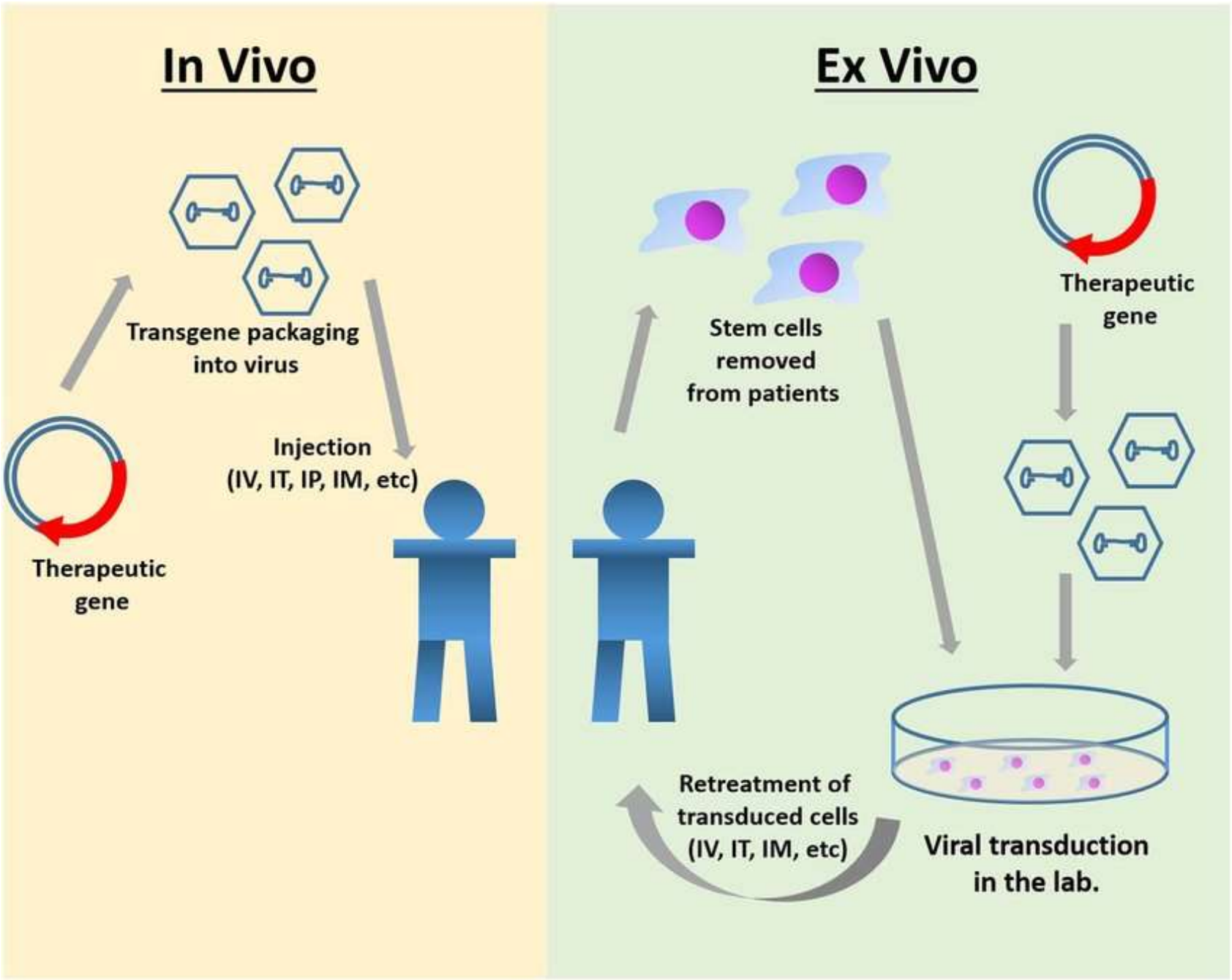


Gene Structure



Variation


Normal sequence
Mutated protein
Type of mutation
PROTEIN
PROGIEN
PROT
PRIEN
PETORIN
PROOOTEIN
Missense
Nonsense
Deletion
Inversion
Insertion


In Vivo
Ex Vivo
Transgene packaging into virus
Therapeutic gene
Injection (IV, IT, IP, IM, etc)
Stem cells removed from patients
Therapeutic gene
Retreatment of transduced cells (IV, IT, IM, etc)
Viral transduction in the lab.

Somatic mutations

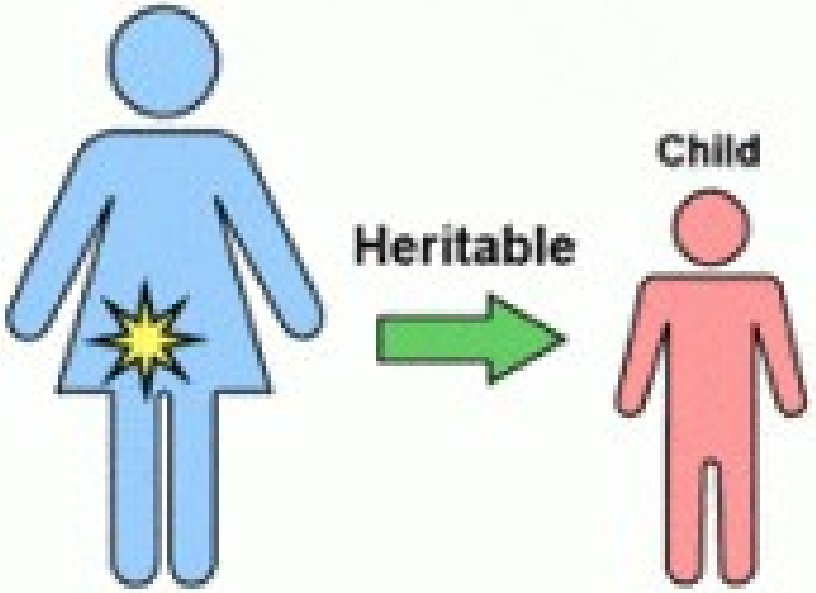
- Occur in *nongermline* tissues
- Cannot be inherited

Nonheritable

Mutation in tumor only
(for example, breast)

Germline mutations

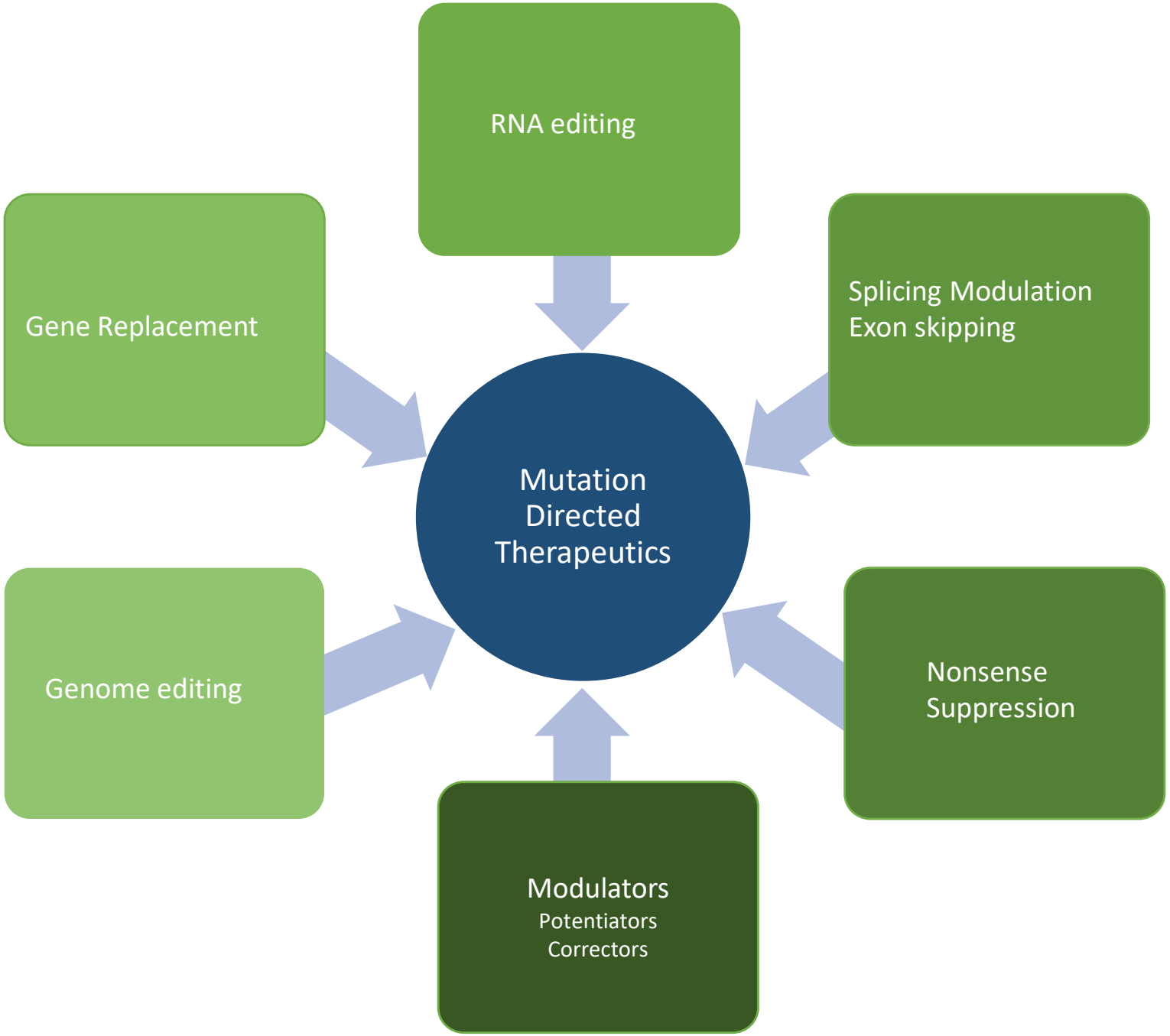
- Present in egg or sperm
- Can be inherited
- Cause cancer family syndrome

