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Escola de Artes, Ciências e Humanidades
Universidade de São Paulo

Biotecnologia **ACH5533 – Fisiologia Humana I**

2º Semestre 2022

Docentes responsáveis:

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Créditos: 4

Período: Quarta-feira, 14:00 às 18:00 h

Local: sala 102, edifício I1

Neurociência e Biotecnologia

Clostridium tetani (tétano)

AÇÃO DA TOXINA TETÂNICA (neurotoxina):



Impede a liberação de glicina

Excesso Acetilcolina
Paralisia espástica

- Tetanospamina
- Espasmos musculares, morte.
- Vacina antitetânica
- Soro Antitoxina
- Relaxante muscular: Diazepam

Clostridium botulinum (botulismo)

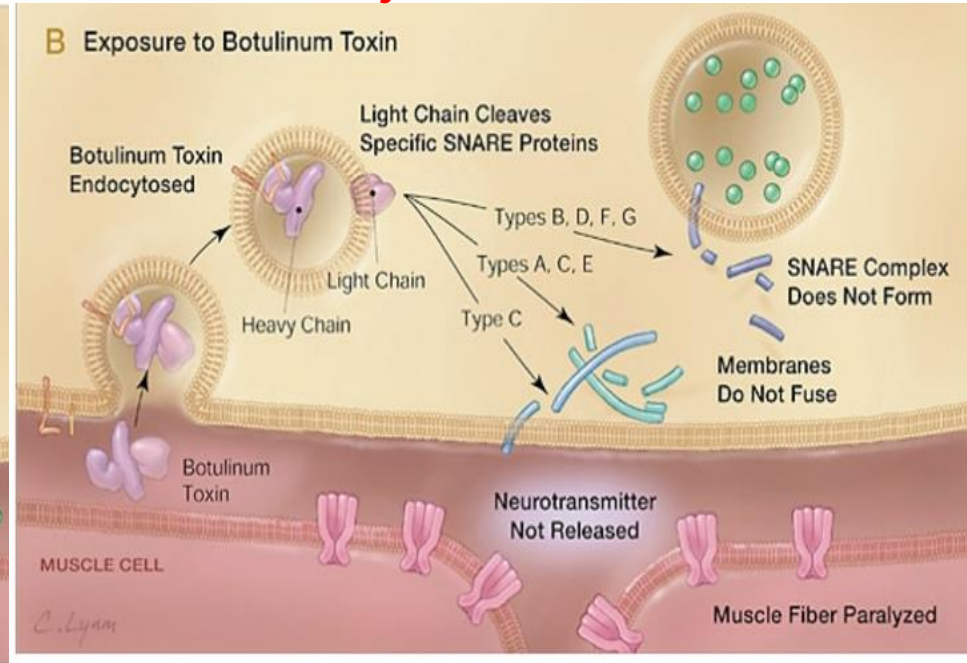
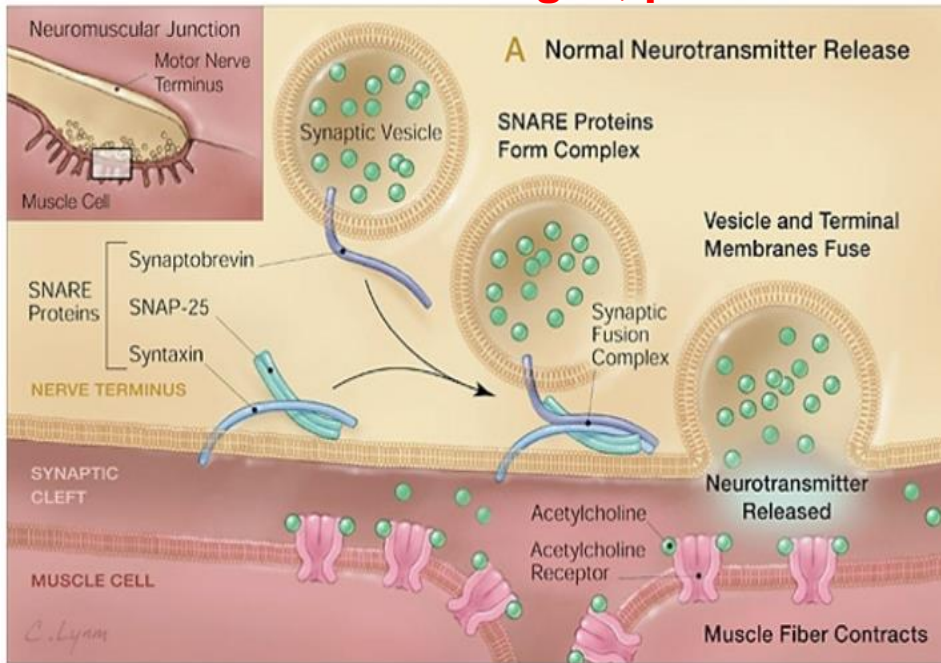
AÇÃO DA TOXINA BOTULÍNICA (neurotoxina):



Pouca Acetilcolina
Paralisia flácida

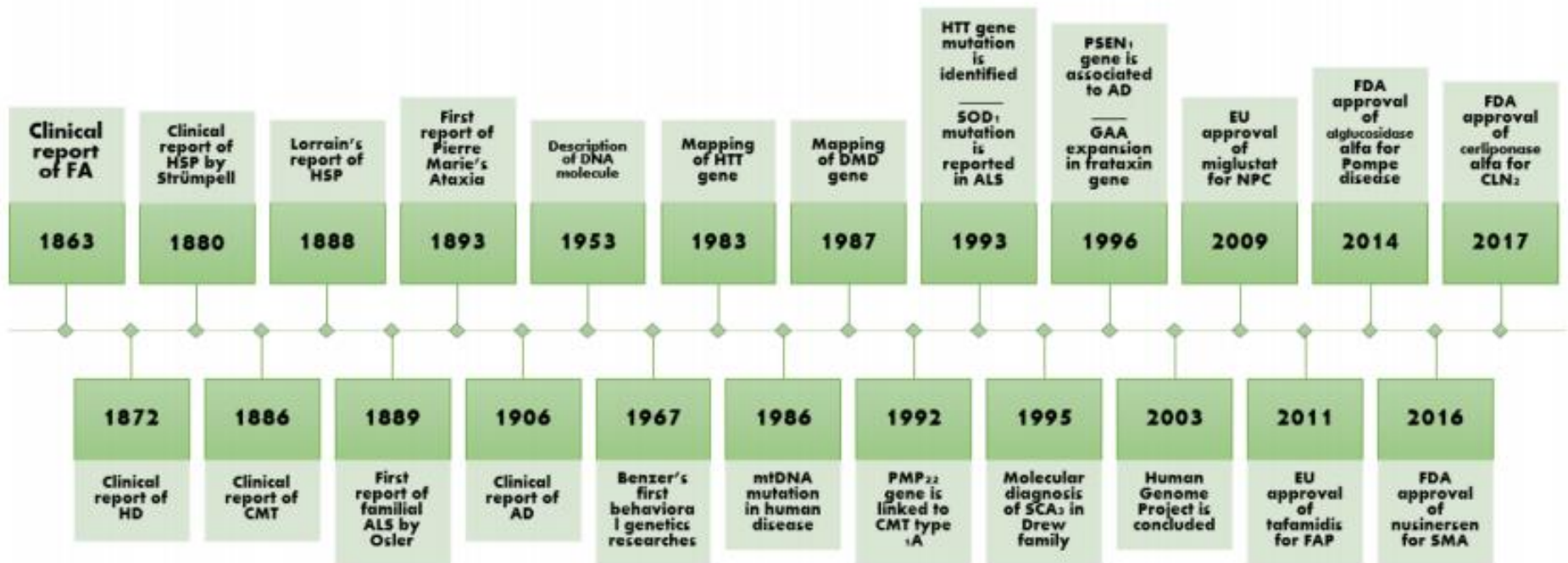
- Toxina botulínica: A-G
- Intoxicação alimentar
- Paralisia de músculo cardíaco ou respiratório
- Antitoxina botulínica
- Penicilina

Tratamento cosmético da toxina botulínica (BoTox), para reduzir linhas finas e rugas, paralisando os músculos subjacentes



A **Neurogenética** integra dois campos da ciência e da prática clínica: Neurologia e Genética. A Neurogenética é o campo que estuda a correlação entre o código genético e o desenvolvimento e a função do sistema nervoso, incluindo comportamento, personalidade e doenças neurológicas.

Linha do Tempo



FA: Friedreich ataxia; HD: Huntington disease; HSP: hereditary spastic paraplegia; CMT: Charcot-Marie-Tooth; ALS: amyotrophic lateral sclerosis; AD: Alzheimer disease; DMD: Duchenne muscular dystrophy; EU: European Union; NPC: Niemann-Pick type C disease; FDA: Food and Drug Administration; SMA: spinal muscular atrophy; FAP: familial amyloid polyneuropathy; CLN2: ceroid neuronal lipofuscinosis type 2.

Mais de 5.500 doenças ou, mais precisamente, fenótipos, são conhecidos por estarem associados a várias perturbações de genes específicos no genoma humano, com quase 50% deles apresentando envolvimento neurológico

Doenças neurológicas degenerativas são os distúrbios que afetam o sistema nervoso, elas não têm cura, o que significa que a condição avança com o passar do tempo, trazendo um grau de comprometimento ainda maior. Elas causam a destruição gradual e irreversível dos neurônios, de forma que as pessoas afetadas apresentam sintomas progressivamente mais intensos.

