Újabb terápiás lehetőségek neurológiai kórképekben: mit hoz 2021?

Salamon András, Klivényi Péter

Neurológiai Kerekasztal

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Génterápia

- **Non-virális stratégiák:**
  - Antisens oligonucleotidok (ASO)
- **Virus alapú stratégiák:**
  - RNS interferencia (RNAi)
  - CRISP/Cas9
  - Adenovírus-asszociált gén bejuttatás (AAV-mediated gene delivery)

Amado et al., 2021

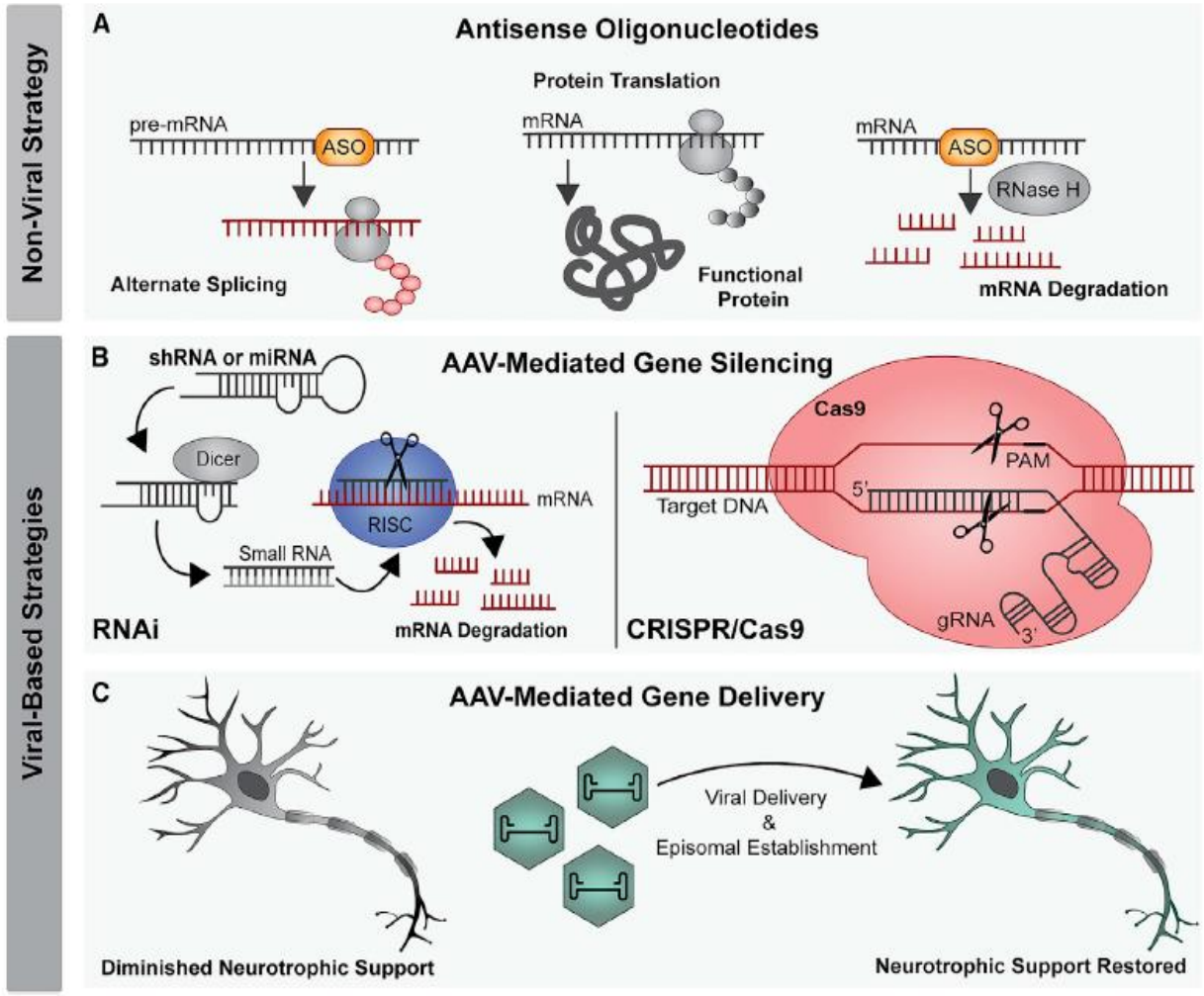
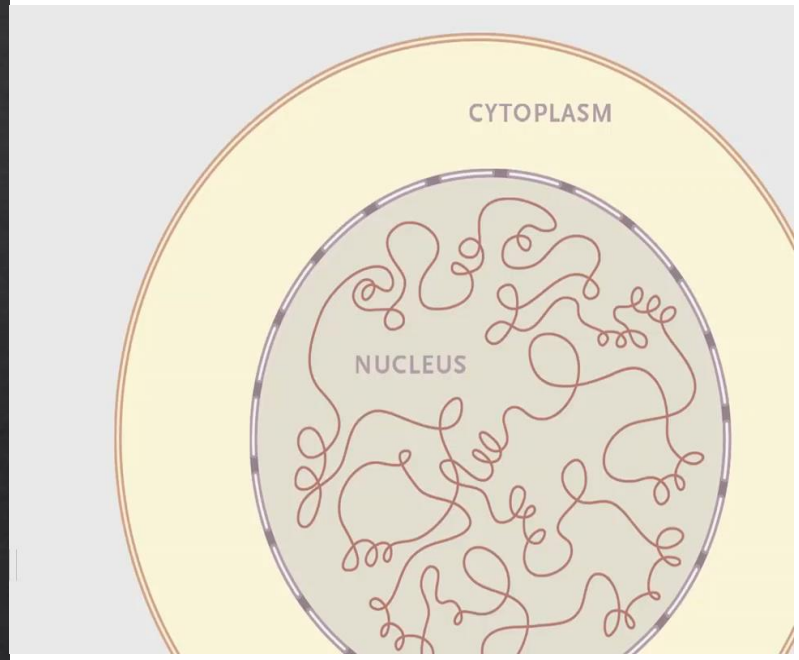
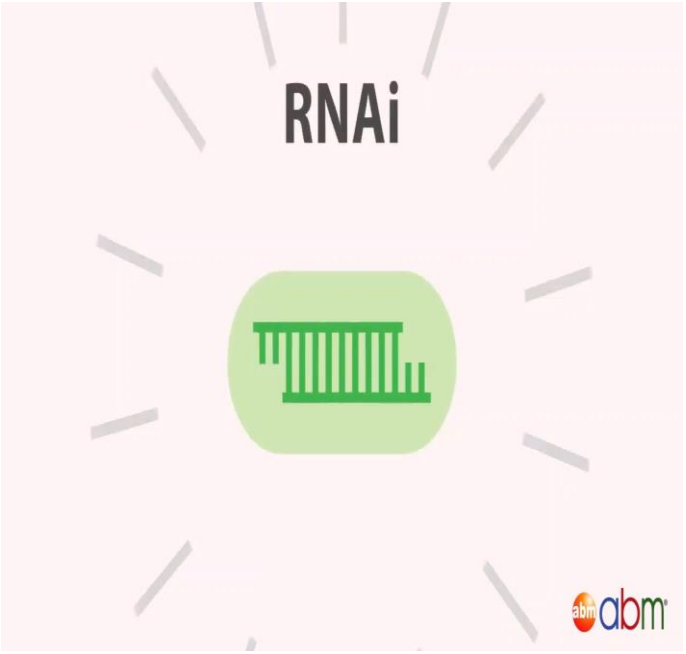
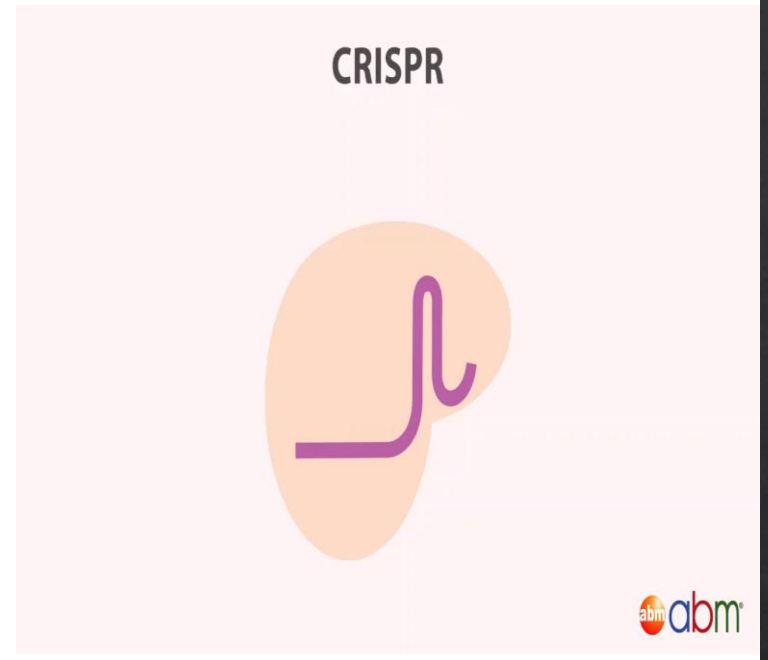