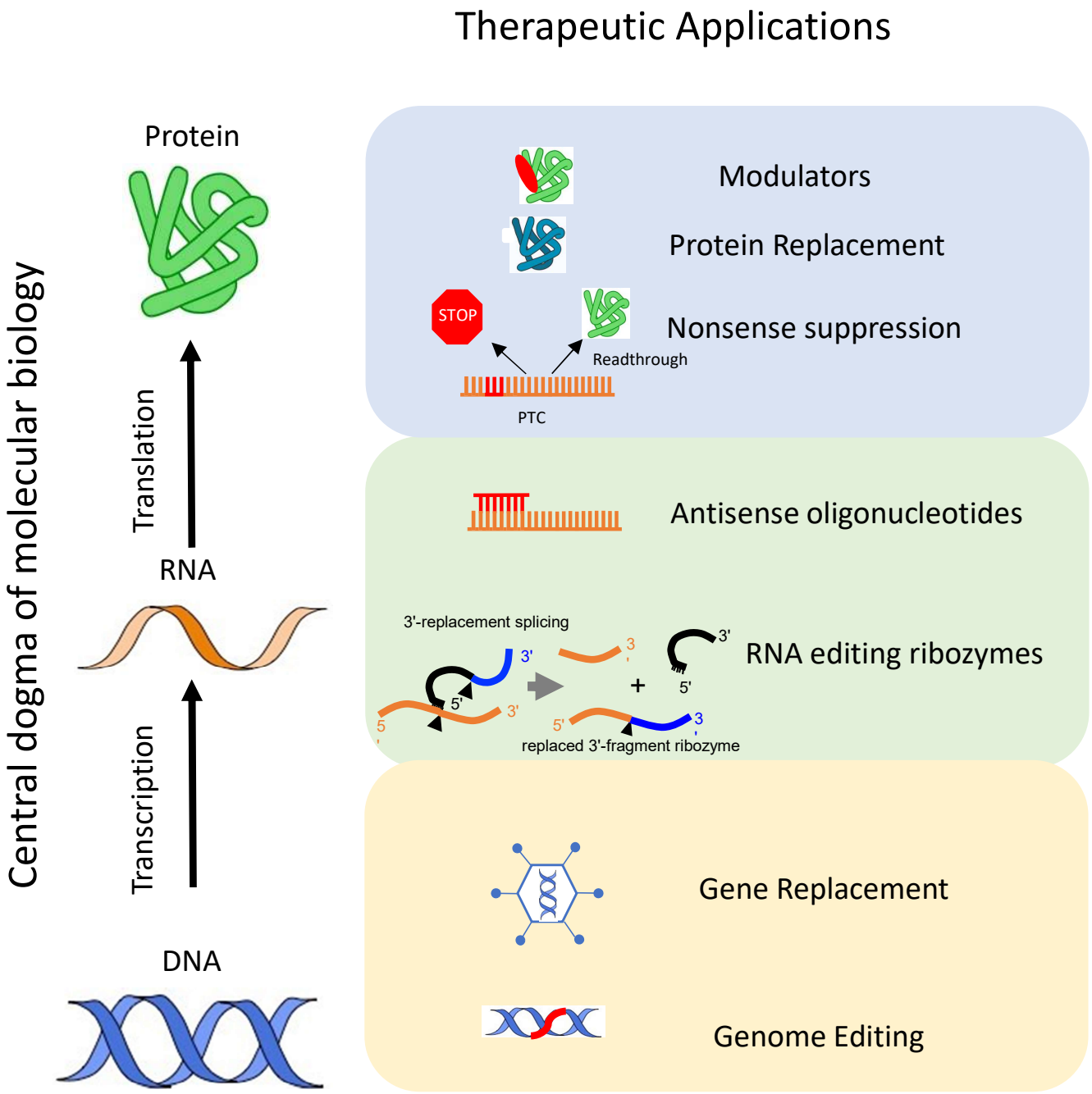


Mutation in
egg or sperm

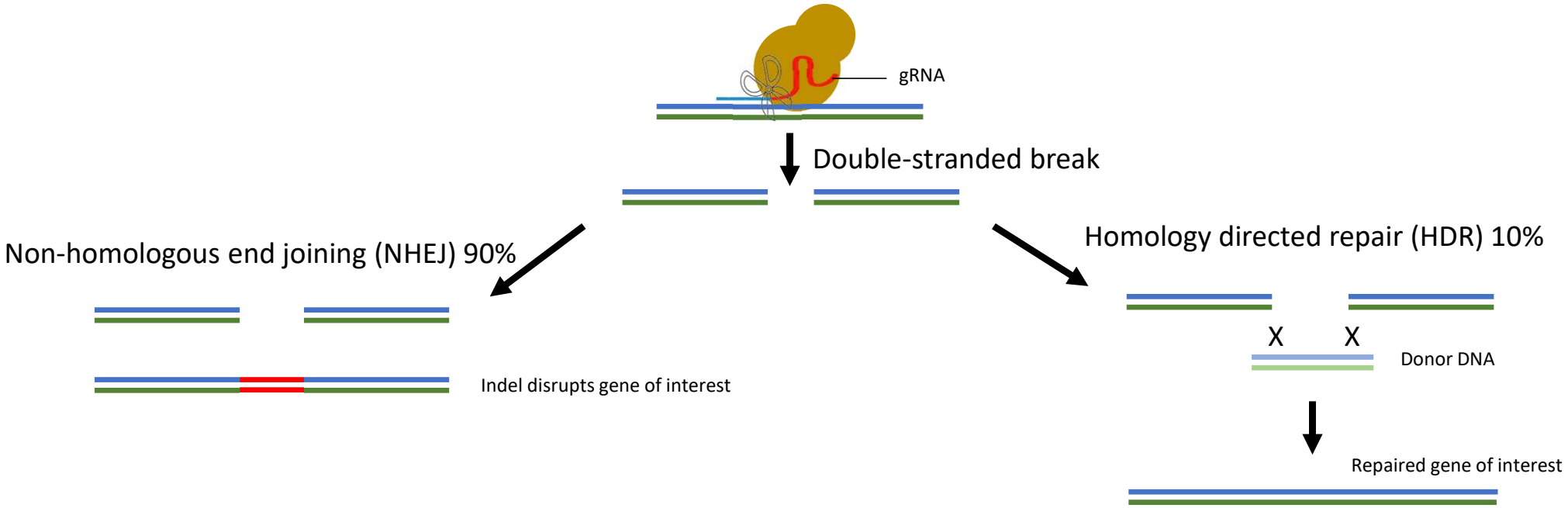
All cells
affected in
offspring

Adapted from the National Cancer Institute and the American Society of Clinical Oncology