Os sintomas dessas doenças variam muito conforme a natureza da condição, podendo incluir dores de variadas origens, transtornos do sono, alteração da consciência, distúrbios dos sentidos (audição, visão, olfato, tato e paladar), mau funcionamento dos músculos, prejuízo da função mental, entre outros.

(A) **Mendelian disease**

One gene : one phenotype



Disease

Huntington disease

Genotype:phenotype correlation

Larger repeat size leads to earlier onset and more severe disease

One gene : multiple phenotypes



Fragile X syndrome

Full mutation (repeat size >200)

Fragile X tremor
Ataxia syndrome

Premutation
(repeat size ~55–200)

Multiple genes : one phenotype



Spinocerebellar
ataxia

Larger repeat size leads to earlier age of onset

(B) **Mendelian disease**

One gene : one phenotype



Disease

Hexosaminidase A
deficiency
(Tay–Sachs disease)

Genotype:phenotype correlation

Severity of disease correlates with effect of mutations on enzymatic activity

One gene : multiple phenotypes



Ataxia with
oculomotor apraxia
type 2 (AOA2)

Mutation effect determines
phenotype (loss-of-function)
and severity

Amyotrophic lateral
sclerosis, type 4
(ALS4)

Mutation effect determines
phenotype (gain-of-function)

Multiple genes : one phenotype



Hereditary spastic
paraplegia

Varies by gene/disease

Complex disease

Multiple genes : genetic risk



Parkinson disease

Additive, variants have differing effect sizes

Major Neurodegenerative Diseases

Parkinson's Disease

01



Alzheimer's Disease

02



Amyotrophic lateral sclerosis

03



Parkinson

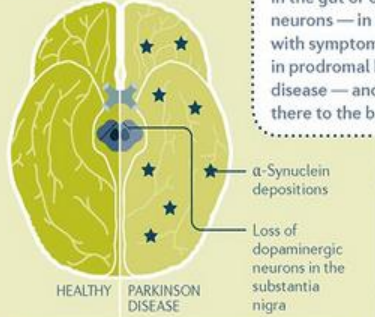
A DP é uma doença neurodegenerativa causada por lesões na via nigroestriatal, e a principal alteração patológica é a degeneração e necrose dos neurônios dopaminérgicos na substância negra.

For the Primer, visit doi:10.1038/nrdp.2017.13

→ Parkinson disease is a neurodegenerative disorder associated with dopamine depletion in the basal ganglia. Although best known as a movement disorder, non-motor symptoms, such as depression and cognitive impairment, are also frequent.

MECHANISMS

The motor symptoms associated with Parkinson disease result from loss of dopaminergic neurons in the substantia nigra, which ultimately inhibits neuronal output from the basal ganglia to the thalamus. Damage to dopaminergic neurons is caused by a complex interplay between toxic α -synuclein aggregates (owing to impaired protein degradation), mitochondrial dysfunction, oxidative stress, impaired intracellular calcium homeostasis and neuroinflammation.



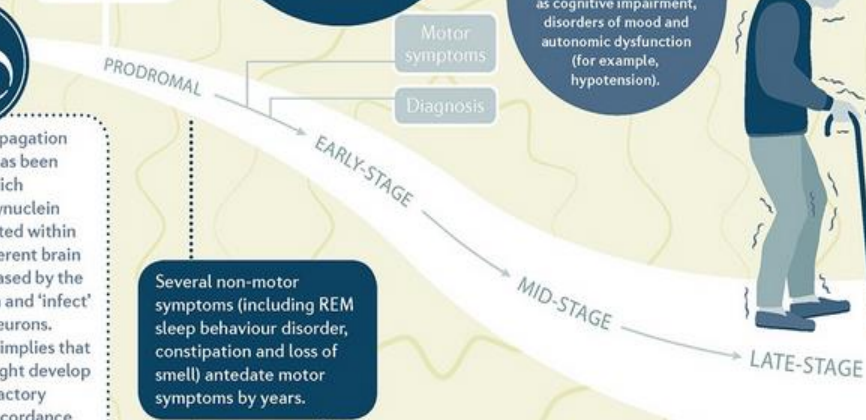
! Combined loss of specific neurons and widespread, intracellular α -synuclein aggregations are the pathophysiological hallmarks of Parkinson disease.

DIAGNOSIS

! Parkinson disease is defined by the presence of motor symptoms, including bradykinesia (a slowness of movement) and one or more additional cardinal motor feature (rigidity or rest tremor).

Although sporadic (also known as idiopathic) Parkinson disease is the most common type (>90% of patients), heritable forms have also been described. Genetic abnormalities identified in the latter have greatly contributed to understanding the processes involved in Parkinson disease.

In addition to the motor symptoms, patients show persistent and new non-motor symptoms, such as cognitive impairment, disorders of mood and autonomic dysfunction (for example, hypotension).



A prion-like propagation of α -synuclein has been proposed, in which aggregated α -synuclein can be transported within a neuron to different brain regions, be released by the affected neuron and 'infect' neighbouring neurons. This hypothesis implies that initial insults might develop in the gut or olfactory neurons — in accordance with symptom development in prodromal Parkinson disease — and spread from there to the brain.

Several non-motor symptoms (including REM sleep behaviour disorder, constipation and loss of smell) antedate motor symptoms by years.



200 years after James Parkinson's seminal essay on 'the shaking palsy', Parkinson disease can now effectively be managed with sustained symptom control and improvement of quality of life for many years.

In addition to clinical evaluation, diagnostic tests are mainly based on imaging, for example, of striatal dopamine levels.

OUTLOOK

The identification of markers for prodromal disease and the development and optimization of curative treatments remain considerable challenges. New

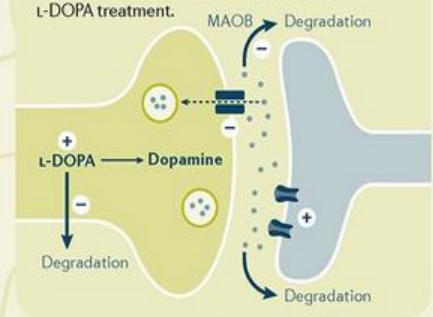
treatments might involve gene therapy, fetal cell transplantation, stem cell treatment or new disease-modifying drugs.

EPIDEMIOLOGY

Parkinson disease is the second-most common neurodegenerative disorder. The incidence of the disease sharply increases with age. Indeed, the global prevalence across all age groups is estimated at 0.3%, whereas levels increase to 2–3% in the population ≥ 65 years of age. Parkinson disease is more common in men than in women. The incidence varies depending on ethnicity, environmental factors (such as exposure to organic pollutants), lifestyle factors (for example, smoking and caffeine intake) and genetic factors.

MANAGEMENT Rx

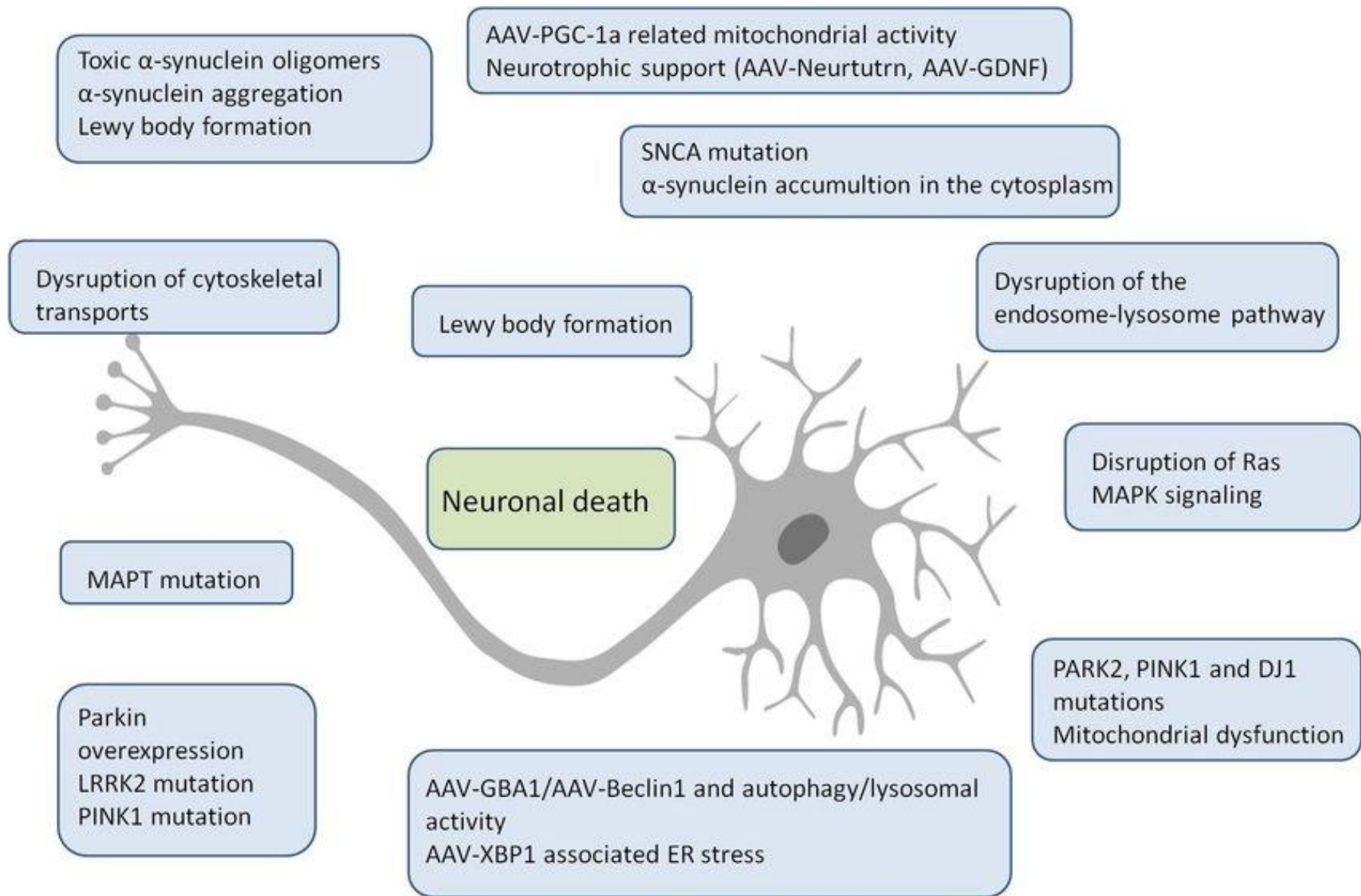
Systemic administration of the dopamine precursor L-DOPA is the gold-standard treatment, although chronic use can result in adverse effects, such as motor fluctuations or L-DOPA-induced dyskinesia (involuntary movement). Inhibitors of dopamine metabolism can complement L-DOPA therapy, whereas L-DOPA-degrading enzymes or dopamine receptors provide alternative targets. Non-dopaminergic pharmacological treatments can improve motor and non-motor symptoms. Deep brain stimulation of the subthalamic nucleus is a valid option for patients with advanced Parkinson disease who experience adverse effects to L-DOPA treatment.