Figure 1. Summary of gene therapy strategies

(A) Non-viral strategies include using ASOs to induce alternate splicing or RNase H-mediated degradation. (B and C) Viral strategies include (B) AAV-mediated gene silencing, through RNA interference or CRISPR-Cas9 or (C) AAV-mediated gene delivery including neurotrophic factors. AAV, adeno-associated virus; ASO, antisense oligonucleotide; Cas, CRISPR-associated system; miRNA, microRNA; PAM, protospacer adjacent motif; RISC, RNA-induced silencing complex; RNAi, RNA interference; shRNA, small hairpin RNA.







Génterápia

Főbb betegségek

ALS

Transthyretin familialis amyloid neuropathia

SMA

Huntington-kór

Alzheimer-kór

ALS

- "Gold Coast„ kritériumok:
  - Progresszív felső és alsó motoneuron tünetek, eltérések egy végtagon, vagy testrészen

    VAGY
  - Progresszív alsó motoneuron tünetek és eltérések legalább két végtagon vagy testrészen

    ÉS
  - Egyéb betegségekre utaló elektrophysiológiai, képalkotói és patológiai evidenciák hiánya, amelyek az alsó és/vagy felső motoneuron tüneteket magyarázhatják
- 2-5 éven belül halálos, légzési elégtelenség
- Prevalencia: 5/100.000
- Több mint 80 klinikai vizsgálat során 2 molekula volt eddig hatékony: **riluzole** (glutamát antagonista) + **edaravone** (antioxidáns és szabadgyök scavanger) – excitotoxicitás, oxidatív stressz

