



Gene Therapy for Neuromuscular Diseases

Pathogenic mechanism • molecular intervention • clinicopathologic interpretation

From molecular lesion to therapeutic design, tissue response, and clinical evidence

Dr Abu Suraih Sakhri

MBBS, MD (Pathology)

www.abusuraihsakhri.com

Learning outcomes

- Match loss-of-function and toxic gain-of-function mechanisms to appropriate genetic treatment classes.
- Explain AAV cargo limitations, mini-dystrophin, immune barriers, and hepatic toxicity.
- Interpret exon skipping, read-through, RNA silencing, and genome editing without equating molecular response with proven benefit.
- Evaluate genotype eligibility, tissue biology, clinical endpoints, and contemporary safety changes.

Therapeutic logic begins with pathogenic mechanism

Molecular pathogenic lesion

```
graph TD; A[Molecular pathogenic lesion] --> B[Loss of functional protein]; A --> C[Toxic gene product or RNA]; A --> D[Disease-causing DNA sequence]; B --> B1[Gene replacement / gene modulation]; C --> C1[Targeted knockdown / silencing]; D --> D1[Genome correction: Investigational];
```

Loss of functional protein

Gene replacement / gene modulation

Toxic gene product or RNA

Targeted knockdown / silencing

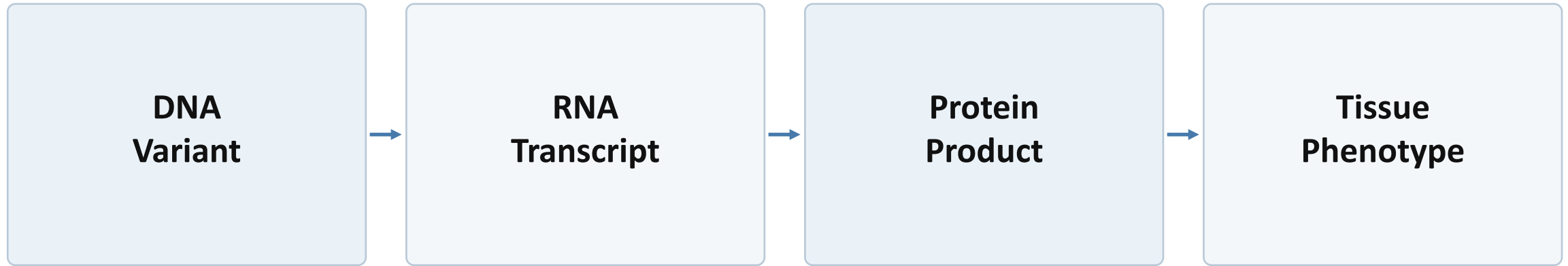
Disease-causing DNA sequence

Genome correction:
Investigational

Four therapeutic verbs mean different things

Approach	What changes	What remains
Replace	Introduce a functional transgene	Native mutant locus usually remains
Modulate	Change RNA splicing or translation	Underlying DNA variant persists
Silence	Reduce pathogenic RNA/protein	Mutated DNA generally persists
Correct	Edit the causative DNA sequence	Potentially alters endogenous locus

DNA → RNA → protein: Three sites of action



Gene addition / editing

Exon skipping / silencing

Read-through / restored
protein

Functional outcome needs
testing

Genotype-specific treatment is not optional detail

- A mutation class may predict whether gene addition, splice modulation, or read-through is mechanistically plausible.
- Exon skipping is defined by the exact DMD exon configuration; A diagnosis of Duchenne muscular dystrophy alone is insufficient.
- Toxic gain-of-function is distinct from simple protein deficiency.
- Molecular confirmation precedes claims of patient eligibility.

Broad replacement versus narrow mutation matching

Gene replacement

- Designed to supply functional protein independently of some variant locations.
- Still limited by vector capacity, tissue delivery, and immune biology.