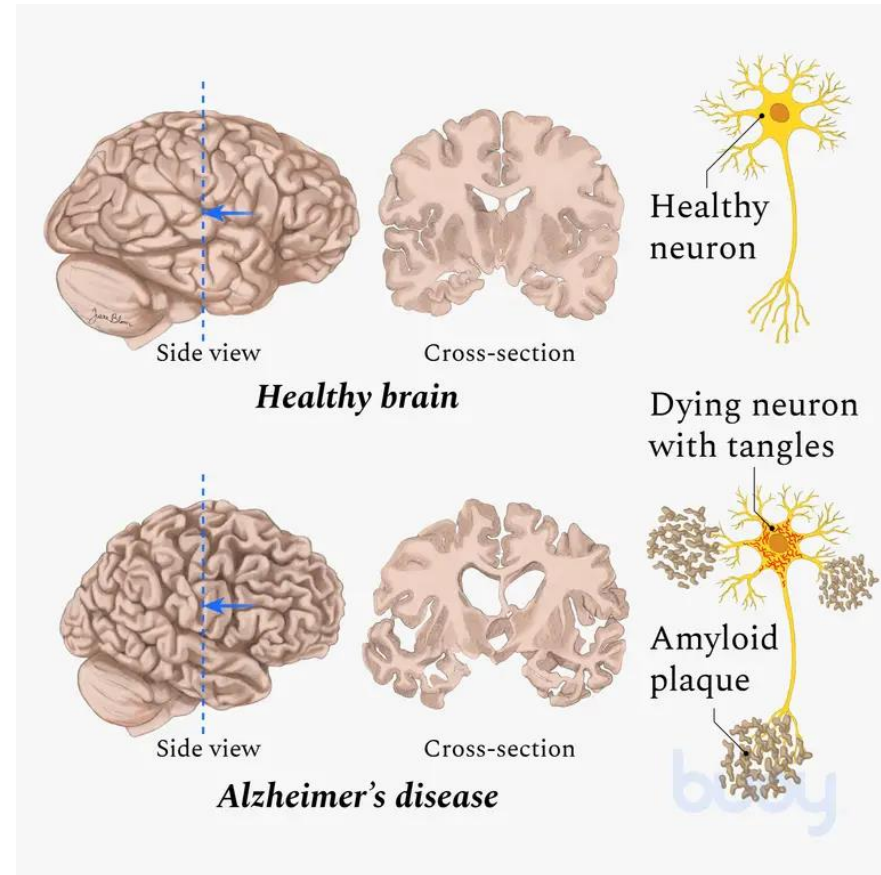
Written by Liesbet Lieberc designed by Laura Marshall

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Article number: 17014; doi:10.1038/nrdp.2017.14; published online 23 Mar 2017



A **doença de Alzheimer** é um distúrbio cerebral que destrói lentamente a memória e as habilidades de pensamento e, eventualmente, a capacidade de realizar as tarefas mais simples. Na maioria das pessoas com a doença – aqueles com sintomas do tipo de início tardio aparecem pela primeira vez em meados dos anos 60. Acredita-se que a doença de Alzheimer seja causada pelo acúmulo anormal de proteínas dentro e ao redor das células cerebrais. Uma das proteínas envolvidas é chamada de amilóide, cujos depósitos formam placas ao redor das células cerebrais. A outra proteína é chamada tau, cujos depósitos formam emaranhados dentro das células cerebrais.



	Gene symbol	Description	Category	Gene ID	
1	APP	Amyloid beta (A4) precursor protein	Protein-coding	GC21M027252	
2	COL25A1	Collagen, type XXV, alpha 1	Protein-coding	GC04M109731	
3	BPTF	Bromodomain PHD finger transcription factor	Protein-coding	GC17P065821	
4	PSEN1	Presenilin 1	Protein-coding	GC14P073603	
5	PSEN2	Presenilin 2	Protein-coding	GC01P227058	
6	CLSTN1	Calsyntenin 1	Protein-coding	GC01M009789	
7	APOE	Apolipoprotein E	Protein-coding	GC19P045408	
8	GSK3B	Glycogen synthase kinase 3 beta	Protein-coding	GC03M119540	
9	CHAT	Choline O-acetyltransferase	Protein-coding	GC10P050817	
10	APBB1	Amyloid beta (A4) precursor protein-binding, family B, member 1	Protein-	GC11M006414	
	11	PSENE1	Presenilin enhancer gamma secretase subunit	Protein-coding	GC19P036236
	12	LRP1	Low density lipoprotein receptor-related protein 1	Protein-coding	GC12P057497
	13	NCSTN	Nicastrin	Protein-coding	GC01P160313
	14	CDK5R1	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	Protein-coding	GC17P030813
	15	GSK3A	Glycogen synthasekinase 3 alpha	Protein-coding	GC19M042734
	16	CASP3	Caspase 3, apoptosis-related cysteine peptidase	Protein-coding	GC04M185548
	17	APBA1	Amyloid beta (A4) precursor protein-binding, family A, member 1	Protein-coding	GC09M072042
	18	APBA2	Amyloid beta (A4) precursor protein-binding, family A, member 2	Protein-coding	GC15P029213
	19	CASP2	Caspase 2, apoptosis-related cysteine peptidase	Protein-coding	GC07P142985
	20	MAPT	Microtubule-associated protein tau	Protein-coding	GC17P043971

AD is a complex disease caused by a combination of age, genetic, and environmental factors. These factors trigger or increase the risk of developing AD

ELA - Esclerose Lateral Amiotrófica

Esclerose Lateral Amiotrófica (ELA), uma doença do neurônio motor fatal caracterizada por fraqueza muscular progressiva, atrofia e fasciculações, bem como hiperreflexia do tendão sem parestesia. Como 20% dos casos de ELA são familiares, a ELA tem sido associada à mutação da superóxido-dismutase-1 (SOD1), que leva à degeneração dos neurônios motores

For the Primer, visit [doi:10.1038/nrdp.2017.71](https://doi.org/10.1038/nrdp.2017.71)

➔ Amyotrophic lateral sclerosis (ALS; also known as motor neuron disease) is a rare, neurodegenerative disease that is characterized by the degeneration of upper and lower motor neurons, leading to muscle weakness and paralysis.

MECHANISMS

ALS can be classified as either sporadic or familial. Familial ALS has been associated with mutations in >30 genes, but mutations in four genes — *C9orf72*, *TARDBP*, *SOD1* and *FUS* — account for >70% of cases. Proteins encoded by these genes are involved in several aspects of motor neuron function, including protein homeostasis, DNA repair, RNA metabolism, vesicle transport, mitochondrial function and glial cell function. Several of these mechanisms probably interact to contribute to the degeneration of motor neurons in ALS. In general, the proteins encoded by these genes are ubiquitously expressed, so why these mutations lead to the selective degeneration of motor neurons, and not other cell types, is unknown. The pathological hallmark of ALS is the accumulation of intraneuronal protein aggregates, which, in most individuals, contain TAR DNA-binding protein 43. However, other proteins can form aggregates, including superoxide dismutase 1 and neurofilament. Whether these protein aggregates or the protein complexes that precede their formation are toxic to neurons is poorly understood.