ALS genetika

- Az esetek 10%-a herediter
- 120 gén
- Legfontosabbak:
  - SOD1 (superoxide dismutase-1)
  - C9orf72 (G4C2 expanzió)
  - TARDBP (TDP-43 kódoló gén)
  - FUS (fused in sarcoma)
  - ATXN2 (ataxin-2) CAG nukleotid expanzió

ALS (gén)terápia

SOD1:

- Toxikus funkciónyeréses mutáció, ami a SOD1 misfolded proteinek lerakódásához vezet
- 150 különböző mutáció, de a leggyakoribb a G93A
- BIIB067/**Tofersen** Fázis 3: NCT02623699, extension study: NCT03070119
  - Sorozatos LP 12 héten keresztül, ALSFRS romlás lecsökken
- AAVrh10 ASO kifejlesztése folyamatban

C9orf72:

- Hexanukleotid repeat expanzió elleni ASO – fázis 1

Neurotróp faktorok szintjének növelése:

- AVV - IGF1, GDNF, DOK7

Amado et al. 2021

Table 1. Gene therapy-based therapeutic strategies for ALS

Gene therapy	Date	Model	Route	Outcome	Citation
Antisense oligonucleotides					
SOD1	2006	rat; NHP SOD1^{G93A}	i.c.v. (rat), i.t. (NHP)	increased survival; good biodistribution in NHP	Smith et al.[74]
	2013	human SOD1-ALS phase I	i.t.	safe; no benefit	Miller et al.[75]
	2017	mouse SOD1^{G93A}	i.c.v. + i.v.	increased survival, strength, and weight	Biferi et al.[77]
	2020	human SOD1-ALS phase I/II	i.t.	safe; reduced CSF SOD1	Miller et al.[76]
	current	human SOD1-ALS phase III	i.t.	TBD	ClinicalTrials.gov: NCT02623699
C9orf72	2013	human iPSC C9-ALS		reduced nuclear RNA foci and excitotoxicity; reversed aberrant gene expression	Donnelly et al.[78]
	2013	human iPSC C9-ALS		reduced nuclear RNA foci and excitotoxicity; reversed aberrant gene expression	Sareen et al.[81]
	2013	human iPSC C9-ALS; mouse (WT)		reduced nuclear RNA foci; persistent aberrant expression; safe in mice	Lagier-Tourenne et al.[80]
	2016	mouse $C9_{(450)}$	i.c.v.	sustained reduction in nuclear RNA foci and DPRs in motor neurons; cognitive benefit	Jiang et al.[82]
	current	human C9-ALS phase I	i.t.	TBD	ClinicalTrials.gov: NCT03626012
ATXN2	2017	mouse hTDP-43	i.c.v.	reduced Atxn2 mRNA; increased survival; improved gait	Becker et al.[93]
	2018	human iPSC C9-ALS		reduced nuclear RNA foci and excitotoxicity; reversed aberrant gene expression	Zhang et al.[27]
	Current	human ATXN2-ALS & sporadic ALS phase I	i.t.	TBD	ClinicalTrials.gov: NCT04494256
FUS	2019	human FUS-ALS	i.t.	patient passed; further clinical data not reported	Arnold[96]
	Current	human FUS-ALS	i.t.	TBD	Figueiredo[97]

AAV-mediated gene silencing					
AAV-RNAi					
SOD1	2013	mouse SOD1^{G93A} & SOD1^{G37R}; NHP	i.v. (mice), i.t. (NHP)	increased survival, decreasing with age of administration; reduced *SOD1* mRNA in NHP spinal cord	Foust et al.[116]
	2014	rat SOD1^{G93A}	cortex	delayed disease onset and increased survival; preserved motor function	Thomsen et al.[118]
	2016	mouse SOD1^{G93A}	i.c.v.	increased survival; delayed paralysis; increase in motor neurons	Stoica et al.[119]
	2016	mouse SOD1^{G93A}; NHP	i.v. (mice), i.t. (NHP)	delayed onset; increased survival; preserved motor and respiratory function in mice; reduced *SOD1* mRNA in NHP spinal cord	Borel et al.[120]
	2018	NHP	i.t.	reduced *SOD1* mRNA in spinal cord	Borel et al.[121]
	2019	mouse SOD1^{G93A}	tongue, subplural	increased survival; lowered *SOD1* mRNA in muscle, tongue, and diaphragm	Keeler et al.[125]
Gene therapy	Date	Model	Route	Outcome	Citation
	2020	human SOD1-ALS phase I	i.t.	possible clinical stabilization in 1 patient; lowered spinal cord SOD1 in second, with DRG toxicity and no clear clinical benefit	Mueller et al.[122]
	2020	mouse SOD1^{G37R}; pig; NHP	lumbar subpial	stopped disease progression in mice; strong LMN targeting in large animals	Bravo-Hernandez et al.[127]
C9orf72	2015	human iPSC C9-ALS		marked reduction in nuclear foci	Hu et al.[132]
	2017	human iPSC C9-ALS		marked reduction in nuclear foci	Hu et al.[133]
	2019	(G4C2)44 cells; human iPSC C9-FTD		reduced nuclear foci; silenced *C9orf72* in iPSC	Martier et al.[134]
	2019	human iPSC C9-FTD; mouse C9orf72_3	striatum	reduced nuclear foci in iPSCs, reduced *C9orf72* mRNA in mouse striatum	Martier et al.[135]