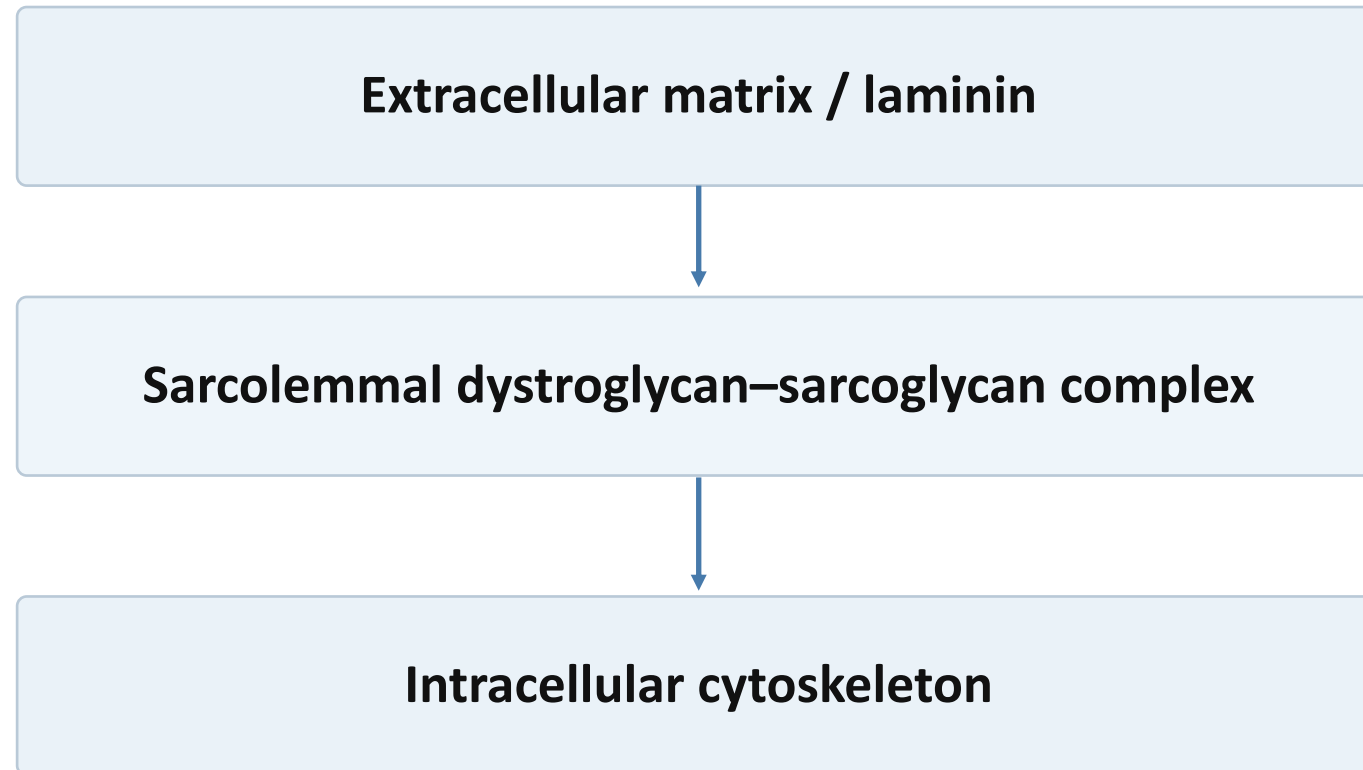
Sequence-specific modulation

- Requires a variant that is amenable to the particular RNA intervention.
- Can preserve endogenous gene regulation but does not repair DNA.

Dystrophinopathy: A model for genetic precision

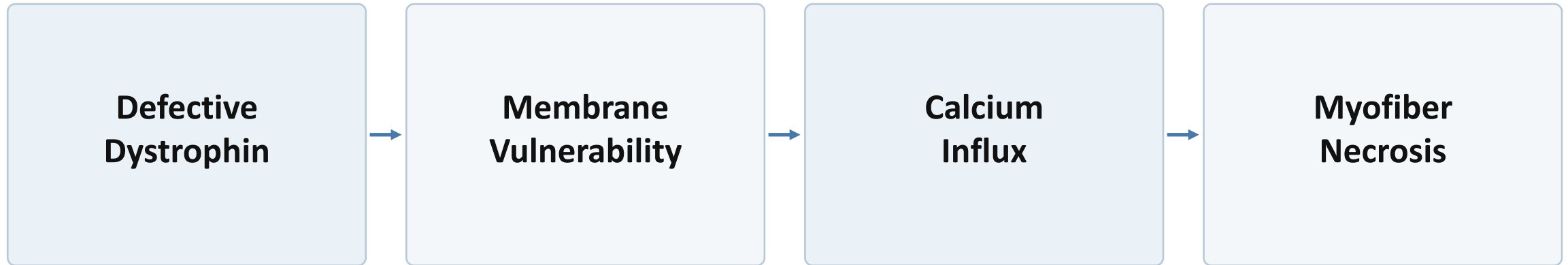
- Duchenne and Becker muscular dystrophies result from loss-of-function DMD variants on the X chromosome.
- Dystrophin is a structural component of the sarcolemmal protein complex.
- The amount and functionality of remaining dystrophin help explain phenotype severity.
- This genotype–protein–phenotype relationship motivates targeted treatment.

Dystrophin links muscle cytoskeleton to extracellular matrix



Dystrophin provides linkage and stability

Loss of dystrophin destabilizes the sarcolemma



Loss of mechanical linkage

Contraction-related
membrane injury

Damage-triggering
intracellular events

Progressive tissue injury

Disrupted reading frame often produces severe DMD

- Frameshift variants disrupt the coding frame and commonly abolish useful dystrophin synthesis.
- The full-length DMD gene encodes a very large protein.
- Severe Duchenne disease is associated with absent dystrophin rather than merely reduced normal function.
- Frameshift status explains why RNA-level restoration is a rational target.

Becker dystrophy demonstrates partial protein function

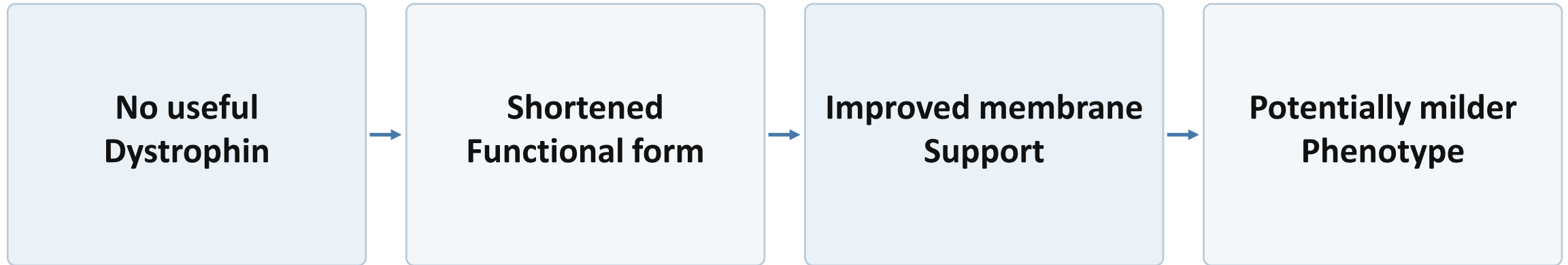
Duchenne pattern

- Absent or profoundly deficient dystrophin
- Earlier, more severe progressive muscle damage

Becker pattern

- Truncated dystrophin with retained activity
- Often later onset and less severe progression

Restoring some function is biologically meaningful



Typical severe loss-of-function

Exon skipping may yield this

Functional effect is not binary

Clinical size of benefit must be proven

Young DMD muscle: Necrosis and regeneration coexist

- Muscle damage outpaces compensatory repair.
- Biopsies may show segmental myofiber necrosis and clusters of regenerating fibers.
- Myofiber size varies even while fascicular architecture remains recognizable.
- Myophagocytosis may accompany degeneration without prominent primary inflammation.