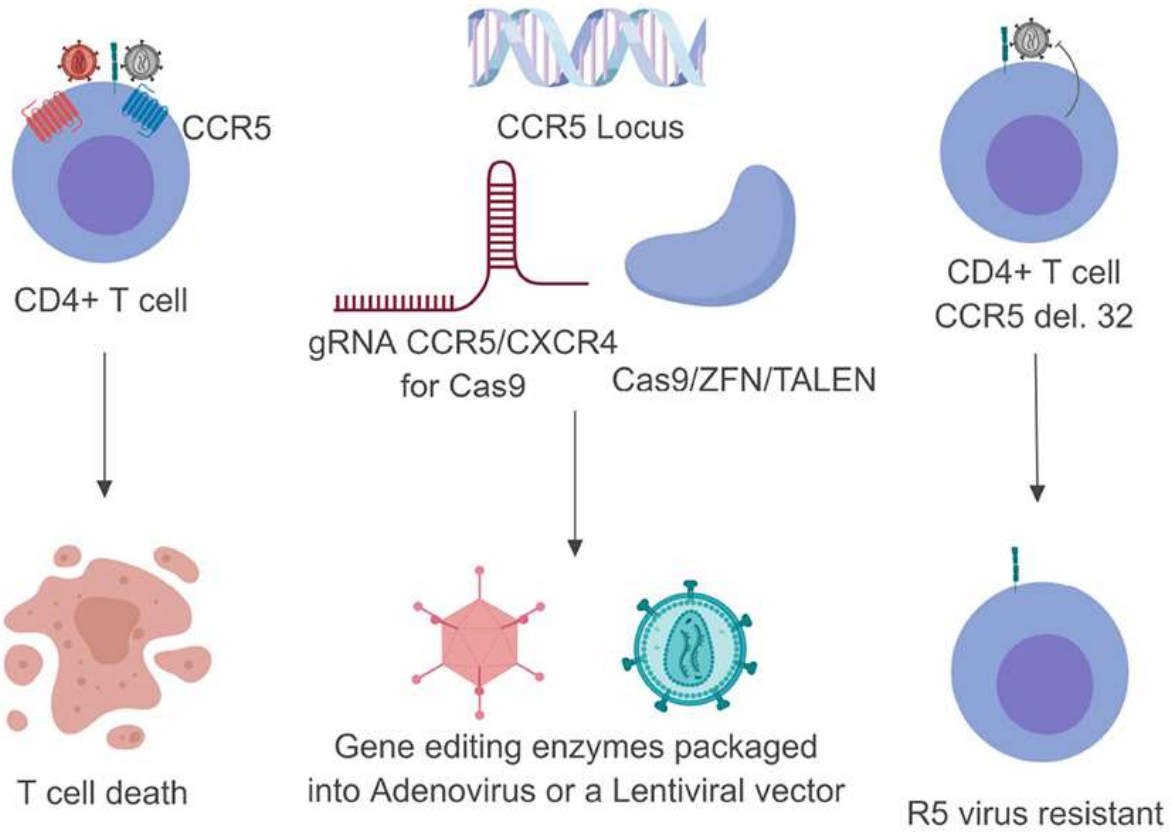

RNA editing
Gene Replacement
Splicing Modulation
Exon skipping
Mutation
Directed
Therapeutics
Genome editing
Nonsense
Suppression
Modulators
Potentiators
Correctors


Therapeutic Applications
Central dogma of molecular biology
Protein
Translation
RNA
Transcription
DNA
Modulators
Protein Replacement
STOP
Readthrough
PTC
Nonsense suppression
Antisense oligonucleotides
3'-replacement splicing
RNA editing ribozymes
replaced 3'-fragment ribozyme
Gene Replacement
Genome Editing

CRISPR/Cas9 Gene Editing



Example CRISPR/Cas9



CRISPR/Cas9

Advantages

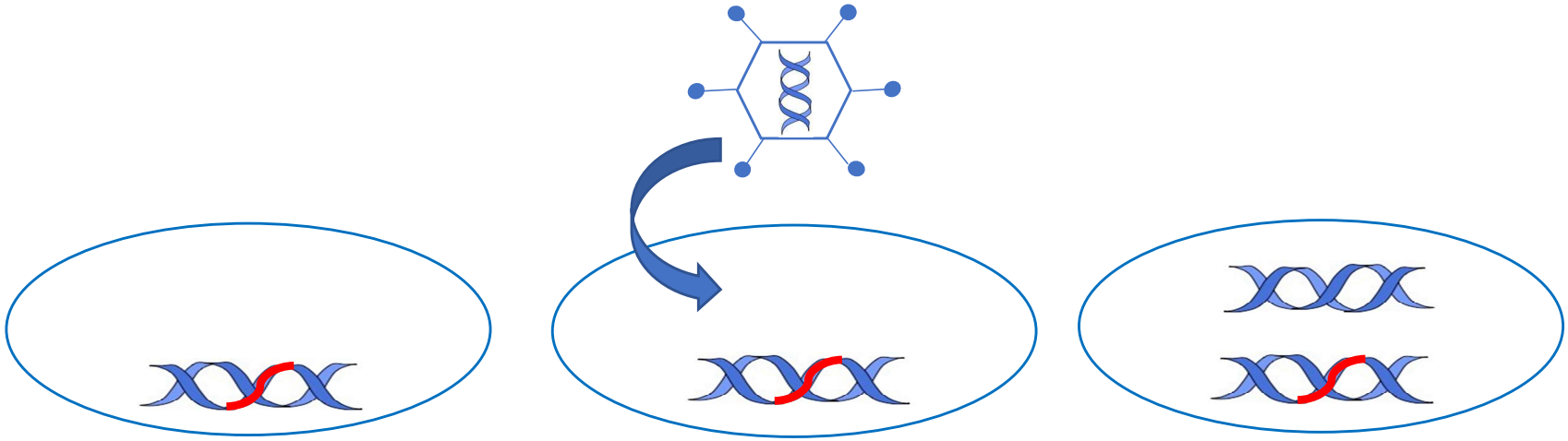
- Permanent cell editing
- Extreme ease of design
- Low cost as a reagent

Disadvantages

- Low efficiency of editing
- Non-specific gene editing (off-target)
- Each guide is generally mutation specific and not applicable to all mutations in a given gene
- Requires exogenous protein expression, which adds to complexity of clinical applications