AAV-CRISPR					
SOD 1	2017	mouse SOD1^{G93A}	i.v.	increased survival and strength with decreased SOD1 protein in spinal cord	Gaj et al.[128]
	2020	mouse SOD1^{G93A}	i.c.v.	increased survival and strength with decreased SOD1 protein in spinal cord	Duan et al.[129]
	2020	mouse SOD1^{G93A}	i.t.	increased survival and strength with decreased SOD1 inclusions in spinal cord	Lim et al.[130]
C9orf72	2019	Drosophila C9; human iPSC C9-ALS		reduced apoptotic pathway activation; reduced nuclear foci	Lopez-Gonzalez et al.[137]
	2020	human iPSC C9-ALS		reduced *C9orf72* expression; near elimination of dipeptide repeats; reduced axonal degeneration	Krishnan et al.[136]
AAV-mediated gene delivery					
Neurotrophic support					
IGF	2010	mouse SOD1^{G93A}	i.c.v.	improved motor function and increased survival	Dodge et al.[151]
	2016	mouse SOD1^{G93A}	i.m.	preserved motor neurons; increased survival	Allodi et al.[148]
	2018	mouse SOD1^{G93A}	i.m.	preserved motor neurons; increased survival	Lin et al.[149]
	2018	mouse SOD1^{G93A}	i.v.	preserved motor neurons; increased survival	Wang et al.[150]
VEGF	2010	mouse SOD1^{G93A}	i.c.v.	improved motor function and increased survival	Dodge et al.[151]
	2013	feline Lix1$^{-/-}$ (LMN disease)	i.c.v., i.v., or i.c.m.	only i.c.m. delivery resulted in sustained VEGF in spinal cord, without therapeutic benefit	Bucher et al.[155]
	2016	mouse SOD1^{G93A}	i.t.	improved motor function and increased survival	Wang et al.[154]
GDNF	2017	rat SOD1^{G93A}	i.v.	improved strength, but no effect on survival; worsened cognitive function, decreased activity	Thomsen et al.[158]
G-CSF	2011	mouse SOD1^{G93A}	intra-spinal	preserved motor units; increased survival	Henriques et al.[159]
HGF	2019	mouse SOD1^{G93A}	i.t.	improved motor function and increased survival	Lee et al.[160]
	2019	mouse SOD1^{G93A}	i.t.	improved motor function and increased survival	Lee et al.[161]

ALS immunológiai terápiák

- **Mastinib**: fázis 2/3 (+ riluzole) – tyrosine kináz inhibítor – microgliák, macrophágok – fázis 3 folyamatban

- **Ravulizumab-cwvz** – humanizált monoklonális antitest – C5 komplement aktiváció gátlása – neuroinflammáció csökkentése – FDA engedély: HUS + PNH. Fázis 3

Transthyretin familialis amyloid neuropathia

- Progresszív szenzori-motoros és autonóm neuropathia
- Transthyretin protein misfolding – amyloid fibrillumok – amyloidosis (perifériás idegek, autonóm idegrendszer, szív)
- 7-12 éven belül szívelégtelenségben meghalnak
- TTR-FA**P**; TTR-FA**C**

Table 2. ATTReuNET-recommended diagnosis of nonendemic (usually late-onset) TTR-FAP: Key points of note

Typical clinical features of later disease (average 4 years post onset; the usual delay for diagnosis)
Progressive idiopathic polyneuropathy
Early walking difficulties, using aid support
Initial complaint: [20]
Sensory-motor neuropathic symptoms (80%)
Autonomic symptoms (10%)
Examination: All modality sensory deficit
Presence of family history (less than 50%)
Autonomic neuropathy without diabetes (uncommon at the onset)
Neurogenic orthostatic hypotension
Digestive symptoms (e.g., diarrhoea, constipation)
Urogenital symptoms (e.g., erectile dysfunction)
Unintentional major weight loss
Associated cardiac symptomatology (syncope, dyspnoea)
Diagnosis
DNA testing for *TTR* mutation (sequencing) first line in the future
Tissue biopsy confirms amyloid deposition

ATTReuNET, European Network for TTR-FAP; TTR, transthyretin; TTR-FAP, transthyretin familial amyloid polyneuropathy.

Adams et al. 2016

Gyakori téves diagnózisok

- ✗ Chronicus gyulladásos demyelinisatiós polyneuropathia (CIDP)
- ✗ Lumbalis spinalis stenosis
- ✗ Immunglobulin-könnyűlánc (AL) amyloidosis
- ✗ Carpalis alagút szindróma
- ✗ Charcot-Marie-Tooth betegség (CMT)
- ✗ Motoneuron betegségek

CIDP	ATTR-PN
primer demyelinisatio	axonalis károsodás
dysautonomia NEM jellemző	dysautonomia
elsősorban vastag, myelinisált idegrostok károsodása	elsősorban vékony idegrostok károsodása
emelkedett CSF* fehérjeszint	emelkedett CSF* fehérjeszint NEM jellemző
megnagyobbodott kereszt-metszetű idegek	NEM jellemző az idegek kereszt-metszetének megnagyobbodása


DPN
ATTR-PN
elsődlegesen vékony idegrostok érintettsége
elsődlegesen vékony idegrostok érintettsége
dysautonomia
dysautonomia
motoros tünet nem jellemző
distalis motoros tünet jellemző
jellemzően lassan progrediál
jellemzően gyorsan progrediál