The gross macroscopic features of ALS include atrophy of skeletal muscle and the motor cortex, and sclerosis of the pyramidal tracts

DIAGNOSIS

Diagnosis includes clinical investigation to rule out other causes of the symptoms and to identify evidence of disease progression

Symptoms of upper motor neuron degeneration include spasticity and muscle weakness, whereas fasciculations (twitching), muscle cramps and wasting are indicative of lower motor neuron degeneration

OUTLOOK

One barrier to the development of effective treatments for ALS is the poor understanding of how the pathology of disease affects the overall integrity and function of brain networks. Improvements in model systems to study ALS and better ways to study the disease in humans will enhance our understanding of ALS and enable us to target therapies to specific aspects of the pathophysiology.

EPIDEMIOLOGY

Up to 50% of patients develop cognitive and/or behavioural impairments during the course of the disease

In Europe, incidence is in the range of 2–3 cases per 100,000 individuals; incidence is lower in east Asia (0.8 cases per 100,000 individuals) and south Asia (0.7 cases per 100,000 individuals). The phenotype of ALS varies between populations. For example, the age at onset of symptoms and diagnosis is higher in Europe than in Asia or South America. In addition, the proportion of individuals with bulbar-onset disease is much lower in Asia than in Europe and patient survival is lower in Europe (24 months from the onset of disease) than in central Asia (48 months).

Some forms of ALS share a genetic overlap with neuropsychiatric conditions, such as schizophrenia

MANAGEMENT Rx

Few randomized controlled trials assessing symptomatic therapies in patients with ALS have been conducted; accordingly, many therapies are based on the management of other diseases. For example, anticholinergic drugs can be used to treat sialorrhoea (hypersalivation), baclofen can be used to treat spasticity and muscle relaxants can be used to treat cramps. The management of dysphagia includes dietary changes, swallowing manoeuvres and exercise and, if severe, use of a gastrostomy tube. Other available symptomatic treatments include speech therapy for dysarthria and noninvasive ventilation for respiratory failure.

Two disease-modifying therapies — riluzole and edaravone — have been approved by the US FDA

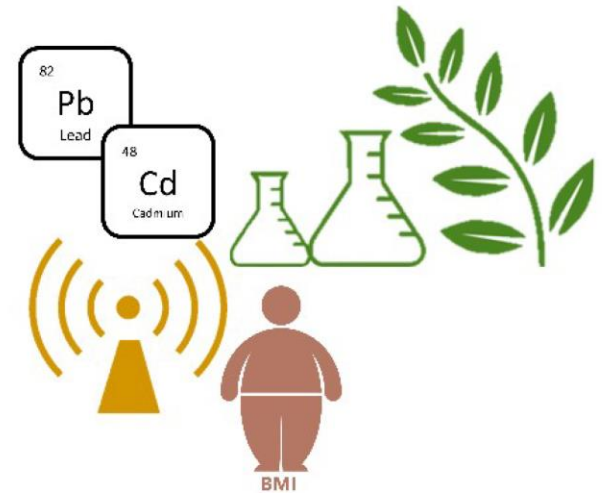
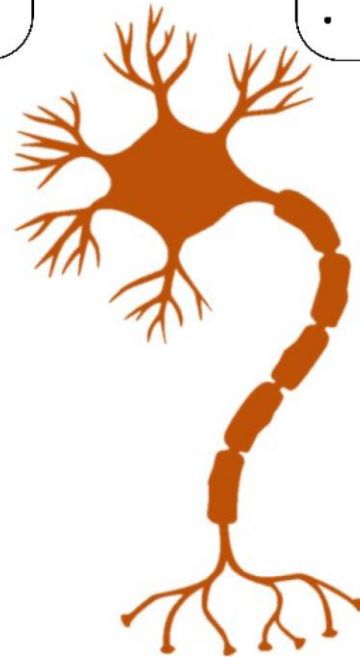
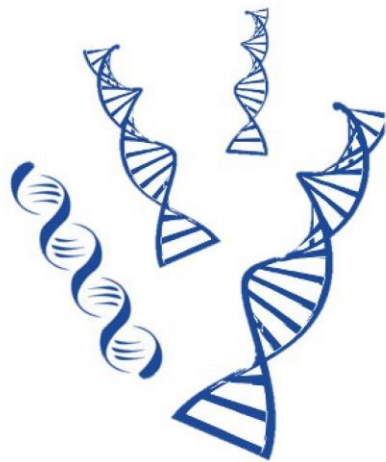
Risk factors predisposing to Amyotrophic Lateral Sclerosis

Genetic factors

- mutation in Superoxide Dismutase 1 (SOD1 gene)
- TARDBP mutation
- C9orf72 mutation
- TBK1 mutation

Environmental factors

- exposure to heavy metals (lead, iron, cadmium, selenium, mercury)
- exposure to pesticides
- exposure to electromagnetic fields (EMF)
- BMI and nutritional state





Advances in gene therapy for neurogenetic diseases: a brief review

Duchenne muscular dystrophy (DMD)
Huntington's disease (HD)
Adrenoleukodystrophy (ALD):
Doenças PolyQ
Atrofia Muscular Espinhal (SMA)
ELA - Esclerose Lateral Amiotrófica (ALS)
Doença de Parkinson (PD)

① SMA, DMD, ALS

② PD, SMA, ALS

③ SMA, DMD, ALD

④ HD, PolyQ diseases

Examples of gene therapy strategies in neurogenetic diseases

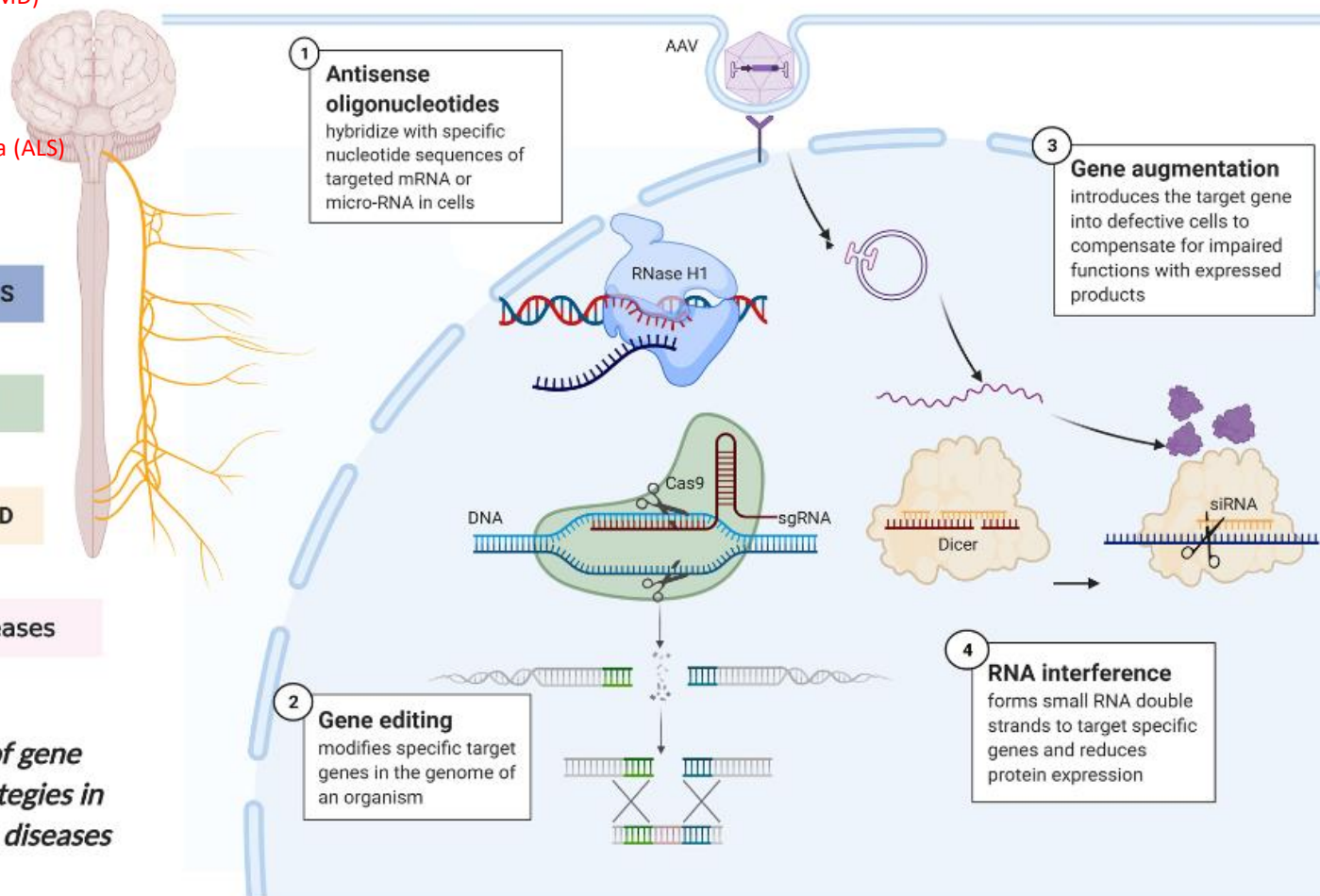


Fig. 1 Examples of gene therapy strategies used in the treatment of neurogenetic diseases. The schematic diagram shows different gene therapy approaches used for treating a sampling of neurogenetic disorders, such as ASO therapy, gene editing, gene augmentation, and RNA interference