Stage for NF1: Testing CRISPR in vitro and in vivo after nanoparticle delivery

Gene Replacement

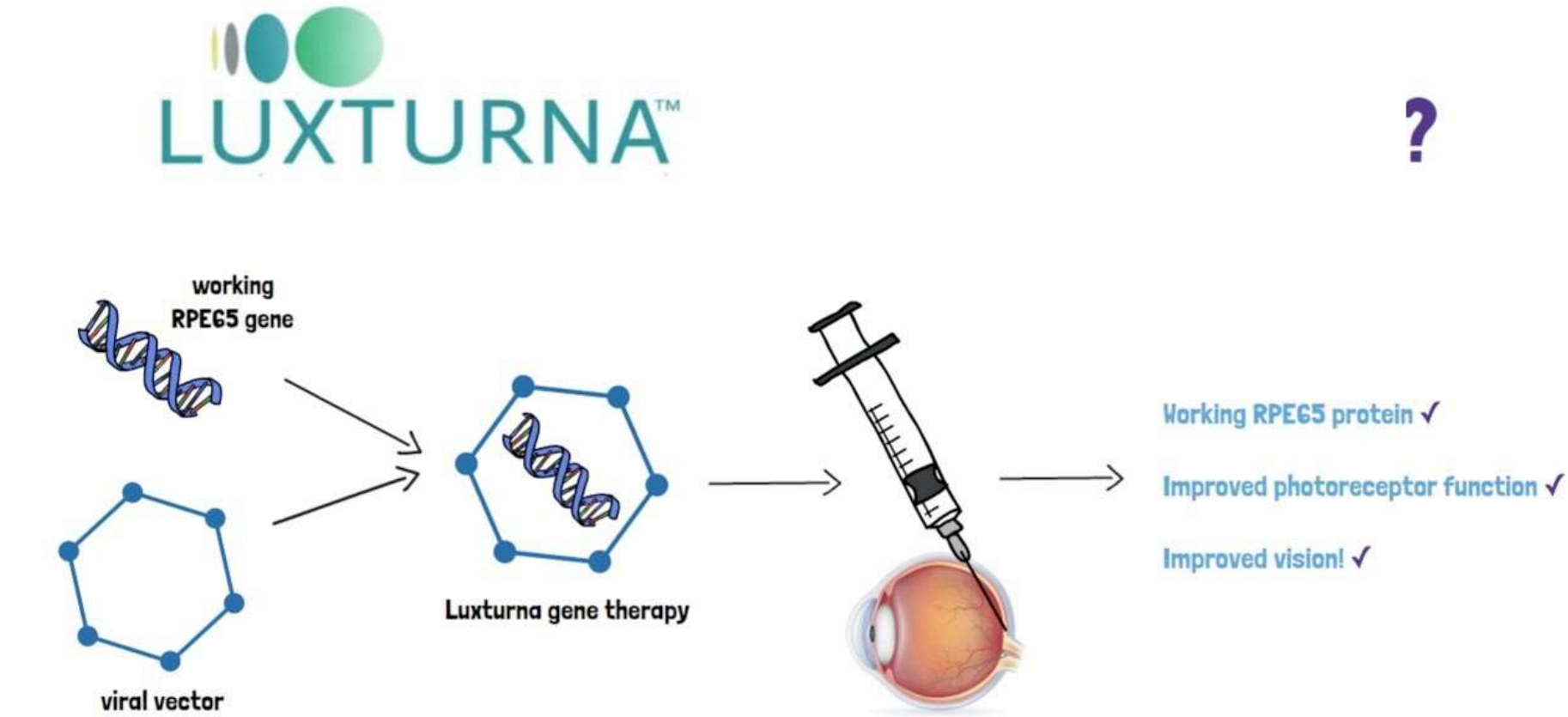


Cell with mutant gene

Normal gene copy is introduced

Gene function is replaced

Example Gene Replacement



Gene Replacement

Advantages

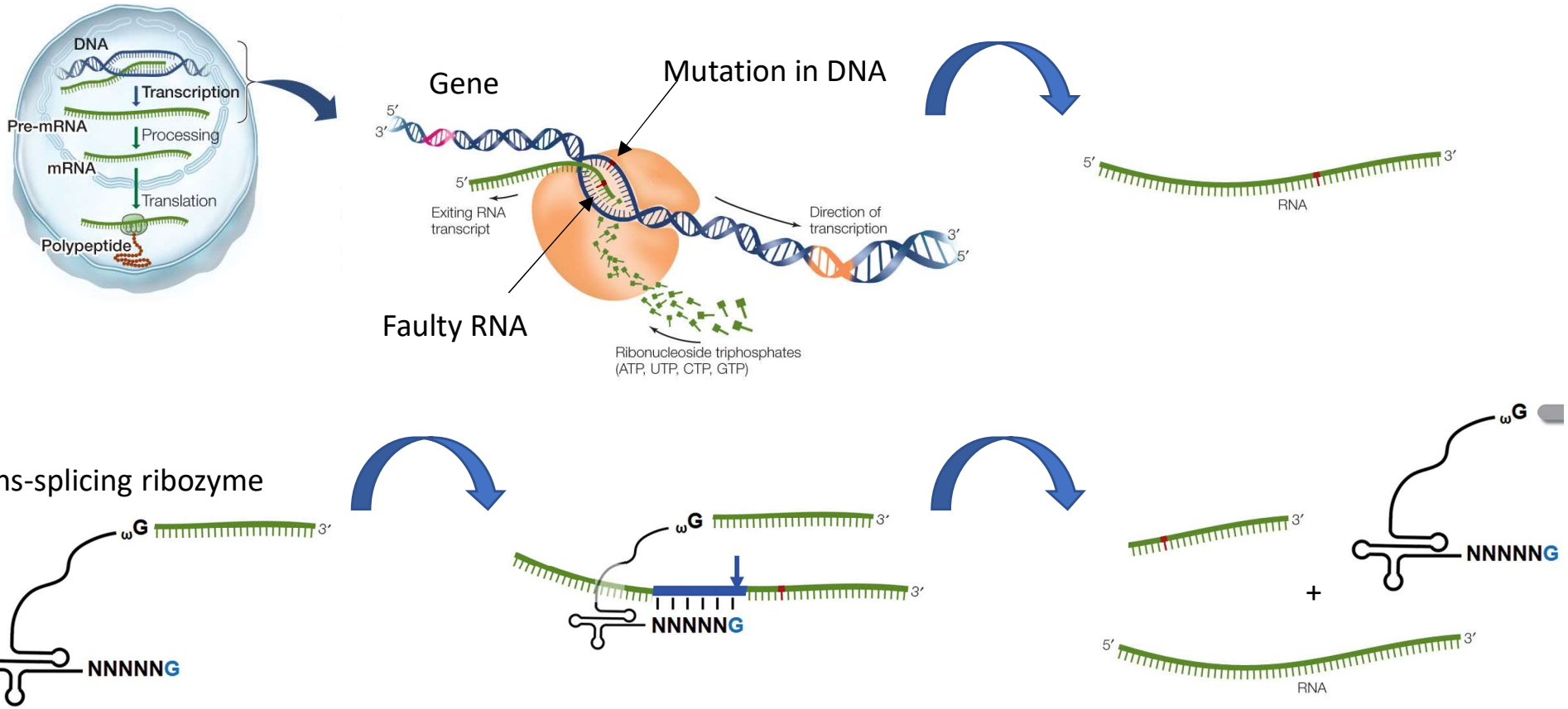
- Targets large mutation spectrum

Disadvantages

- Inefficient cargo delivery
- May not be permanent
- Still at early stage clinically
- Complex and costly to manufacture
- Payload is limited