Terápiás lehetőségek ATTR-ben

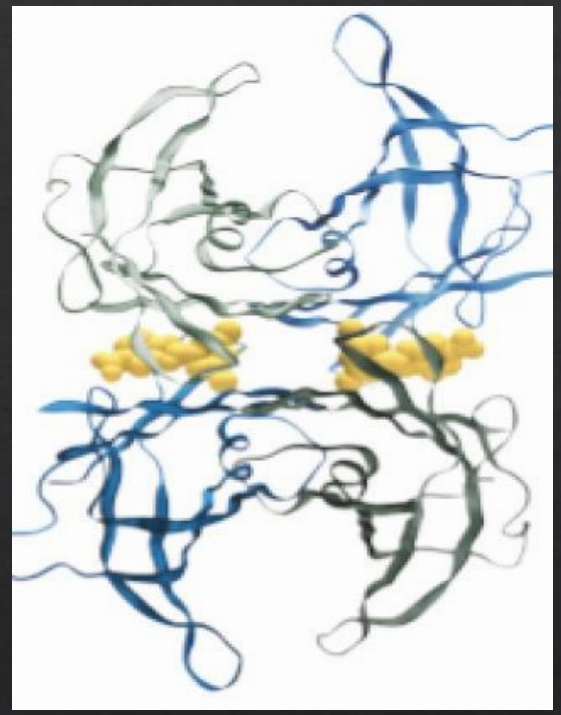
Tafamidis (Vyndaqel 20 mg caps.):

- a mutáns tetramer (transthyretin) stabilizációja, ezáltal az amyloidogenesis csökkentése
- járóképes betegek (Stage I kaphatják) – csökkenti a perifériás neurológiai károsodást

ALN-TTR02 – fázis III. – RNS interferencia

ISIS 420915 ASO

Diflunsal – NSAID



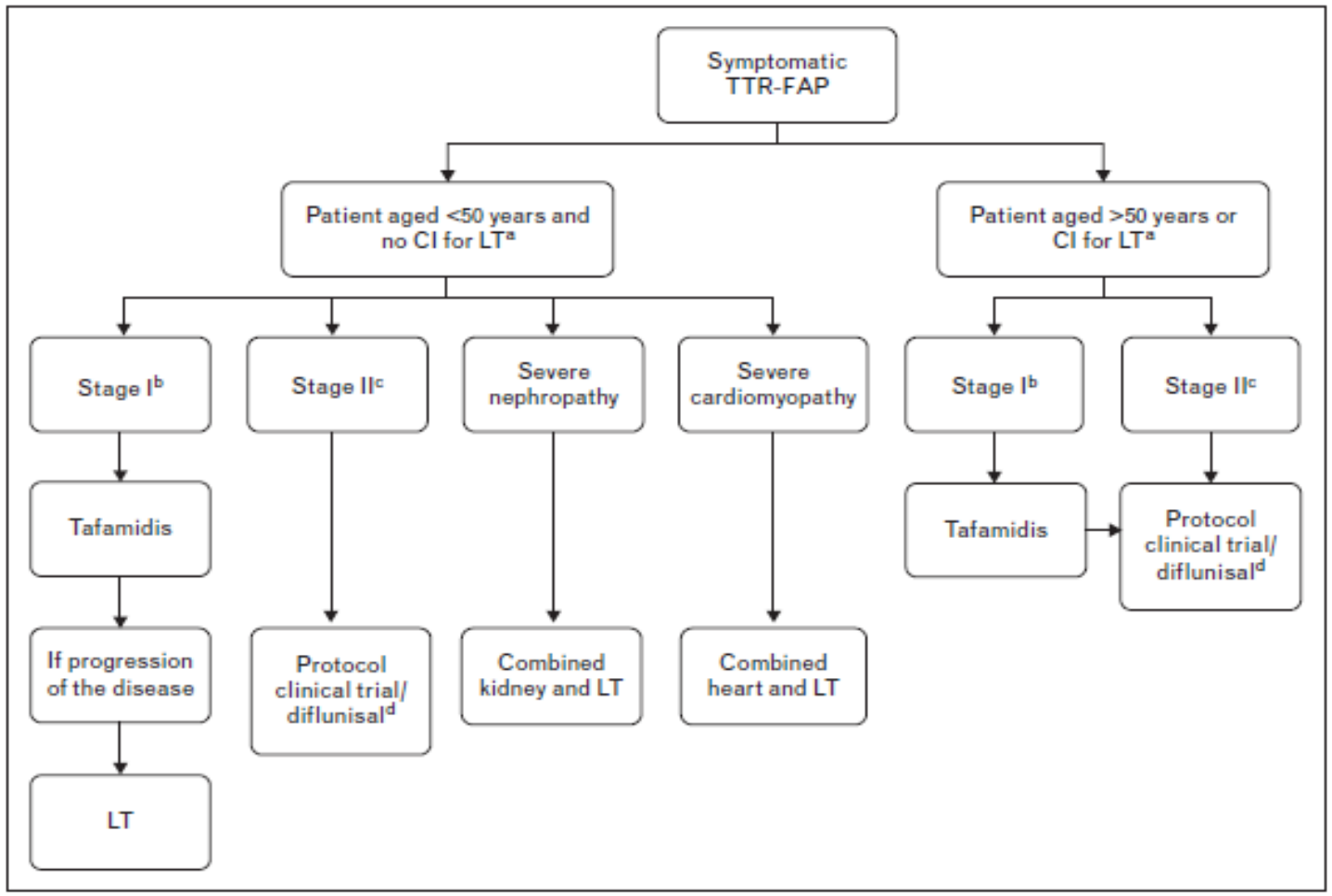


FIGURE 1. Strategy for specific therapy in TTR-FAP. CI, contraindications; LT, liver transplantation; TTR-FAP, transthyretin familial amyloid polyneuropathy. [a]CI for LT include: active and uncontrolled cancer; aged > 50 years for males and > 70 years for females [39▪▪,77], except for Italy (aged >65 years); modified body mass index below 800 kg/m^2·g/L; some non-Val30Met *TTR* mutations; cardiac insufficiency. [b]Stage I: walking unaided outside. [c]Stage II: walking with aid. [d]Protocol clinical trial for antisense oligonucleotides, small interfering RNA, combination doxycycline–tauroursodeoxycholic acid; or diflunisal off-label. Adapted from [1▪].