Table 1 Gene therapy trials for neurogenetic diseases

Disorder	Therapy	Strategy	Delivery	Vector	Model	Refs
ALS	CBEs	Nonsense-coding substitution to SOD1	Intrathecal	Dual AAV	SOD1 ^{G93A} mouse	[33]
	ASOs	Mutant SOD1 knockdown	Intrathecal	N/A	SOD1 Gly93Ala rat	[72]
	ASOs	Reduce the build-up of C9ORF72 repeat expansion RNA	Co-culture	N/A	iPSC-derived motor neuron	[73]
	ASOs	Mediate RNase H-dependent degradation of SOD1 mRNA to reduce the synthesis of SOD1 protein	Intrathecal	N/A	<i>SOD1</i> mutant ALS adult	[17]
DMD	RNAi-shRNA	Mutant SOD1 knockdown	Intracerebral	AAV	SOD1 ^{G93A} transgenic rat	[74]
	CRISPR-Cas9	Deletion of <i>Dmd</i> exon23	Intrathecal	AAV	Mdx mouse	[75]
	ABEs	Correct a nonsense mutation in <i>Dmd</i> gene	Embryo Microinjection	AAV	DMD mouse	[76]
HD	siRNA & Di-siRNA	Repress the expression of <i>HTT</i> gene	Intraventricular	N/A	HD mouse/HD Patient	[77]
	shRNA	Repress the expression of <i>Htt</i> gene	Intracerebral	AAV	HD mouse	[59]
HTI	ABEs	Correct a point mutation in <i>Fah</i> exon8	Tail-vein injection	Lipid-nanoparticle	FAH ^{mut/mut} mouse	[78]
SMA	CRISPR-Cas9	Enhancement of <i>SMN2</i> Exon 7 Inclusion	Zygote Co-injection	N/A	Human iPSC & SMA mouse (Smn ^{-/-} , SMN2 ^{tg/-})	[79]
	ABEs	Splicing correction of ESS-A and ESS-B of <i>SMN2</i> exon 7	Zygote Co-injection	N/A	Human iPSC & SMA mouse (Smn ^{-/-} , SMN2 ^{tg/-})	[22]
	ASOs	Increase expression of <i>SMN2</i> Exon 7	Intraventricular	N/A	SMA mouse	[80]
	ASOs	Enhancement of <i>SMN2</i> Exon 7 Inclusion	Co-culture	N/A	HEK293 cells	[81]
X-ALD	LV	Correct mutant <i>ABCD1</i> gene	Intracerebral	Lentivirus	X-ALD patient	[82]

Atrofia Muscular Espinhal (AME)

A atrofia muscular espinhal (AME) é uma doença neurodegenerativa com herança genética autossômica recessiva. É a principal desordem fatal com esse caráter genético depois da fibrose cística (1:6.000), com uma incidência de 1:6.000 a 1:10.000 nascimentos¹. A frequência de indivíduos portadores (heterozigotos) da doença é de um para cada 40 a 60 indivíduos².

A doença é causada por uma deleção ou mutação homozigótica do gene 1 de sobrevivência do motoneurônio (SMN_1), localizado na região telomérica do cromossomo 5q13, sendo que o número de cópias de um gene semelhante a ele (SMN_2), localizado na região centromérica, é o principal determinante da severidade da doença³.

Essa alteração genética no gene SMN_1 é responsável pela redução dos níveis da proteína de sobrevivência do motoneurônio (SMN). O gene SMN_2 não compensa completamente a ausência da expressão do SMN_1 porque produz apenas 25% da proteína SMN⁴. A falta da proteína SMN leva à degeneração de motoneurônios alfa (α) localizados no corno anterior da medula espinhal, o que resulta em fraqueza e paralisia muscular proximal progressiva e simétrica

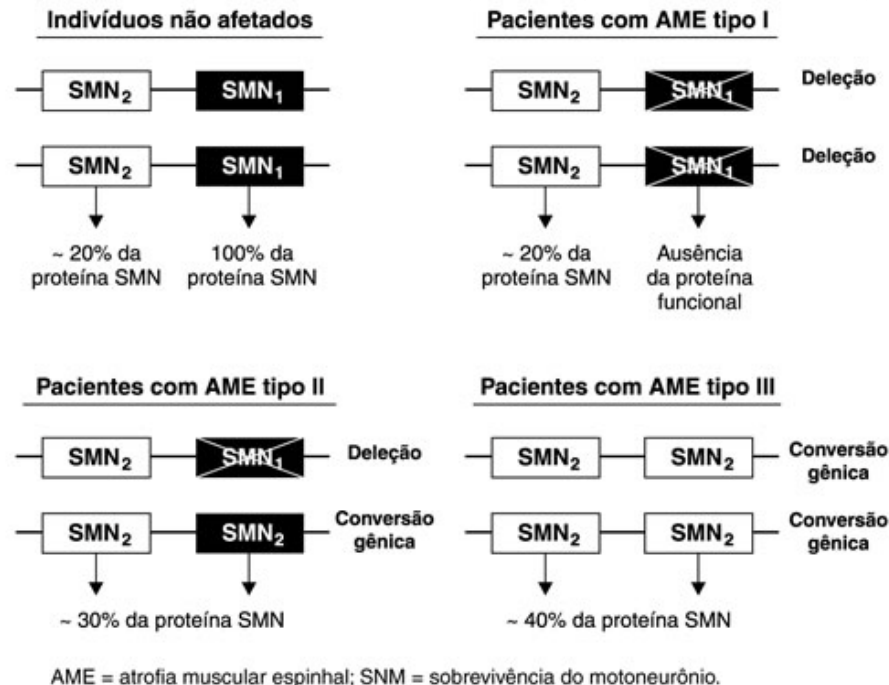


Figura 2 - Genótipo de indivíduos não-afetados e afetados pela AME

Spinal muscular atrophy (SMA; AME)

A AME é uma doença genética neuromuscular manifestada como miastenia simétrica e atrofia muscular das extremidades proximais. A deleção ou mutação do gene do neurônio motor de sobrevivência 1 (SMN1) leva à falta da proteína SMN. A gravidade da doença é negativamente correlacionado com o número de cópias do gene SMN2, que é geneticamente semelhante ao gene SMN1 e pode compensar a perda de SMN1, produzindo assim a proteína SMN completa. No entanto, o gene SMN2 apresenta uma variante C a T no exon 7, resultando em apenas 10% da proteína SMN completa sendo produzida devido ao splicing alternativo.

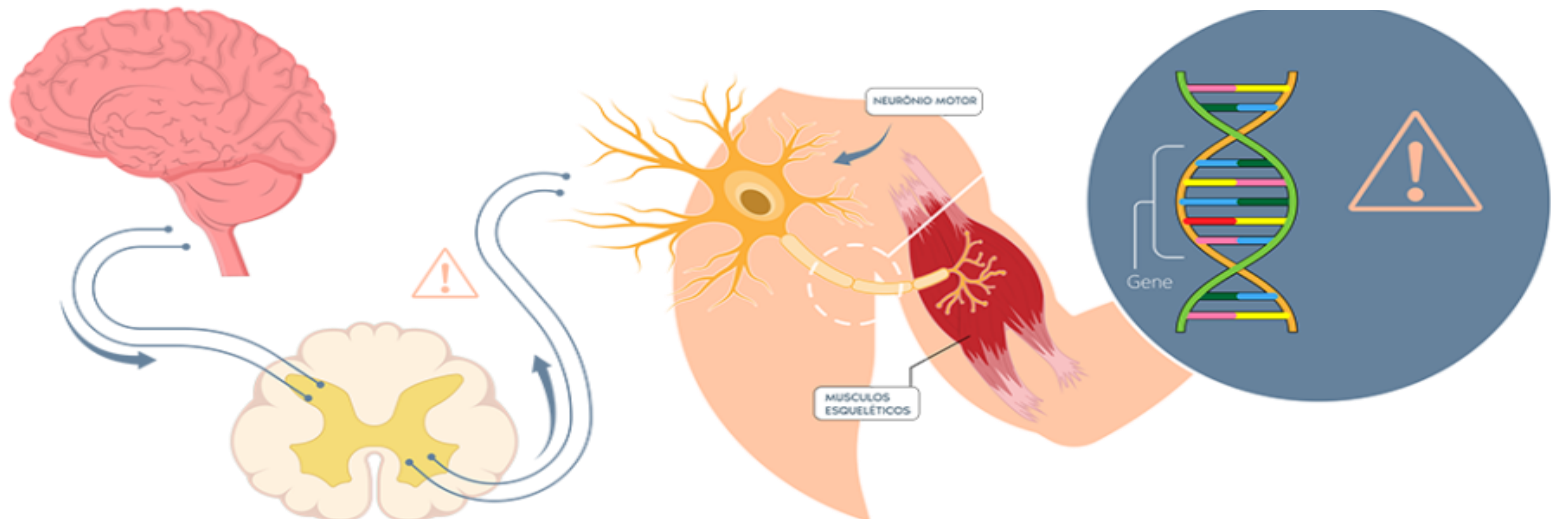
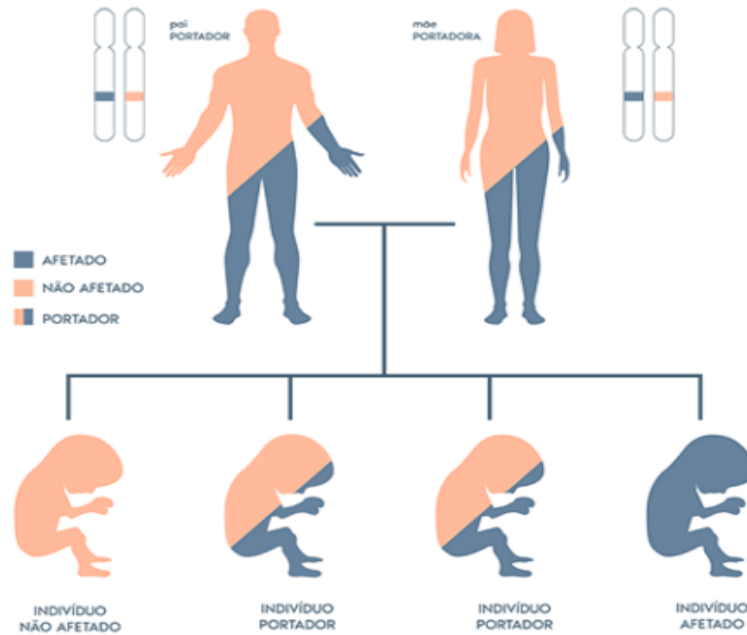