Advanced DMD muscle: Tissue replacement becomes dominant

**Repeated
Necrosis**



**Repair
Capacity exceeded**



**Endomysial
Fibrosis**



**Fatty tissue
Replacement**

Persistent injury

Incomplete regeneration

Architectural distortion

Reduced contractile muscle
mass

Dystrophin immunostaining has a distinct interpretive role

Finding	Pathology interpretation	Therapeutic caution
Absent sarcolemmal stain	Supports severe dystrophin deficiency	Baseline expression is not a treatment endpoint
Reduced/patchy expression	May reflect residual protein	Expression pattern requires careful validation
Post-treatment expression	Evidence of protein production	Does not independently prove functional benefit

Muscle biopsy and sequencing answer different questions

Molecular analysis

- Identifies pathogenic variant and reading-frame consequences.
- Determines potential exon-skipping or nonsense-mutation eligibility.

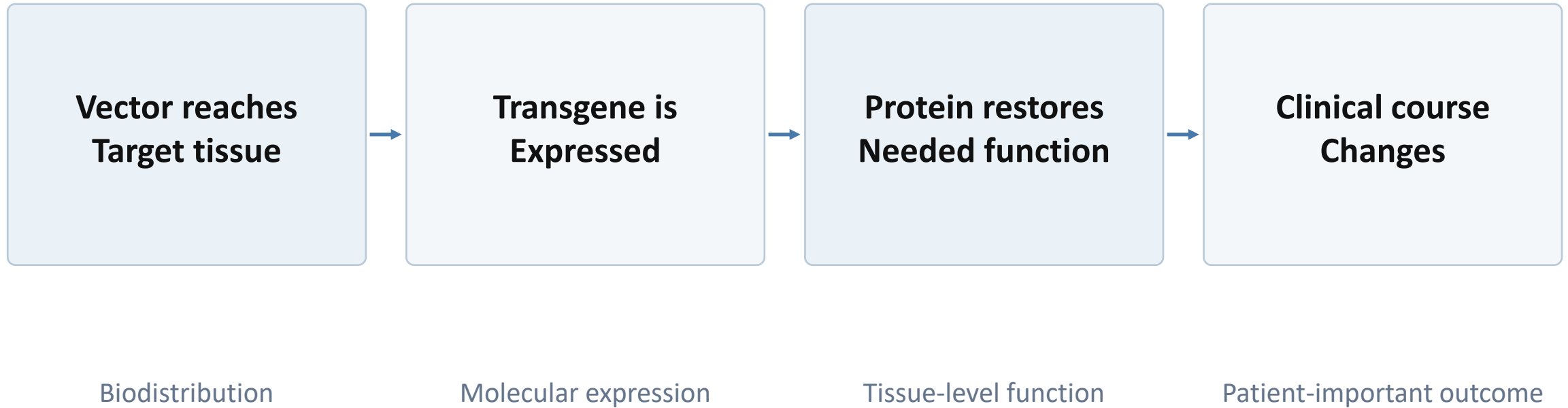
Tissue analysis

- Documents degeneration, fibrosis, and dystrophin expression.
- Provides context for protein-expression biomarkers.

Functional decline involves more than limb strength

- DMD begins with proximal weakness but progresses to major mobility limitation.
- Respiratory muscles may become weak, producing impaired ventilation.
- Cardiac dystrophin deficiency contributes to cardiomyopathy and arrhythmias.
- A tissue-specific benefit should not be assumed to protect every affected organ.

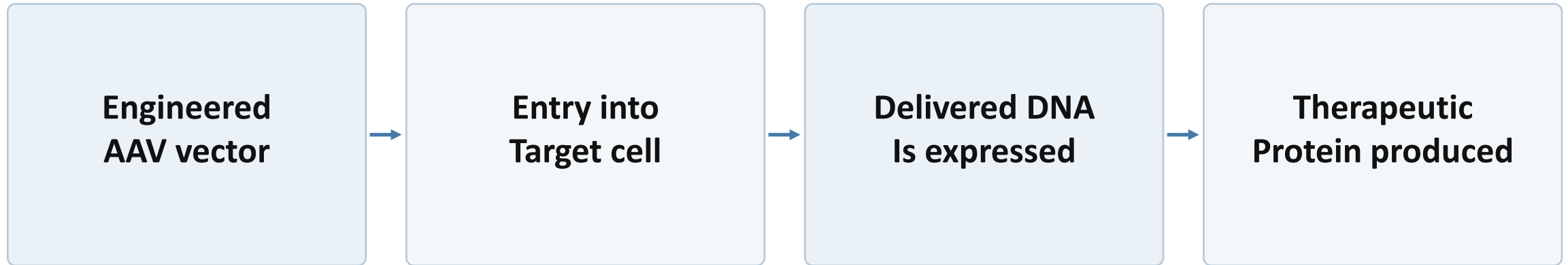
Gene delivery addresses one step in a longer chain



AAV is a vehicle for DNA delivery

- Adeno-associated virus vectors can carry DNA constructs encoding a functional protein.
- The delivered construct is not automatically a correction of the patient's genomic locus.
- Therapeutic success depends on both cargo design and target-tissue expression.
- AAV delivery raises vector-specific immune and safety constraints.

AAV delivery: Vector → target cell → gene expression



Package transgene

Tissue exposure

Transcription / translation

Downstream activity must
be assessed

AAV capacity conflicts with the size of DMD

AAV carrying capacity

Approximate AAV packaging limit



4.7 kb

DMD coding sequence



14 kb

The 14 kb coding sequence exceeds a single conventional AAV cargo budget.