Stage for NF1: Testing in vitro and in vivo in NF1 deficient rat mammary tumors

RNA Editing - therapeutic ribozymes



Ribozymes

Advantages

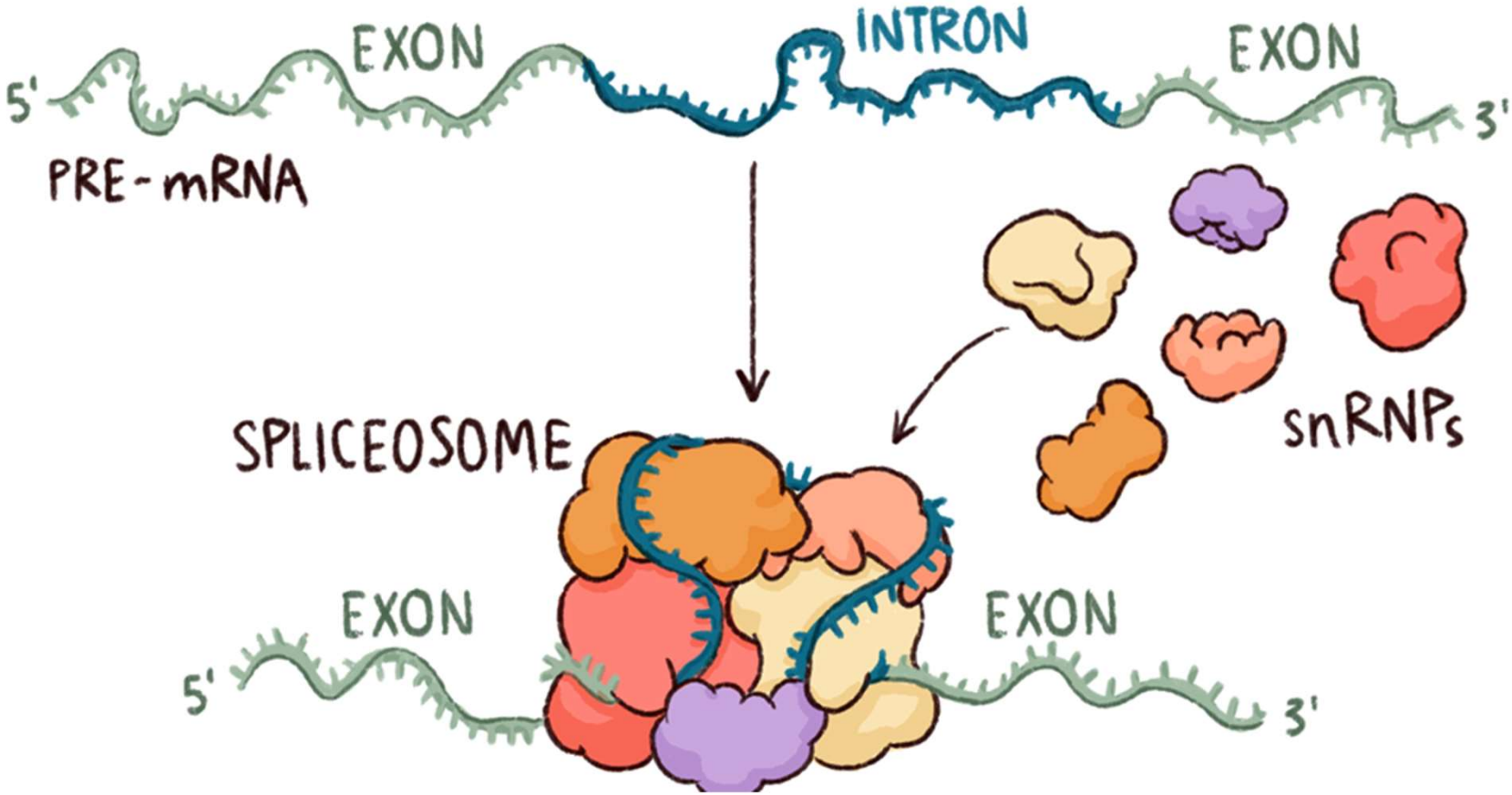
- Does not change DNA

Disadvantages

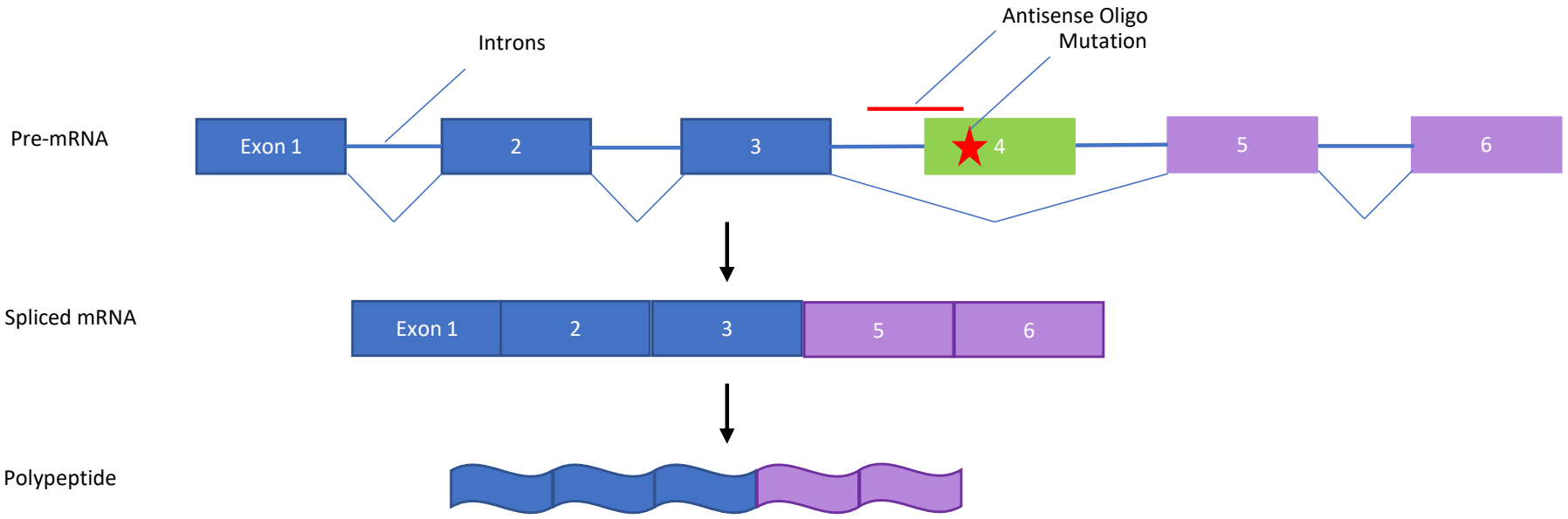
- Low efficiency of editing
- Non-permanence
- Off-target effects
- Early stage clinically

Stage for NF1:Testing in vitro and evolving ribozymes in human cells for increased efficiency

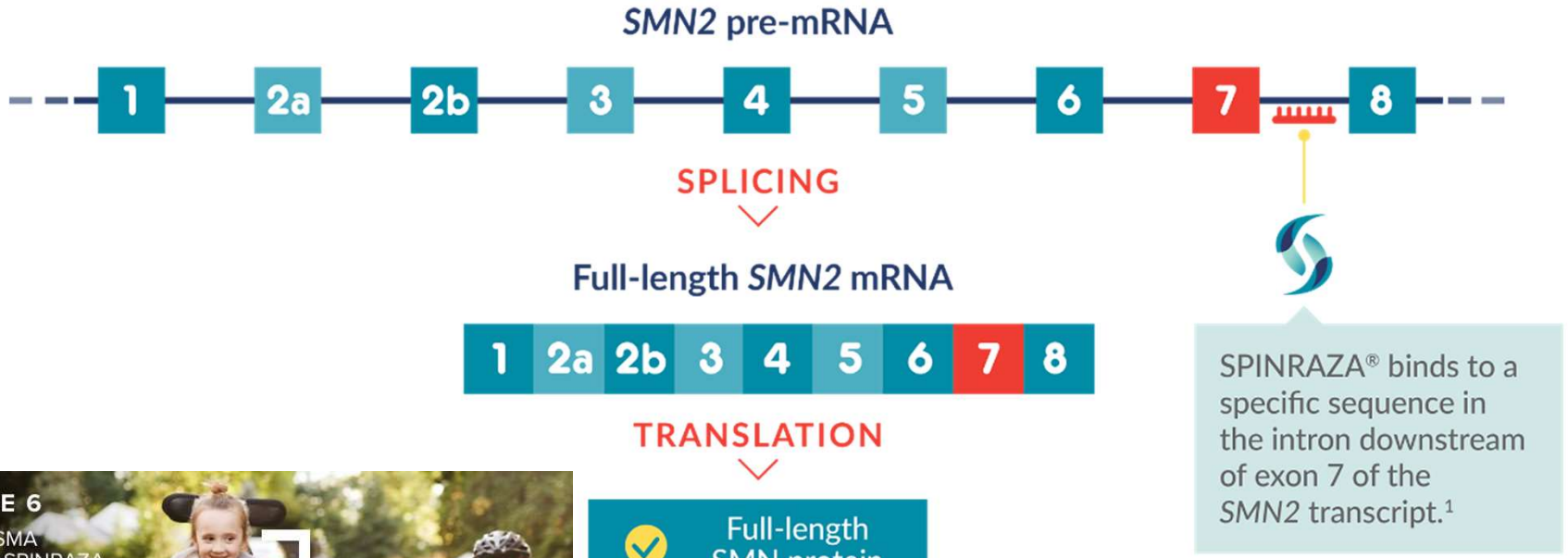
Intron Splicing and the Spliceosome



Antisense Oligonucleotides and Exon skipping



Spinraza for SMA



ASOs

Advantages

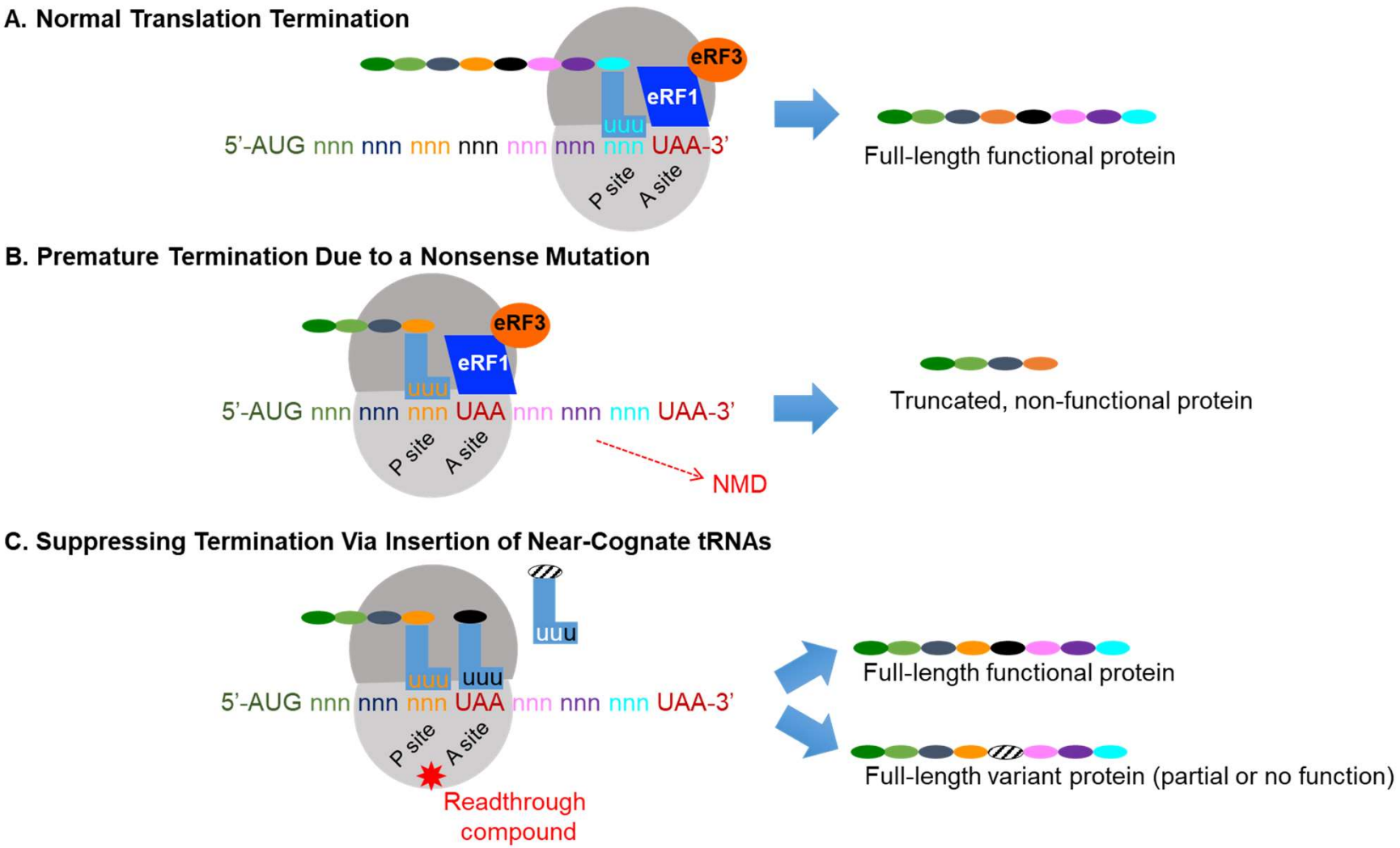
- Virtually any gene can be targeted
- Address targets otherwise inaccessible with traditional therapies
- Reduced toxicity
- Already in clinic