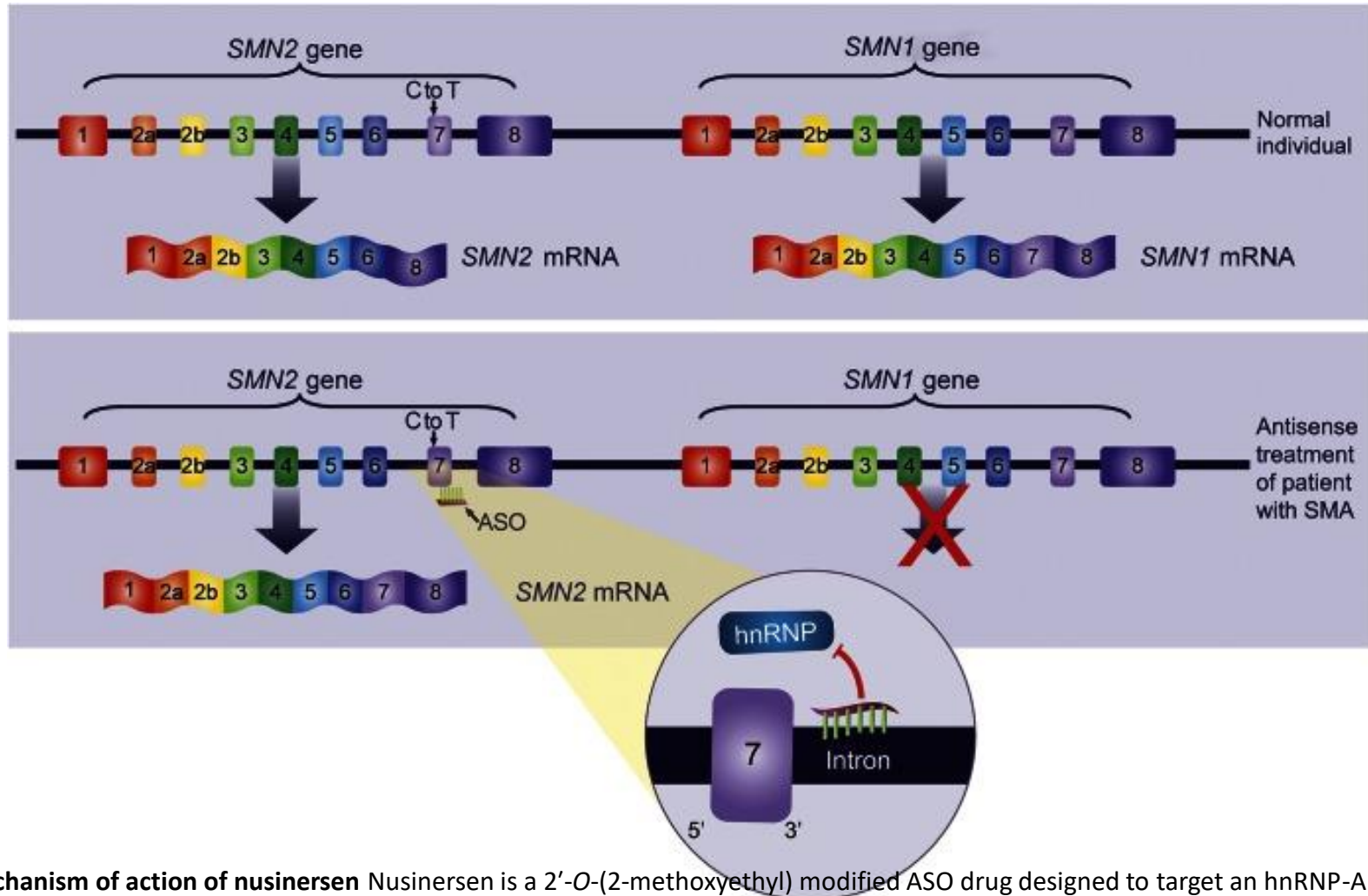


Table 1. Main clinical developments in spinal muscular atrophy (SMA).

Approach /Compound	Sponsor	Mechanism	Trials' Phase (SMA Type)	Administration	FDA Approval
Splicing modifiers of <i>SMN2</i> 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 <i>SMN1</i> 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.

Nusinersen



Mechanism of action of nusinersen Nusinersen is a 2'-O-(2-methoxyethyl) modified ASO drug designed to target an hnRNP-A1/A2-dependent splicing silencer, ISS-N1, in intron 7 of the *SMN* pre-mRNA. Nusinersen displaces hnRNP proteins from the ISS-N1 site on the *SMN2* pre-mRNA, facilitating accurate splicing of *SMN2* transcripts (e.g., increasing the synthesis of transcripts containing exon 7) and resulting in increased production of full-length SMN protein. ASO = antisense oligonucleotide; hnRNP = heterogenous nuclear ribonucleoprotein; ISS = intronic splicing silencer; mRNA = messenger RNA; SMA = spinal muscular atrophy; SMN = survival of motor neuron.

Zolgensma (Onasemnogene abeparvoveque)

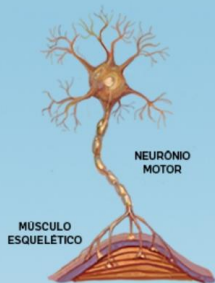
Como o remédio funciona?



1. Os cientistas retiram o material genético de um vírus, o **Adeno-associado 9**, ou AAV9, que recebe no lugar uma cópia saudável do gene SMN1.



2. Por meio de **uma sonda**, o vírus modificado é introduzido na corrente sanguínea em um procedimento hospitalar que leva uma hora.



3. O AAV9 chega às células do sistema nervoso e deposita o SMN1 saudável, que substitui a cópia com defeito. Como o vírus não pode se replicar, acaba desaparecendo no corpo.



4. Após alguns dias, as células começam a produzir a proteína SMN, dando aos músculos suas funções. O movimento perdido até o recebimento do remédio, no entanto, não volta.

O que se gasta com R\$ 9,8 milhões* daria para tratar:

uma pessoa com AME que toma o Zolgensma

ou 105 mil pessoas com diabetes**



ou 4.260 portadores de HIV**



*Média por paciente

**Por ano



78 brasileiros receberam o remédio do governo federal após ação judicial

Brineura

How is Brineura administered to my child?

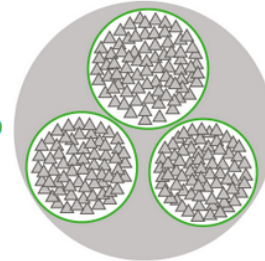
Brineura is a type of treatment called **enzyme replacement therapy (ERT)**. It's administered through **intraventricular infusion**—a method that allows Brineura to be directly delivered into the fluid surrounding the brain, known as the **cerebrospinal fluid**.⁴

Brineura® (cerliponase alfa) is the only treatment that directly addresses the cause of CLN2 disease by helping to replace the TPP1 enzyme

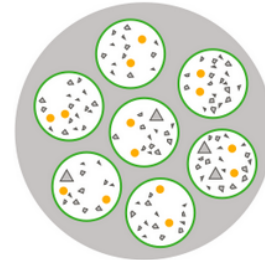
What is Brineura?

Brineura® (**cerliponase alfa**) is a prescription medication used to slow loss of ability to walk or crawl (ambulation) in symptomatic pediatric patients 3 years of age and older with **late infantile neuronal ceroid lipofuscinosis type 2 (CLN2)**, also known as **tripeptidyl peptidase 1 (TPP1) deficiency**.

CLN2 disease is a type of **lysosomal storage disorder** that affects cells in the brain. **Lysosomes** are in every cell and contain **enzymes** that break down materials in the cell. One of these **enzymes** is called **TPP1**, which is missing or not working properly in children with **CLN2 disease**.¹



The **TPP1 enzyme** is missing or not working properly in children with **CLN2 disease**. This leads to a buildup of storage materials in their **lysosomes**, associated with cell damage in the brain.¹⁻³



When Brineura is delivered to a child with **CLN2 disease**, it helps replace the missing **TPP1 enzyme**.⁴

A **doença de Batten** é causada por alterações em diversos tipos de genes, que se tornam incapazes de produzir as proteínas necessárias para o metabolismo das células neurais. Como resultado, os neurônios sofrem danos progressivos, desencadeando uma série de problemas ao sistema nervoso.