A size mismatch forces construct redesign

- The textbook contrasts approximately 4.7 kb AAV packaging capacity with roughly 14 kb of DMD coding sequence.
- Full-length coding sequence cannot fit in a conventional single AAV cassette.
- A smaller dystrophin-derived transgene offers a practical delivery concept.
- The engineered protein is not the intact native dystrophin molecule.

Mini-dystrophin: Selective retention of useful function

Full-length dystrophin

- The native protein has a coding sequence too large for single conventional AAV packaging.
- Multiple structural interactions contribute to muscle stability.

Engineered abbreviated construct

- Removes sequence to accommodate vector constraints.
- Must retain enough relevant functionality for a therapeutic effect.

Replacement does not recreate the original gene

Question	Gene addition using AAV	Genome editing concept
DNA locus	Mutant native locus remains	Causative sequence is the target
Therapeutic expression	Introduced DNA encodes a protein	Edited locus may produce corrected product
Major hurdle	Cargo/delivery/immune effects	Editing accuracy and delivery
Status	Clinically implemented for DMD	Further from clinical implementation

Protein produced $\neq$ normal tissue restored

**Mini-dystrophin
Expression**



**Membrane support
May improve**



**Established fibrosis
May persist**



**Clinical course
Remains uncertain**

Molecular endpoint

Proposed mechanism

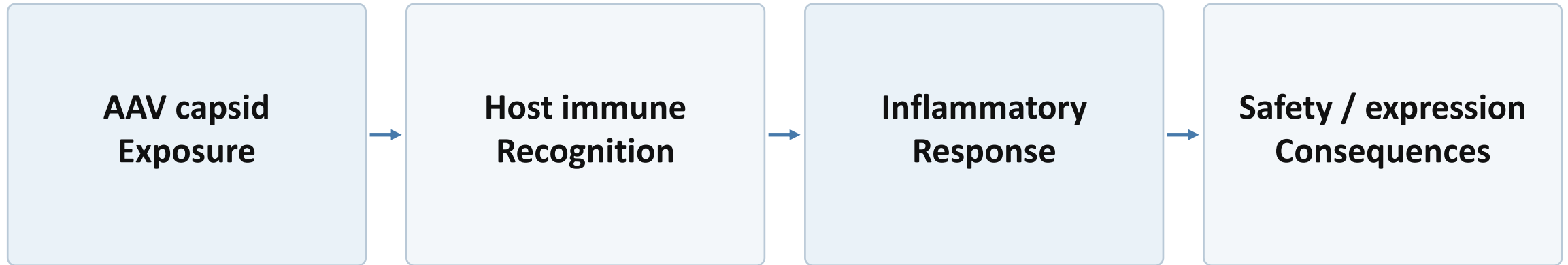
Pre-existing irreversible
pathology

Requires long-term
observation

Neutralizing antibodies can impede AAV

- Naturally occurring antibodies against AAV may neutralize the viral vector.
- An otherwise suitable transgene cannot act if delivery to its target is blocked.
- Serologic vector considerations differ from the causative disease variant.
- Eligibility therefore has both disease-genetic and delivery-related dimensions.

Immune responses may target the vector itself



Viral proteins

Immune barriers

Potential adverse reactions

Monitor beyond the
intended transgene

Immune responses may target the new protein

- The transgene can produce a protein that the host has not previously expressed in normal amounts.
- The expressed transgene product itself may become an immune target.
- Immunity can complicate safety as well as durability of expression.
- Gene construct design and host biology cannot be evaluated independently.

Hepatotoxicity is a major AAV limitation

Mechanistic rationale

- Systemic vector administration exposes organs beyond skeletal muscle.
- Hepatotoxicity as a common AAV-related adverse effect.

Clinical interpretation

- Liver safety requires a separate risk assessment from muscle efficacy.
- Severe post-treatment liver injury can outweigh a plausible molecular benefit.

AAV platform: Delivery advantages and constraints

Potential advantage	Mechanistic constraint
Introduces new protein-coding DNA	Limited payload capacity
Can reach biologically relevant cells	Pre-existing neutralizing antibodies
Targets a causal protein deficit	Capsid / transgene immune responses
Can create measurable protein expression	Hepatic toxicity and uncertain long-term benefit

AAV evidence must be interpreted in layers

After therapy: What was demonstrated?

Construct detected / delivered

Distribution or exposure

Protein measured in tissue

Molecular target engagement

**Durable patient function
improved**

Clinically meaningful benefit

Protein expression is a surrogate endpoint

- Demonstrating newly produced dystrophin supports biological activity of the construct.
- The extent, distribution, and functionality of expressed protein all matter.
- Improved protein expression does not automatically establish improved walking or cardiopulmonary outcomes.
- Long-term functional benefit must be tested independently.

Long-term AAV effectiveness remains an empirical question

- The long-term benefits of DMD mini-dystrophin delivery remain to be determined.
- Short-term biochemical effects cannot establish lifelong muscle protection.
- Progressive baseline disease and already established tissue injury complicate follow-up.
- Clinical trials and extended surveillance address questions that mechanistic plausibility cannot settle.