Adams et al. 2016

SMA

- Autosomalis recesszív, 5q, SMN1 gén
- Progresszív vázizom és légzőizom gyengeség
- Altípusok:

Type	Age of Onset	Maximal Motor Milestone	Motor Ability and Additional Features	Prognosis[c]
SMA 0	Before birth	None	Severe hypotonia; unable to sit or roll[a]	Respiratory insufficiency at birth; death within weeks
SMA I	2 weeks (Ia) 3 months (Ib) 6 months (Ic)	None	Severe hypotonia; unable to sit or roll[b]	Death/ventilation by 2 years
SMA II	6 to 18 months	Sitting	Proximal weakness; unable to walk independently	Survival into adulthood
SMA III	<3 years (IIIa) >3 years (IIIb) >12 years (IIIc)	Walking	May lose ability to walk	Normal life span
SMA IV	>30 years or 10 to 30 years	Normal	Mild motor Impairment	Normal life span

[a]Need for respiratory support at birth; contractures at birth, reduced fetal movements.
[b]Ia joint contractures present at birth; Ic may achieve head control.
[c]Prognosis varies with phenotype and supportive care interventions.

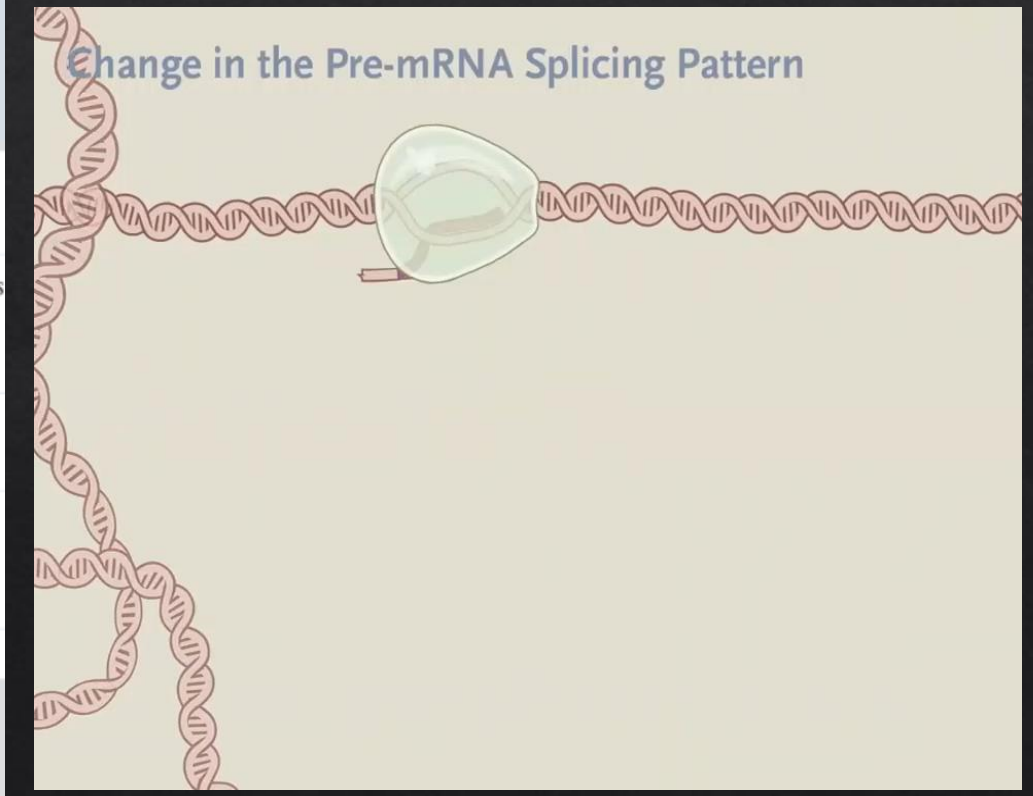


Table 1. Main clinical developments in spinal muscular atrophy (SMA).

Approach /Compound	Sponsor	Mechanism	Trials' Phase (SMA Type)	Administration	FDA Approval
Splicing modifiers of *SMN2* gene					
Nusinersen	Ionis-Biogen	ASO	I, II and III (I, II, III)	Intrathecal	X
Risdiplam	Roche	Small molecule	I, II and III (I, II, III)	Oral	pending
Albuterol		Beta-adrenergic agonist	Off-label	Oral	
Replacing *SMN1* gene					
Onasemnogene abeparvosec	Novartis-Avexis	AAV-9-vector construct	I, II and III (I, II)	Intravenous	X
Onasemnogene abeparvosec	Novartis-Avexis	AAV-9-vector construct	I	Intrathecal	
Muscle enhancing					
Reldesemtiv	Cytokinetics	Troponin activator	I and II (II, III, IV)	Oral	
SRK-105	Scholar Rock	Myostatin inhibitor	I and II (II, III)	Intravenous	
Neuroprotection					
Olesoxime	Hoffmann-La Roche	Anti-apoptotic agent	I and II (II, III) (development ended in 2018)	Oral	

ASO = antisense-oligonucleotide; AAV = adeno-associated virus; FDA= Food and Drug Administration.