Duchenne muscular dystrophy (DMD)

A DMD é uma doença genética recessiva ligada ao cromossomo X causada por uma deficiência de distrofina funcional. As características clínicas são fraqueza muscular simétrica lentamente progressiva e atrofia. As mutações genéticas mais comuns na DMD são deleções de éxons ou duplicações do gene da distrofina, sendo a deleção dos éxons 3-22 e éxons 45-54 a mais comum. As deleções ou duplicações de exons interrompem o quadro de leitura do transcrito e causam a incapacidade de produzir uma proteína distrofina funcional

Huntington's disease (HD)

Huntington's disease (HD), apresenta expansão anormal do fragmentos de repetição de trinucleotídeos CAG no gene da huntingtina (HTT), resultando em acúmulo anormal de cadeias de poliglutamina na proteína huntingtina.

Adrenoleukodystrophy (ALD):

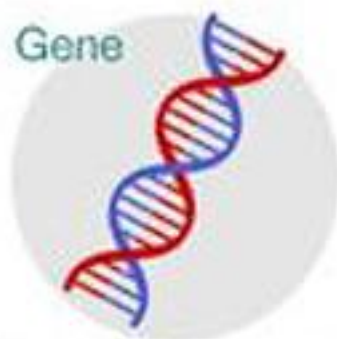
Adrenoleucodistrofia (ALD), uma doença genética ligada ao cromossomo-X, é causada por mutações no gene ABCD1 (ATP-binding cassette, subfamília D, gene do membro 1). Esses defeitos resultam na quebra anormal de ácidos graxos de cadeia longa e afetam principalmente o tecido do sistema adrenal e nervoso, causando transtornos mentais progressivos, perda de visão e auditiva e outros sintomas clínicos

Doenças PolyQ são um grupo de doenças autossômicas hereditárias distúrbios neurológicos dominantes causados pela expansão de repetições CAG instáveis nas regiões traduzidas do genes afetados. Existem nove doenças poliQ conhecidas, ou seja, HD, seis tipos de ataxias espinocerebelares (SCA) (tipos 1, 2, 3, 6, 7 e 17); dentatorrubral-palidolusian atrofia (DRPLA); e atrofia muscular espinhal e bulbar (SBMA). Mutações de repetição CAG expandidas levam a longos tratos de poliglutamina ou proteínas tóxicas que finalmente resultam na disfunção neuronal e morte celular.

Silenciamento gênico

- **Doença de Huntington**
- **Disfunção motora progressiva, falta de equilíbrio, dificuldade para falar, andar e engolir**
- **Danos cognitivos**

Como a droga funciona



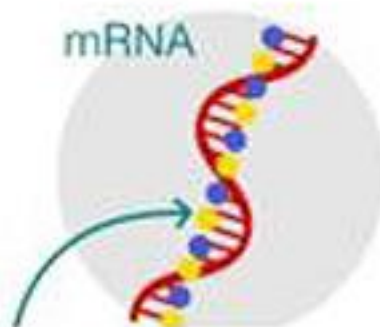
Uma mutação no gene Huntingtina leva à produção de proteínas defeituosas



A receita para fabricar essas proteínas fica guardada em uma molécula de RNA



As proteínas defeituosas atacam o cérebro



A nova droga intercepta e bloqueia o RNA antes que as proteínas sejam produzidas

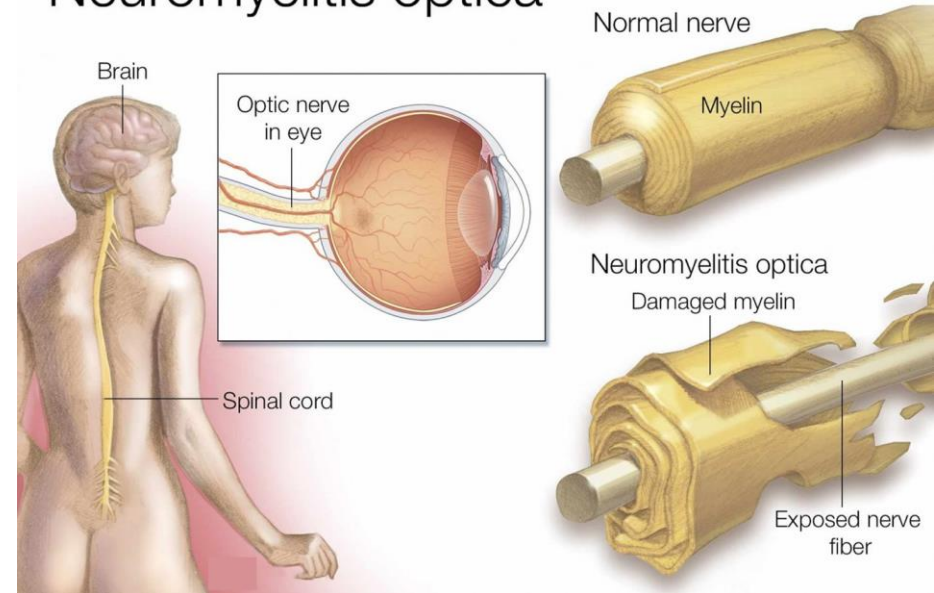


Isso evita que a mutação genética cause a doença

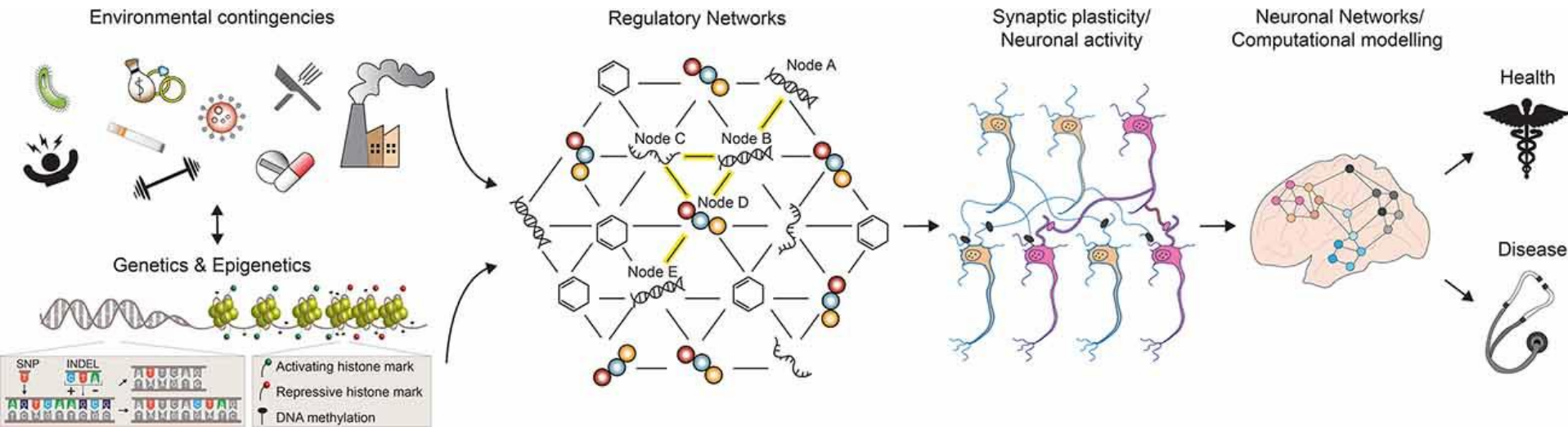
A **Neuromielite óptica (NMO)**, também conhecida como doença de Devic, é uma doença inflamatória, autoimune do sistema nervoso central - que atinge principalmente os nervos ópticos e a medula espinhal, causando a perda da visão; dificuldade para andar; dormência nos braços e pernas; e alterações no controle da urina e do intestino. Os ataques repetidos levam ao acúmulo de deficiência neurológica e incapacidade.

Doença autoimune do sistema nervoso central em que canais de água, chamados aquaporina 4, são atacados por anticorpos produzidos pelo próprio paciente. O aquaporina 4 é fundamental para o funcionamento normal do sistema nervoso central. Quando esse canal é bloqueado pela presença dos anticorpos, existe uma lesão, que acontece principalmente nas estruturas do sistema nervoso central, que são ricas em aquaporina 4, nos nervos ópticos, na medula espinhal e áreas que apresentam contato com o líquido no sistema nervoso central e nos vasos.

Neuromyelitis optica



A epigenética é um campo de pesquisa que investiga como os estímulos ambientais podem ativar determinados genes e silenciar outros



Besides learning and memory, a broad range of experiences ranging from psychological stress to nutrition and lifestyle were also found to induce epigenetic modifications in the central nervous system (CNS), and epigenetic mechanisms have been implicated in neurodevelopmental and neuropsychiatric disorders, neurodegeneration, and aging. Based on these findings, several drugs targeting the epigenome are currently in clinical trials with the hope to reverse genetically and/or environmentally induced aberrant epigenetic changes in the CNS. Examples of such epigenetically targeted drugs include the following: (a) HDAC inhibitors to treat Alzheimer's disease (AD; NCT03056495 and NCT03533257), Parkinson's disease (PD; NCT02046434), schizophrenia [NCT00194025, but note that the same drug—Valproate—has been previously tested for the treatment of AD (NCT00071721) with negative results] and cognitive decline (NCT02457507); (b) natural compounds which target DNA methyltransferase (Dnmt) activity to treat AD (NCT01716637 and NCT00951834).