Exon skipping intervenes during RNA processing



Reading frame disturbed

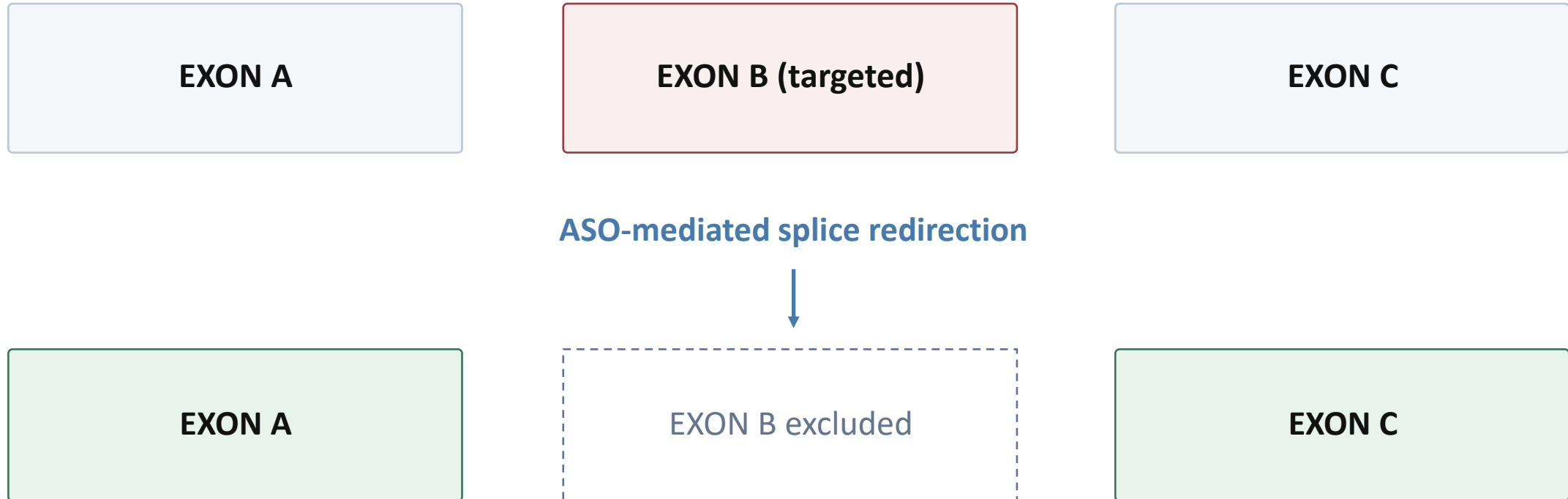
Binding at RNA level

Alternative splicing

Shortened dystrophin may
be produced

Frameshift and reading-frame rescue

Illustrative pre-mRNA before splice modification



Schematic: Exon exclusion can restore a frame only for a variant with suitable exon lengths.

Exon skipping does not repair the DNA variant

Changes

- Splicing pattern of the DMD transcript
- Potential production of shortened partly functional dystrophin

Does not change

- The pathogenic variant in genomic DNA
- The need for variant-specific therapeutic selection

Specific exons identify eligible molecular subgroups

- Exon 45-, 51-, and 53-skipping approaches for selected DMD variants.
- The relevant criterion is whether the individual variant is amenable to the specified exon skip.
- The exon number identifies the target of the oligonucleotide—not every mutation located within that exon.
- Variant annotation and reading-frame prediction are therefore essential.

Exon skipping is only relevant to amenable variants

Confirmed pathogenic DMD variant

```
graph TD; A[Confirmed pathogenic DMD variant] --> B[Frame disrupted; Exon skip can restore frame]; A --> C[Variant not amenable to targeted exon skip]; A --> D[Variant is a premature stop codon];
```

Frame disrupted; Exon skip can restore frame

Evaluate exact matching antisense approach

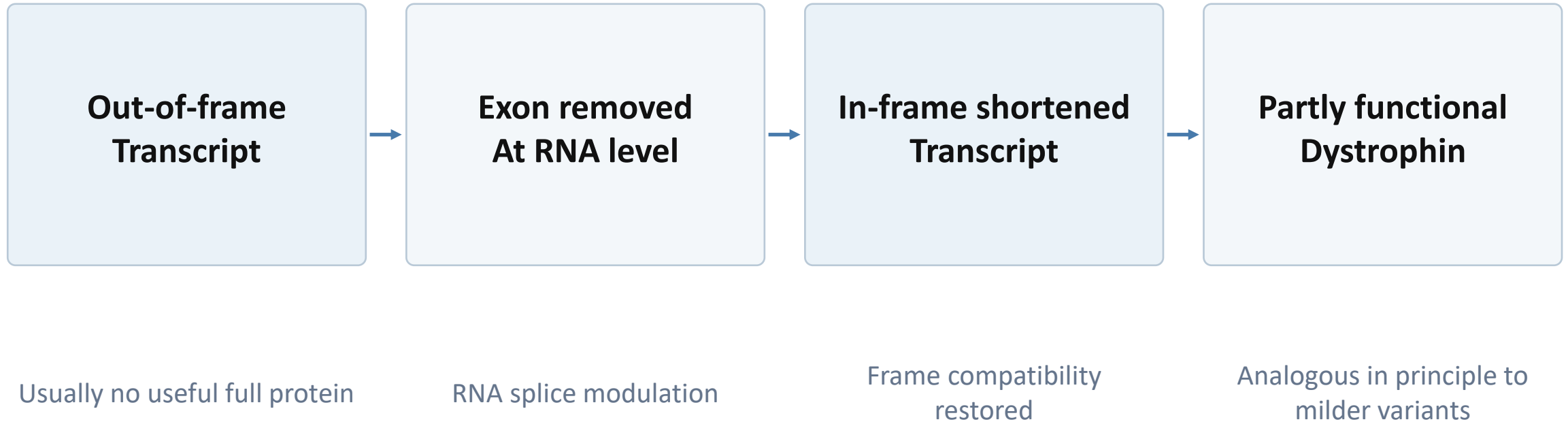
Variant not amenable to targeted exon skip