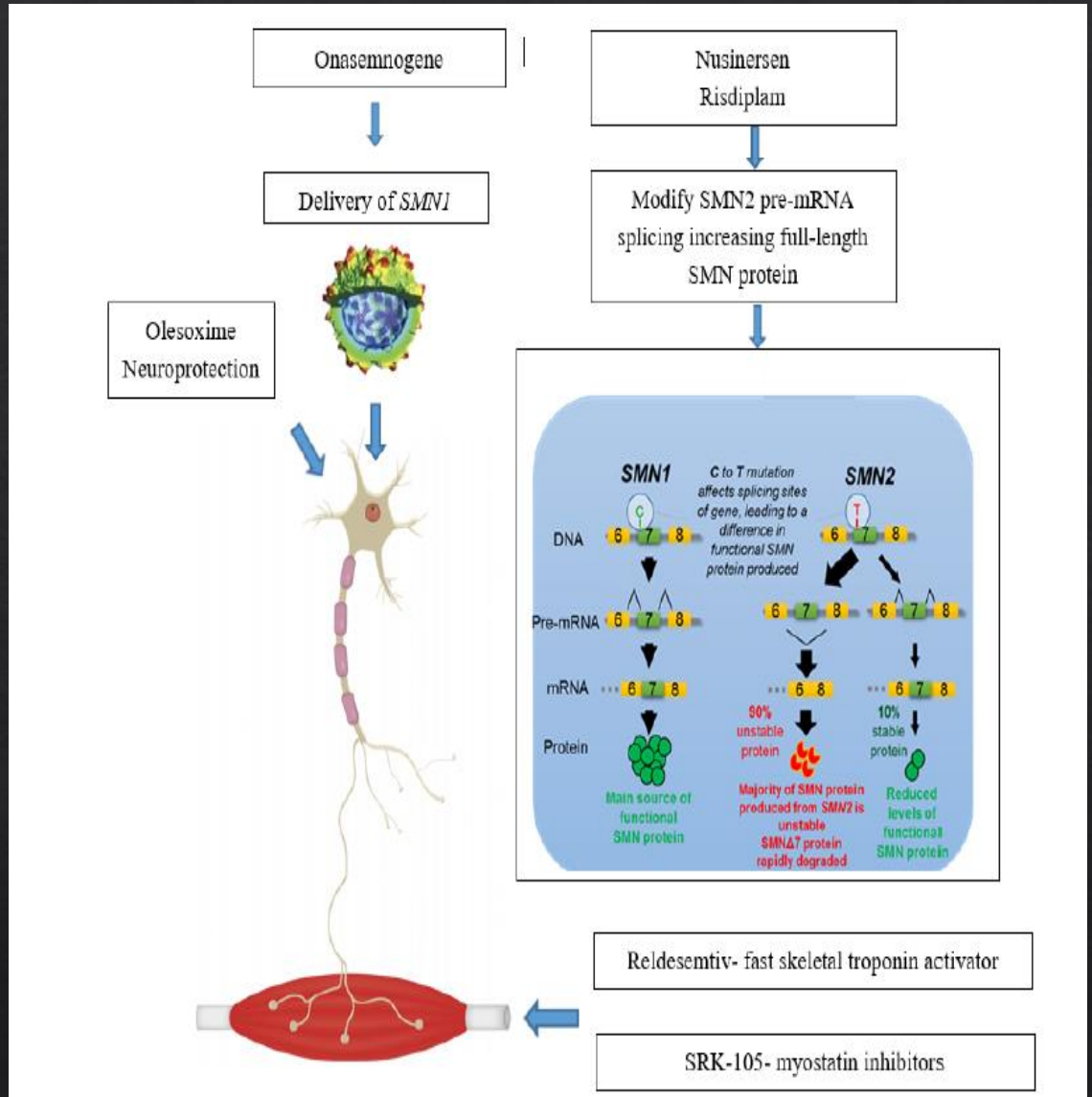


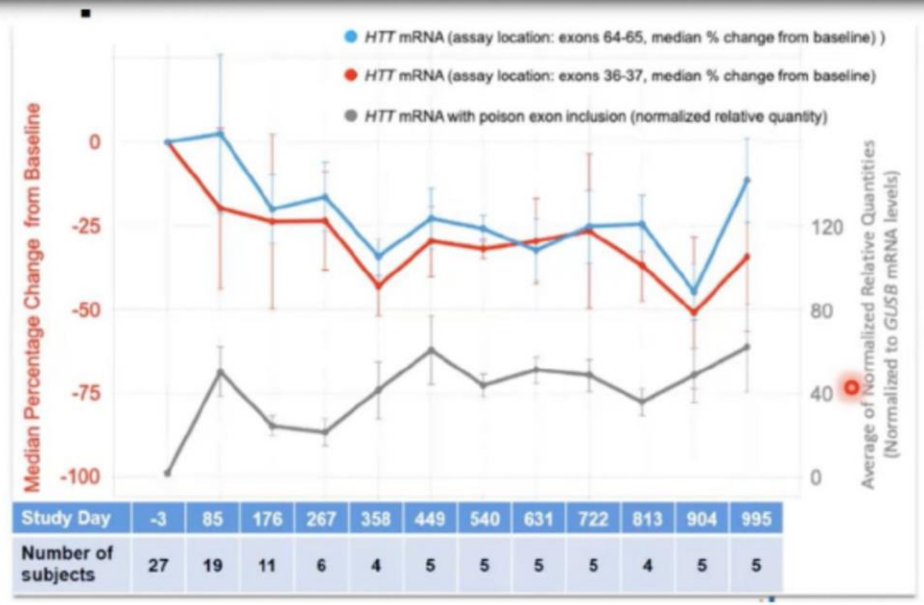
Figure 1. Main available therapeutic approaches and their mechanisms of action. *SMN1* = survival motor neuron 1; *SMN2* = survival motor neuron 2; *SMN* = survival motor neuron.