Disadvantages

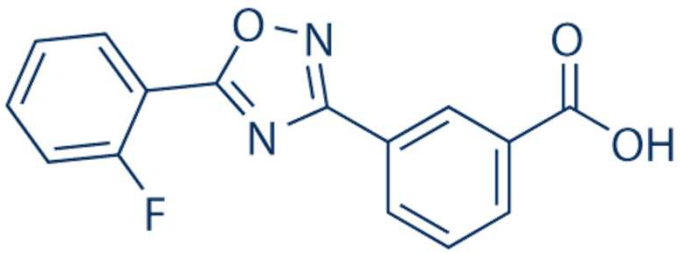
- Stability
- Biodistribution
- Cell penetration
- Endosomal escape
- Off-target effects

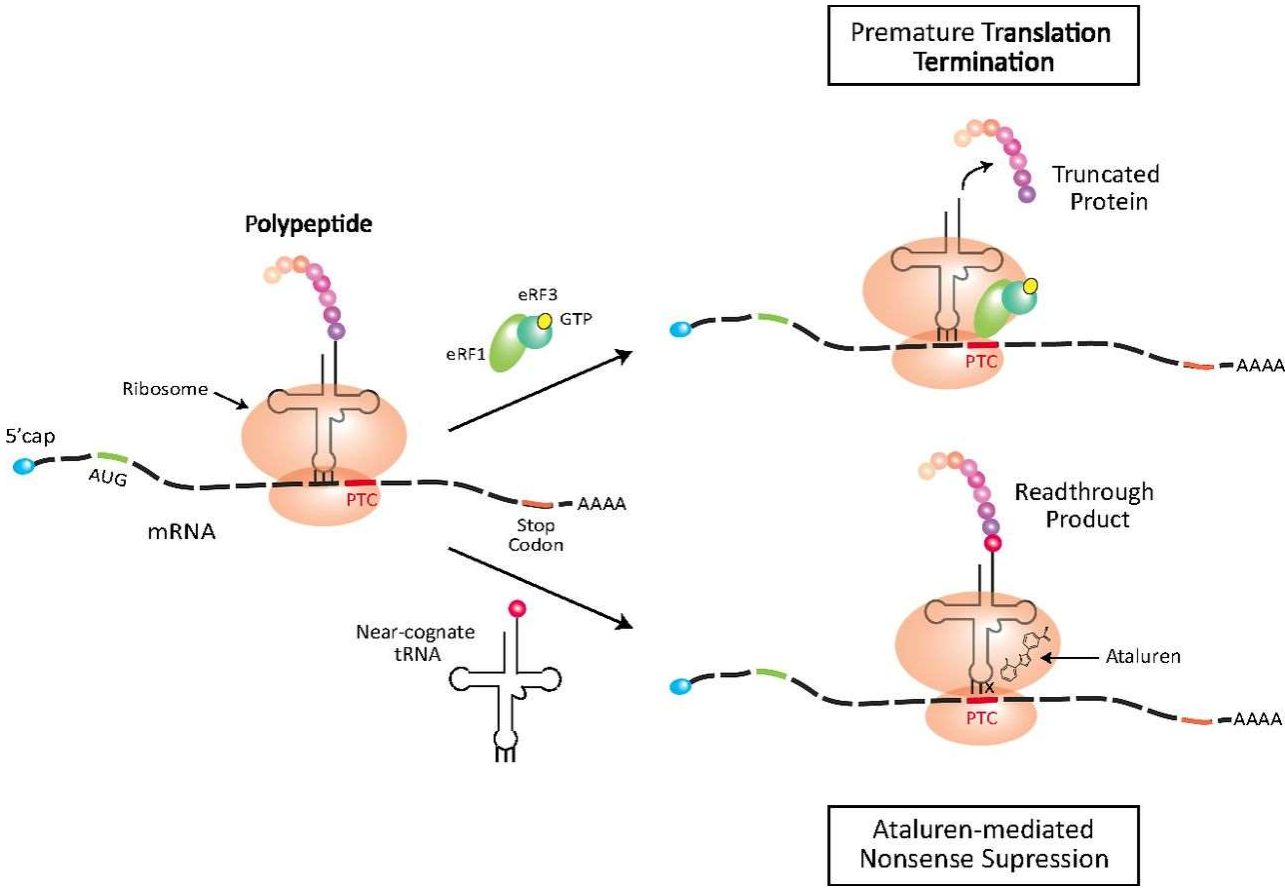
Stage for NF1:In vitro correction of both exon skipping and cryptic splice site repression and moving in vivo in Exon 13 and 17 humanized mouse models

Nonsense suppression



Ataluren for DMD and CF





NSTs

Advantages

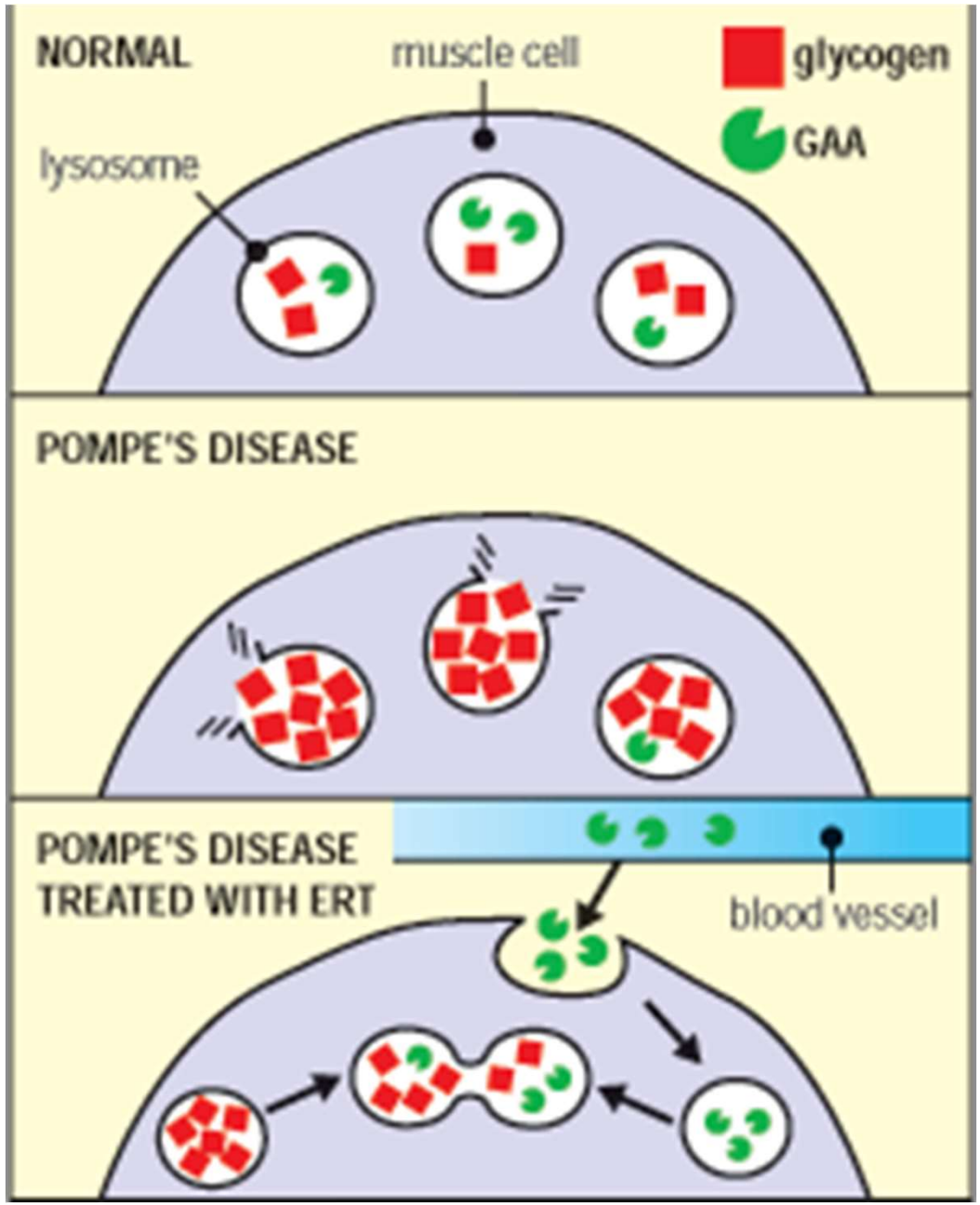
- ~11% of all mutations
- Small Molecules
- May be able to repurpose other drugs
- Potential to be combined with NMD inhibitors

Disadvantages

- Efficiency of readthrough
- NMD
- Off-target toxicity effects of aminoglycosides
- Genomic context effects are unpredictable

Stage for NF1: ~20% of NF1 mutants; Testing in vivo in mouse models

Protein Replacement



Enzyme Replacement Therapy (ERT)