This exon-skipping strategy is not suitable

Variant is a premature stop codon

Read-through is a different proposed mechanism

Why exon skipping evokes a Becker-like mechanism



RNA modulation and DNA delivery have distinct liabilities

Feature	AAV gene addition	Antisense exon skipping
Intervention site	Introduced DNA	Endogenous pre-mRNA
Main selection issue	Vector/construct and host factors	Exact amenable DMD genotype
Product	Engineered transgene protein	Splice-altered endogenous dystrophin
Important caveat	Immune/hepatic toxicity	Restoring frame need not guarantee clinical effect

Skipping an exon is not silencing the gene

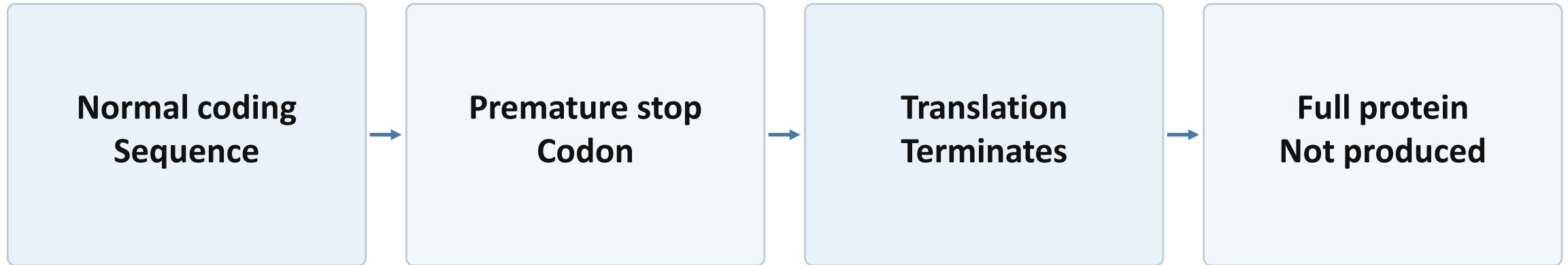
Exon skipping

- Redirects splice-site use in a particular transcript.
- Aims to permit production of a useful shortened protein.

Gene knockdown

- Reduces abundance of pathogenic RNA or protein.
- Aims to diminish toxic gain-of-function.

Nonsense variants disrupt translation



Open reading frame

Nonsense mutation

Truncated translation

Loss of function follows

Read-through aims to overcome premature stop signals

**Nonsense mutation
In mRNA**



**Read-through
Pharmacologic effect**



**Translation may
Continue**



**More full-length
Product possible**

Distinct subgroup

Ribosomal mechanism

Not DNA repair

Clinical effectiveness
requires evidence

Nonsense read-through is subgroup-specific

- That nonsense variants occur in approximately 10–15% of Duchenne cases.
- This mutation class is biologically different from an out-of-frame deletion.
- Read-through drugs aim to modify protein synthesis, not exon selection.
- Patient-specific biological eligibility does not establish clinical efficacy or authorization.

A mechanistic rationale is not regulatory proof

Plausible mechanism

- A ribosome could translate past a premature termination signal.
- Restored product is a testable molecular hypothesis.

Evidence required

- Clinical benefit must be reproducible in affected patients.
- Authorization depends on regulatory review and can change over time.

Toxic gain-of-function demands a different objective

Disease caused by toxic expression

```
graph TD; A[Disease caused by toxic expression] --> B[Reduce toxic RNA]; A --> C[Reduce toxic protein production]; A --> D[Replace a missing protein];
```

Reduce toxic RNA

Antisense oligonucleotide
silencing

**Reduce toxic protein
production**

RNA interference or other gene
knockdown

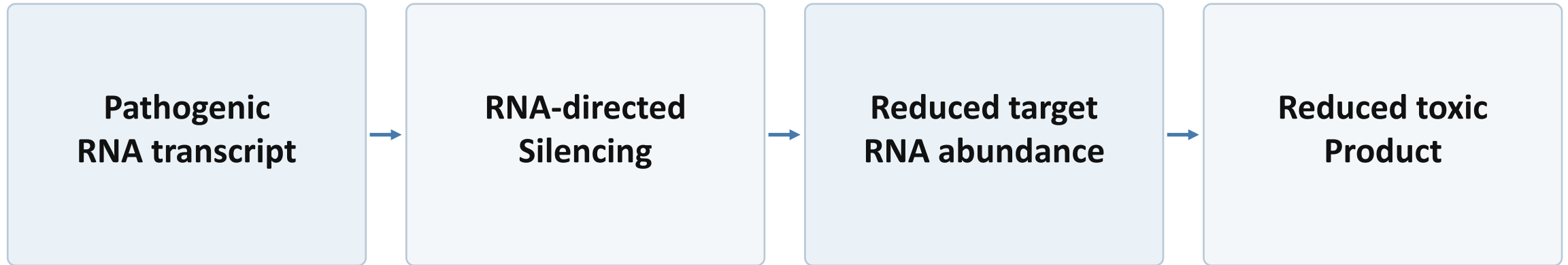
Replace a missing protein

May fail to address the toxic
mechanism

Dominant disease may retain a normal allele

- Dominant inheritance may arise from toxic gain-of-function rather than simple haploinsufficiency.
- Supplying an extra wild-type copy does not necessarily eliminate the toxic product.
- A reduction strategy aims to diminish pathogenic expression.
- Whether allele-selective versus broader suppression is desirable depends on disease biology.

RNA interference concept: Reduce pathogenic transcript



Toxic gain-of-function

Target-complementary
process

Knockdown

Potential tissue benefit

Antisense oligonucleotides can serve two roles

Therapeutic use	RNA effect	Intended result
Exon skipping	Redirect pre-mRNA splicing	Produce partly functional shorter protein
Transcript silencing	Decrease pathogenic RNA	Reduce toxic gain-of-function

“Antisense” describes a molecular tool class, not one uniform biological outcome.