Huntington-kór

- Autoszomális domináns
- Felnőttkori kezdet többnyire, progresszív
- Motoros (chorea) + kognitív + pszichiátriai tünetek
- CAG trinukleotid repeat betegség (HTT)

Terápia Huntington-kórban

- **Tominersen** (IONIS-HTTRx vagy RG6042) – GEN-EXTEND – leállították – phase III
  - A Huntingtin protein (HTT) minden formáját lecsökkentette. ASO
- **Branaplam**
  - LMI070 – fázis 2b
  - Eredetileg SMA kezelésére fejlesztett szer
  - SMA-ban az SMN protein szintjét növeli meg – SMN2 gén serkentésén keresztül hat
  - Jelenleg fázis1/2-ben van SMA1-ben
  - A tesztelés során észlelték, hogy a huntingtin messenger RNS szintjét is képes csökkenteni
- **Pridopidine**
  - PROOF-HF, fázis 3
  - Kis molekula, amely a sigma-1 receptor (S1R) aktivációján keresztül a BDNF szintjét emeli meg, amely a HD betegekben csökkent
  - PRIDE-HD – fázis 2 – 2x45 mg korai HD-ban szignifikánsan lecsökkentette a funkcionális romlást



Here's what happened to the huntingtin levels of the branaplam-treated kids in the SMA trial. The red and blue lines are the amount of huntingtin, falling by about a third then staying low.

Alzheimer-kór

DSM-5 criteria for major neurocognitive disorder due to Alzheimer disease

A. Evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains*:
Learning and memory.
Language.
Executive function.
Complex attention.
Perceptual-motor.
Social cognition.
B. The cognitive deficits interfere with independence in everyday activities. At a minimum, assistance should be required with complex instrumental activities of daily living, such as paying bills or managing medications.
C. The cognitive deficits do not occur exclusively in the context of a delirium.
D. The cognitive deficits are not better explained by another mental disorder (eg, major depressive disorder, schizophrenia).
E. There is insidious onset and gradual progression of impairment in at least two cognitive domains.
F. Either of the following:
Evidence of a causative Alzheimer disease genetic mutation from family history or genetic testing.
All three of the following are present:
1) Clear evidence of decline in memory and learning and at least one other cognitive domain.
2) Steadily progressive, gradual decline in cognition, without extended plateaus.
3) No evidence of mixed etiology (ie, absence of other neurodegenerative disorders or cerebrovascular disease, or another neurological, mental, or systemic disease or condition likely contributing to cognitive decline).

DSM: diagnostic and statistical manual.

* Evidence of decline is based on: Concern of the individual, a knowledgeable informant, or the clinician that there has been a significant decline in cognitive function; and a substantial impairment in cognitive performance, preferably documented by standardized neuropsychological testing or, in its absence, another quantified clinical assessment.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), American Psychiatric Association, Arlington, VA 2013.

Alzheimer-kór kezelés

- **Aducanumab** – humán IgG1 anti-Aβ monoklonális antitest, amely a β-amyloid oligomerek és fibrillumohoz kötődik

- Két fázis 3 vizsgálat volt az elmúlt időszakban, az egyikben úgy tűnt nincs hatás, a másikban viszont pozitív tendenciát észleltek

Egyéb betegségek

Lysosomal storage disorder	Defective enzyme	Enzyme replacement therapies
Type 1 Gaucher disease	β-GCase	Imiglucerase, velaglucerase alfa and taliglucerase alfa
Fabry disease	α-Galactosidase A	Agalsidase beta and agalsidase alfa
Late infantile neuronal ceroid lipofuscinosis type 2 (CLN2 disease)	Tripeptidyl-peptidase 1	Cerliponase alfa
MPS I (Hurler–Scheie and Scheie syndromes)	α-Iduronidase	Laronidase
MPS II (Hunter syndrome)	Iduronidase-2-sulfatase	Idursulfase and idursulfase beta
MPS IV (Morquio syndrome A)	*N*-acetylgalactosamine-6-sulfate sulfatase	Elosulfase
MPS VI (Maroteaux–Lamy syndrome)	*N*-acetylgalactosamine-4-sulfatase (arylsulfatase B)	Galsulfase
MPS VII (Sly syndrome)	β-Glucoronidase	Vestronidase alfa
Pompe disease	α-Glucosidase	Alglucosidase alfa
Wolman disease	Lysosomal acid lipase deficiency	Sebelipase alfa

GCase, glucocerebrosidase; MPS, mucopolysaccharidosis.

NATURE REVIEWS | DRUG DISCOVERY

Konklúzió

- Betegek felismerése
- Genetikai diagnosztika
- Betegregiszterek