Advantages

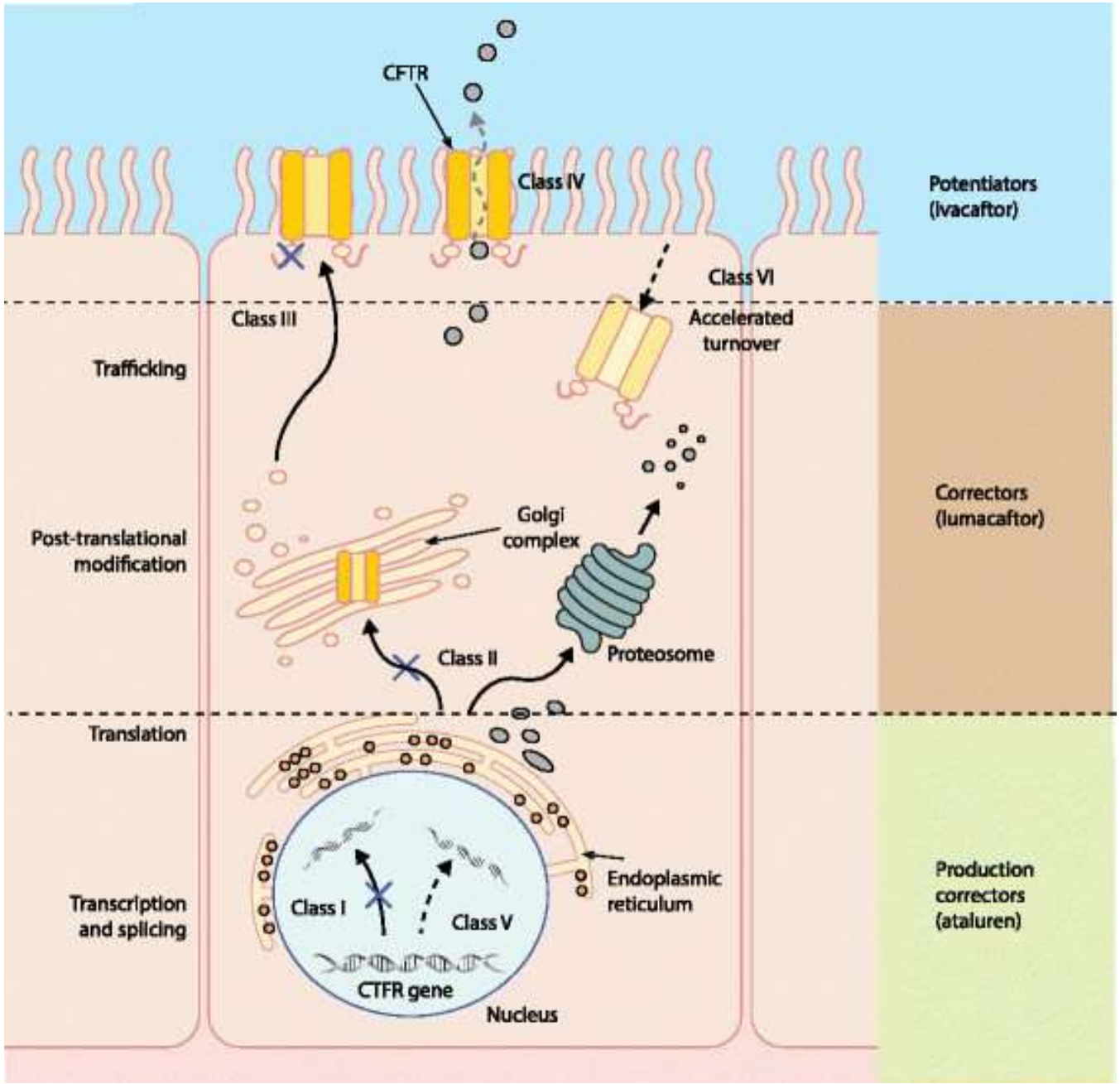
- Well established particularly for Lysosomal Storage Disorders
- Highly selective
- Potent
- Low toxicity

Disadvantages

- Manufacturing cost
- Targeting to correct tissue is limited
- Requires injection
- Poor stability
- Possible immunogenicity

Stage for NF1: Protein production is limited

Modulators


CFTR
Class IV
Potentiators
(ivacaftor)
Class VI
Class III
Accelerated
turnover
Trafficking
Golgi
complex
Post-translational
modification
Correctors
(lumacaftor)
Class II
Proteosome
Translation
Endoplasmic
reticulum
Transcription
and splicing
Class I
Class V
Production
correctors
(ataluren)
CTFR gene
Nucleus