Myotonic dystrophy illustrates toxic RNA biology

**Expanded CTG
Repeat in DMPK**



**Repeat-containing
RNA accumulates**



**Splicing regulators
Sequestered**



**Alternative splicing
Is altered**

DM1 genetic lesion

Toxic RNA behavior

Loss of normal splicing
control

Multisystem manifestations

DM1 provides a rationale for RNA-directed therapy

Underlying lesion

- Expanded repeat-containing transcript binds splicing regulators.
- CLCN1 alternative splicing contributes to myotonia.

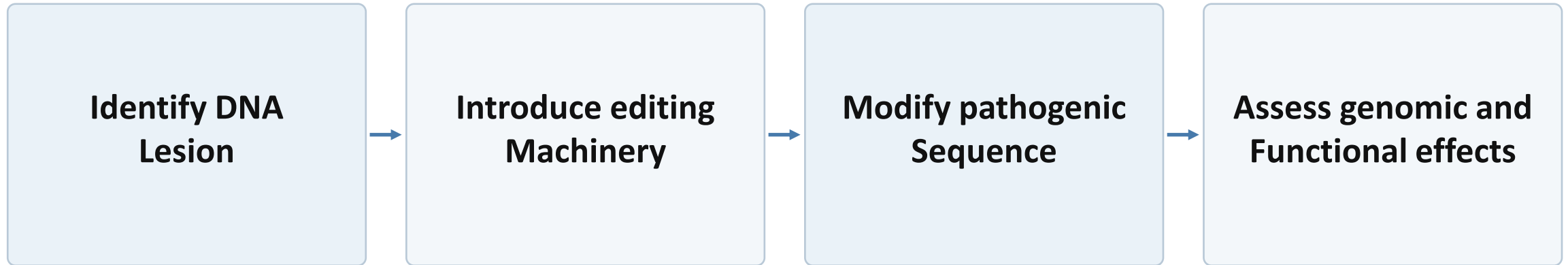
Logical intervention target

- Lower the toxic transcript or interrupt its pathological interactions.
- This is a mechanistic rationale—not an approved therapy claim.

Silencing and exon skipping must not be conflated

Question	Exon skipping	Knockdown
Main problem	Loss of usable protein	Toxic product
Desired RNA change	Different splice isoform	Lower target RNA abundance
Expected protein effect	Partially restore activity	Decrease toxic protein or RNA
Typical mechanism in source	Selected DMD variants	Dominant toxic gain-of-function

CRISPR/Cas9 represents a gene correction strategy



Mutation-specific target

Gene-editing platform

Correction is intended

Research-stage in source

Genome editing and gene addition differ fundamentally

Question	Gene addition	Gene correction
Molecular substrate	DNA introduced by a vector	Endogenous DNA sequence
Typical intention	Compensate for loss of function	Alter causal sequence
Primary limitation	Vector/cargo/immune barriers	Farther from clinical implementation
Shared requirement	Demonstrate safety and patient benefit	Demonstrate safety and patient benefit

Genome editing needs target and consequence verification

- A DNA change must be characterized at the intended target locus.
- An altered sequence matters only if it restores a biologically relevant function.
- Delivery to the affected tissues remains essential.
- Genome correction with CRISPR/Cas9 farther from routine clinical implementation.

Genetic eligibility and clinical eligibility are different

Genetic matching

- Causative gene and pathogenic variant confirmed.
- Predicted relationship between variant class and treatment mechanism.

Clinical matching

- Disease stage and affected organ systems characterized.
- Product-specific indication and safety restrictions independently checked.

Pathology gives context to therapeutic biomarkers

- Early muscle disease can retain fascicular architecture despite substantial necrosis and regeneration.
- Advanced fibrosis and fatty replacement represent loss of functioning muscle tissue.
- Dystrophin immunostaining indicates protein expression but not its entire functional effect.
- Post-treatment tissue findings should be interpreted against disease stage.

Muscle, heart, and respiratory outcomes are distinct

Patient-important treatment outcome

```
graph TD; A[Patient-important treatment outcome] --> B[Skeletal muscle]; A --> C[Respiratory system]; A --> D[Heart]; B --- E[Strength, ambulation, daily function]; C --- F[Ventilatory muscle performance]; D --- G[Cardiomyopathy and rhythm outcomes];
```

The diagram illustrates the relationship between patient-important treatment outcomes and specific organ systems. At the top, a box labeled 'Patient-important treatment outcome' has three arrows pointing down to three separate boxes: 'Skeletal muscle', 'Respiratory system', and 'Heart'. Each of these boxes contains a horizontal line followed by a list of specific outcomes: 'Strength, ambulation, daily function' for skeletal muscle, 'Ventilatory muscle performance' for the respiratory system, and 'Cardiomyopathy and rhythm outcomes' for the heart.

Skeletal muscle

Strength, ambulation, daily
function

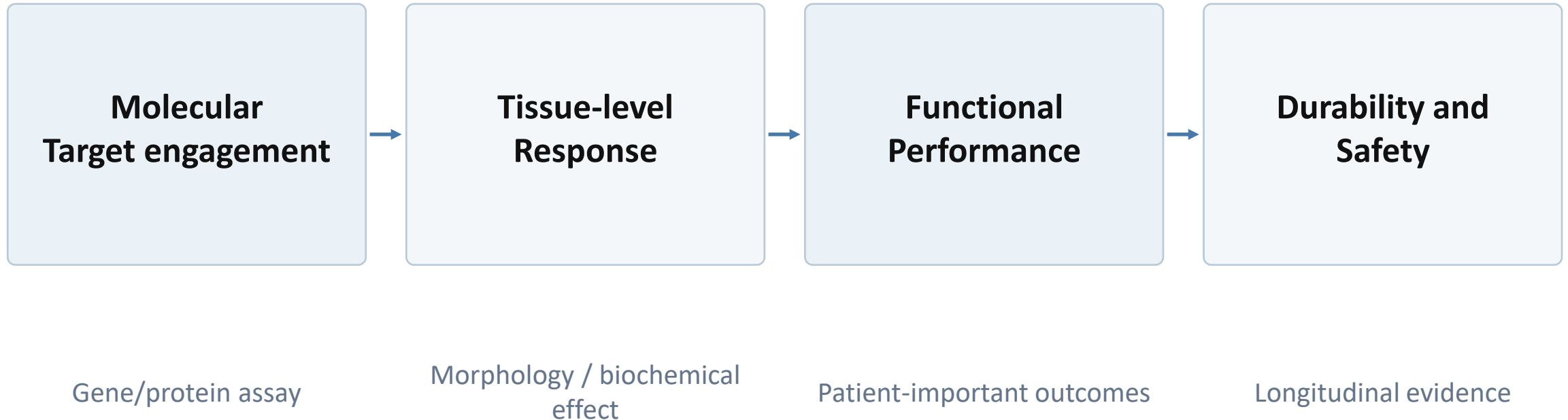
Respiratory system

Ventilatory muscle performance

Heart

Cardiomyopathy and rhythm
outcomes

Clinical endpoints must test the causal hypothesis



AAV DMD therapy: Regulatory context changed in 2025

Robbins baseline statement

- An AAV abbreviated-dystrophin strategy had gained approval.
- The general term mini-dystrophin.

FDA update — Nov 2025

- Elevidys specifically encodes engineered micro-dystrophin.
- FDA indication: Ambulatory DMD, ≥ 4 years, with confirmed DMD mutation.

Fatal liver injury reshaped the Elevidys indication

- FDA reported fatal acute liver failure in non-ambulatory DMD patients treated with Elevidys.
- The November 2025 FDA labeling update removed the non-ambulatory indication.
- The FDA boxed warning highlights serious liver injury and acute liver failure, including fatal outcomes.
- Current use must follow the latest product-specific prescribing information.

Exon-skipping regulatory status is not proof of benefit

- An FDA July 2026 advisory briefing identified four approved DMD antisense drugs targeting exons 45, 51, or 53.
- The document reports that these approvals remain under the accelerated-approval pathway.
- The briefing states that clinical benefit had not yet been verified for these four products.
- Molecular dystrophin production should therefore be separated from demonstrated patient benefit.

EU nonsense read-through status changed in 2025

Textbook historical claim

- Ribosomal read-through medication achieved European approval for nonsense DMD.
- The statement describes a former authorization context.

EMA/EC current correction

- The European Commission did not renew Translarna (ataluren) authorization on 28 March 2025.
- The EMA stated that clinical effectiveness had not been confirmed.

Spinal muscular atrophy: Another loss-of-function model

- Spinal muscular atrophy (SMA) involves pathogenic SMN1 variants and loss of motor neurons.
- The therapeutic target differs from dystrophin deficiency in the muscle fiber.
- FDA-approved AAV gene therapies now provide a contemporary example of gene addition in motor-neuron disease.
- SMA therefore illustrates gene addition directed at motor-neuron disease rather than primary myofiber disease.

FDA-approved SMA gene therapy populations differ

Therapy	FDA population	Principle
Zolgensma (onasemnogene abeparvovec-xioi)	Pediatric SMA, <2 years, biallelic SMN1 mutations	AAV-delivered gene therapy
Itvisma (onasemnogene abeparvovec-brve)	SMA, age ≥2 years, confirmed SMN1 mutation	AAV-delivered gene therapy

Product-specific FDA indications; Consult the latest prescribing information.

Case exercise: Identify a strategy for an out-of-frame DMD variant

Teaching vignette (hypothetical)

An affected child has confirmed DMD frameshift disruption with absent dystrophin on muscle immunostaining.

- What pathogenic mechanism is operating?
- What extra variant information is needed before selecting exon skipping?
- Why would a full-length DMD gene not fit in conventional single AAV?

Loss of function → assess exact exon-skipping amenability; AAV limit ≈4.7 kb versus ≈14 kb DMD coding sequence.

Case exercise: Choose a strategy for toxic repeat RNA

Teaching vignette (hypothetical)

A multisystem myotonic disorder is associated with an expanded repeat-containing transcript that sequesters splicing regulators.

- Is simple gene replacement likely to remove the toxic transcript?
- Which RNA-directed strategy follows from the mechanism?
- What finding would support target engagement?

Knockdown/silencing is the mechanistic candidate; Assess reduced toxic RNA and downstream splicing correction. Do not infer regulatory approval.

Mechanism, evidence, and safety are separate decisions

Should this approach be used?

```
graph TD; A[Should this approach be used?] --> B[Biological fit]; A --> C[Evidence strength]; A --> D[Net clinical suitability];
```

Biological fit

Correct lesion, tissue,
intervention

Evidence strength

Patient benefit beyond surrogate
markers

Net clinical suitability

Safety, indication, disease stage

Three conclusions to retain

- Select genetic treatments from the molecular pathogenesis: Replace, modulate, silence, or correct.
- AAV-mediated DMD treatment is constrained by cargo size, immunity, hepatotoxicity, and uncertain long-term effect.
- Exon skipping, read-through, and knockdown differ in target and mutation specificity.
- Clinical efficacy and authorization status require evidence beyond mechanistic plausibility; Relevant regulatory statements have changed.

Source references and verification boundary

- Robbins, Cotran & Kumar. Pathologic Basis of Disease, 11th ed. (2026), ch. 27, pp. 1121–1125.
- FDA: Elevidys safety/label revision (14 Nov 2025); DMD antisense briefing (29 Jul 2026).
- EMA: Translarna non-renewal (28 Mar 2025).
- FDA: SMA gene therapy product pages, including Itvisma (24 Nov 2025).
- Regulatory status is time-sensitive; reassess current product labels and agency decisions.