Modulators

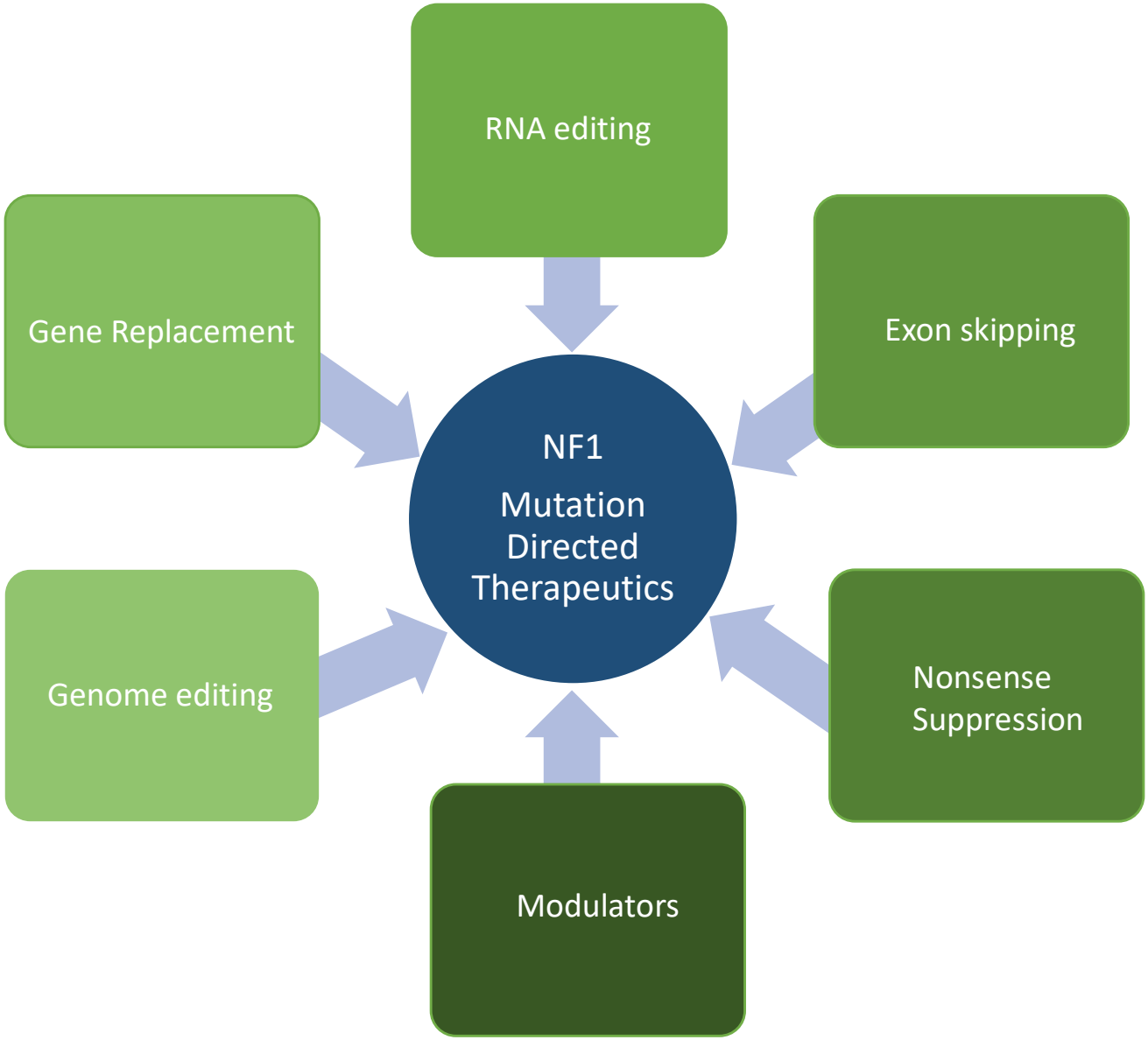
Advantages

- Small molecules
- Low toxicity

Disadvantages

- Limited to select CFTR mutations
- New screens required for other proteins

Stage for NF1: NA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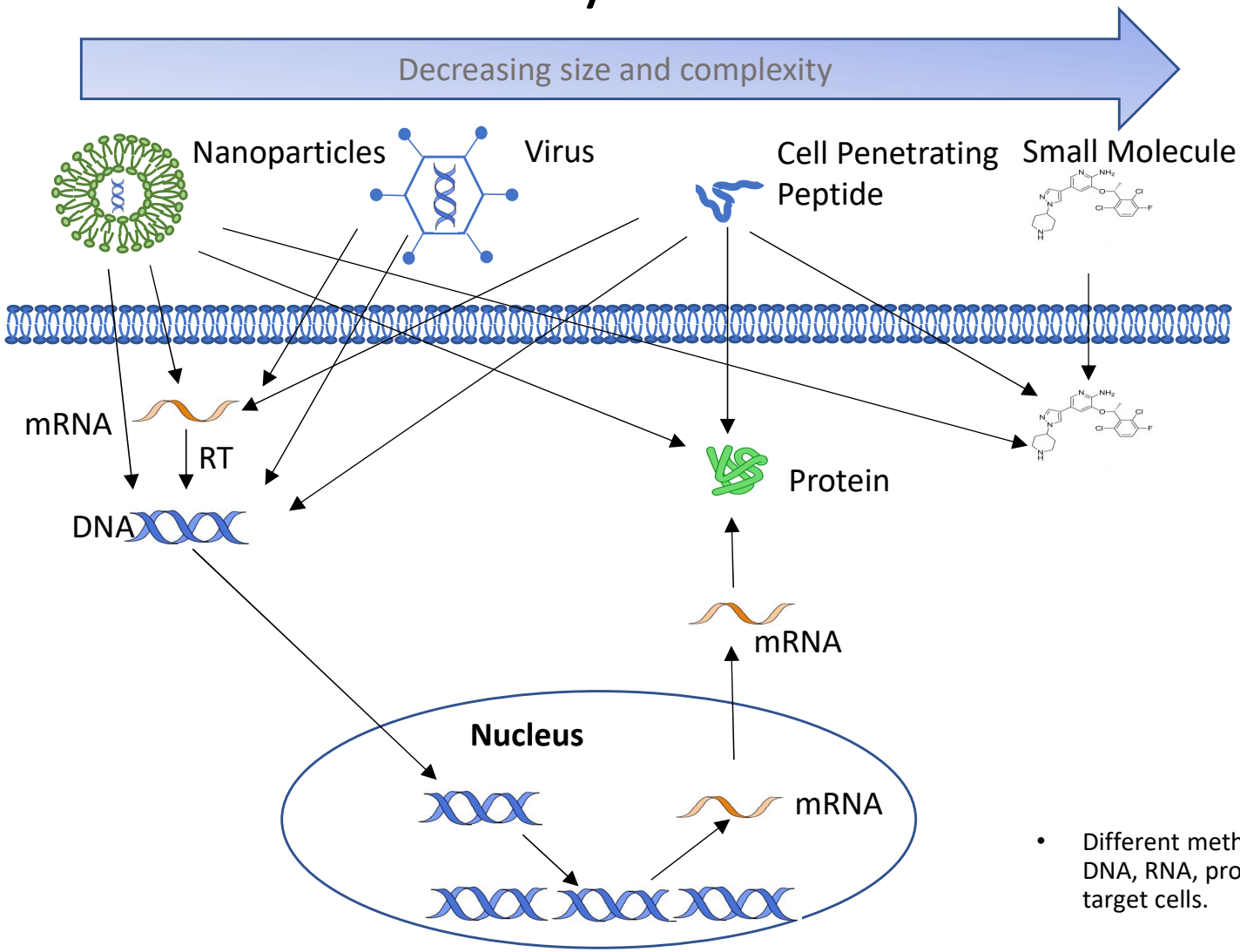
Additional Challenges for NF1

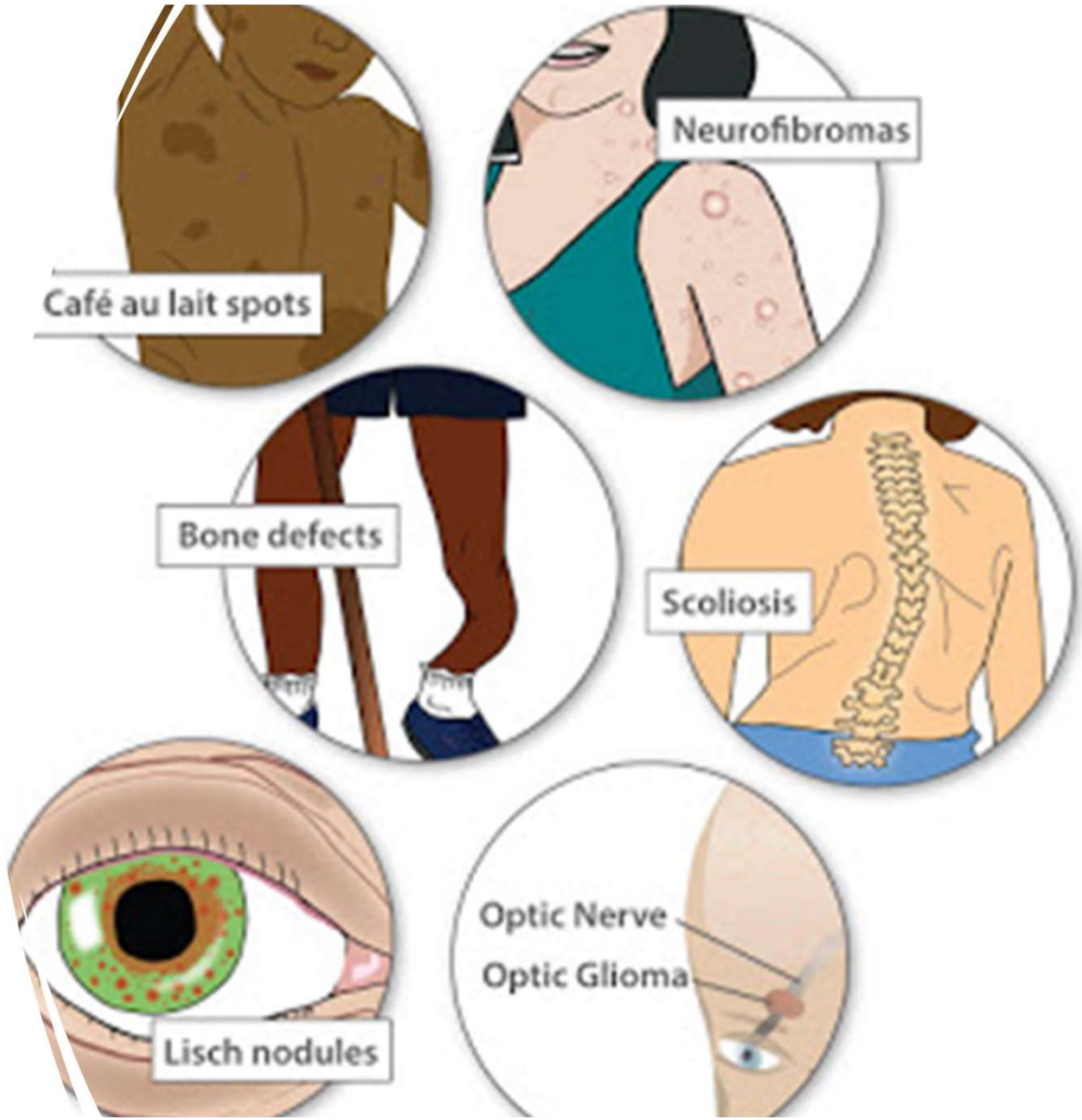
Delivery Methods



- Different methods to deliver therapeutic DNA, RNA, proteins, and small molecules to target cells.

Target Cell/Phenotype

- Schwann cells – cNF or pNF
- Neurons - ADHD
- Melanocytes – Pigment, CALMs
- Osteocytes – bone defects
- Glia – gliomas
- Tumors - MPNST



How much NF1 needs to be restored?

- UNKNOWN
- Affected individuals with the same mutation, may differ in phenotypic severity
- Different heterozygous patient mutations lead to different levels of expression of mutant and normal *NF1* alleles within patient fibroblasts, ranging from 12% to 89% of normal levels
- Neurofibromin has been shown to form dimers
- Protein expression levels do not equate to function
- Restoration of at least 50% neurofibromin function may rescue some *in vivo* phenotypes but not pERK phenotypes
- NF1 phenotypes are cell type-specific, and so are *NF1* expression levels.
- Ras independent functions

Timing and Risk

- When is the best time to initiate treatment?
  - Prophylactic or therapeutic
- Age of onset and variability in severity are confounding
  - Plexiform tumors likely develop early in life and risk becoming MPNST
  - cNFs can develop later in life and are benign
- Different phenotypes carry different risk
- Different approaches confer different risks

#EndNF


UAB
NEUROFIBROMATOSIS


GILBERT
FAMILY FOUNDATION


NIH
NATIONAL
CANCER
INSTITUTE


NTAP
Neurofibromatosis
Therapeutic Acceleration Program
at Johns Hopkins


Response Evaluation in
REiNS
Neurofibromatosis & Schwannomatosis