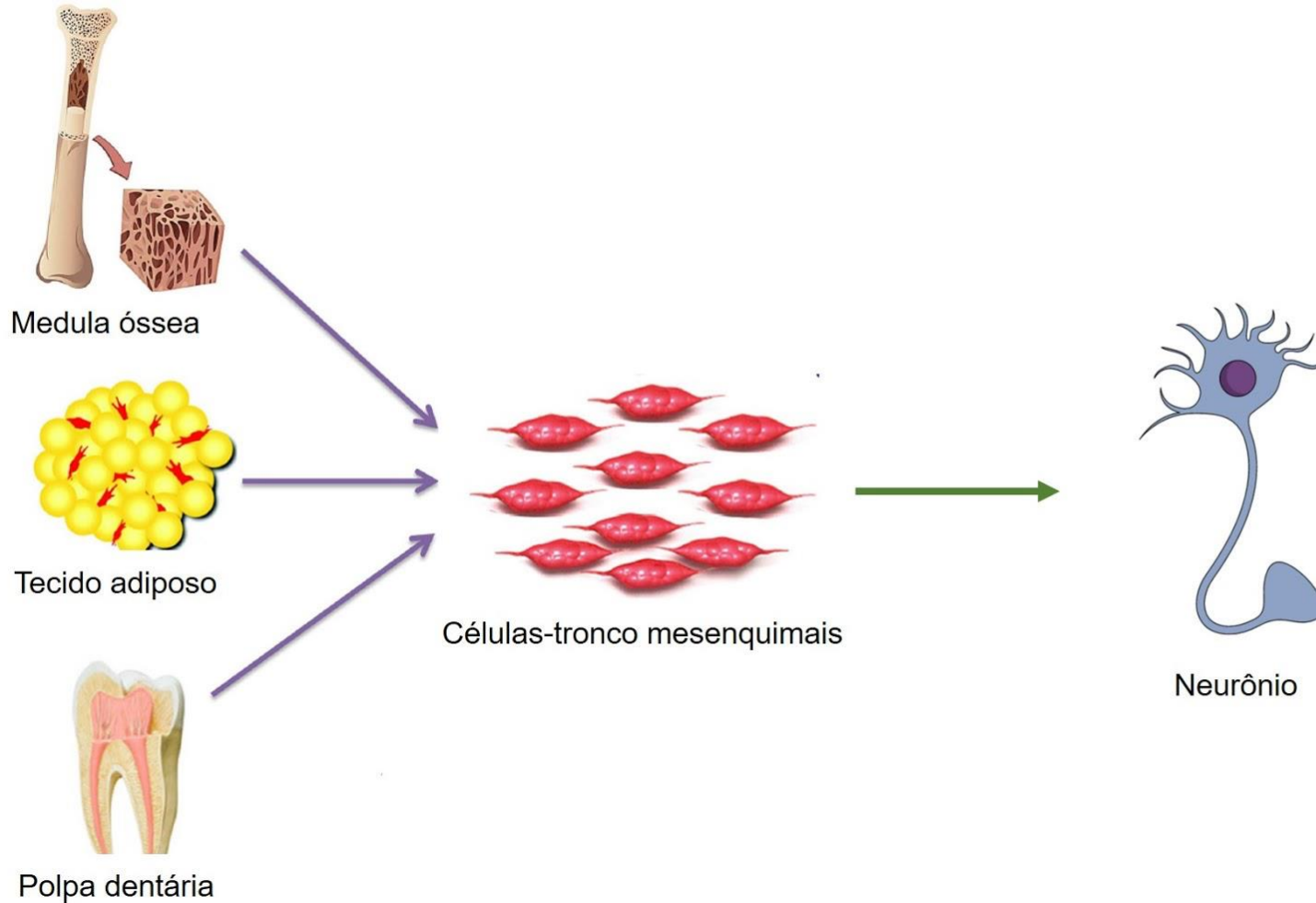
Doenças psiquiátricas

- **7,1% dos gastos destinados ao tratamento de doenças no mundo**
- **Estima-se que o impacto econômico das doenças mentais seja equivalente ao custo com o tratamento do câncer**
- **Entre 1990 e 2010, a prevalência dos transtornos psiquiátricos teve um aumento de cerca de 22%**

Modelos de estudo

- **Animal: limitações e questões éticas**
- **Biópsias: altamente invasivas, problemas éticos e quantidade de amostra**
- **Linhagens celulares: neurônios pós-mitóticos não podem ser expandidos em cultura, pequeno número de linhagens celulares é representativo das doenças humanas**
- **Tecidos *post-mortem*: método muito usado, porém é uma fotografia dos estágios mais tardios da doença, pacientes usualmente intervêm na patofisiologia após o diagnóstico**

Terapia celular



Células-tronco embrionárias

REPORT

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson^{*}, Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Mar...

+ See all authors and affiliations

Science 06 Nov 1998:

Vol. 282, Issue 5391, pp. 1145-1147

DOI: 10.1126/science.282.5391.1145

Induced pluripotent stem cells (iPSC)

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,¹ Koji Tanabe,¹ Mari Ohnuki,¹ Megumi Narita,^{1,2} Tomoko Ichisaka,^{1,2} Kiichiro Tomoda,³ and Shinya Yamanaka^{1,2,3,4,*}

¹Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

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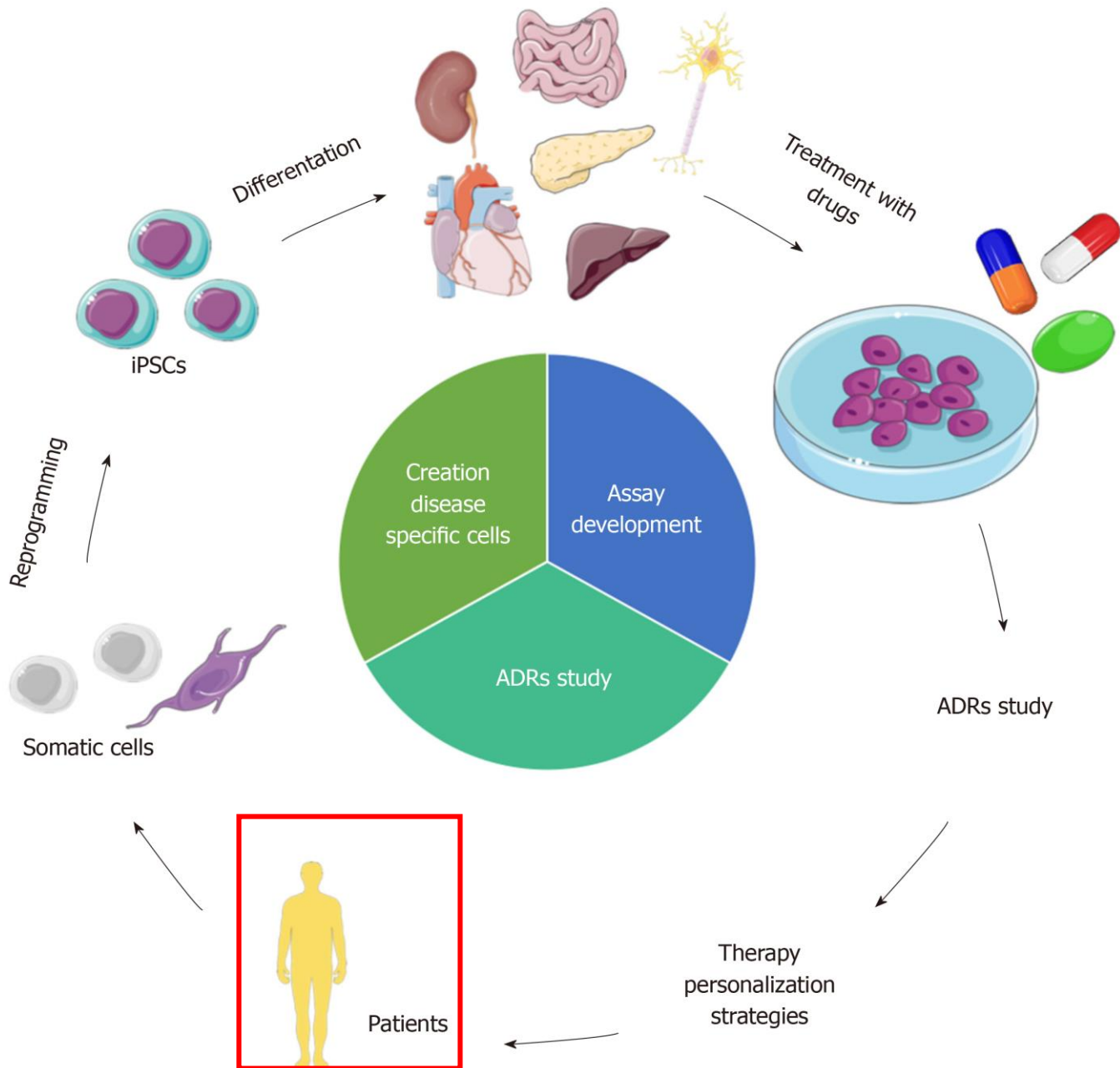
³Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA

⁴Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan

*Correspondence: yamanaka@frontier.kyoto-u.ac.jp

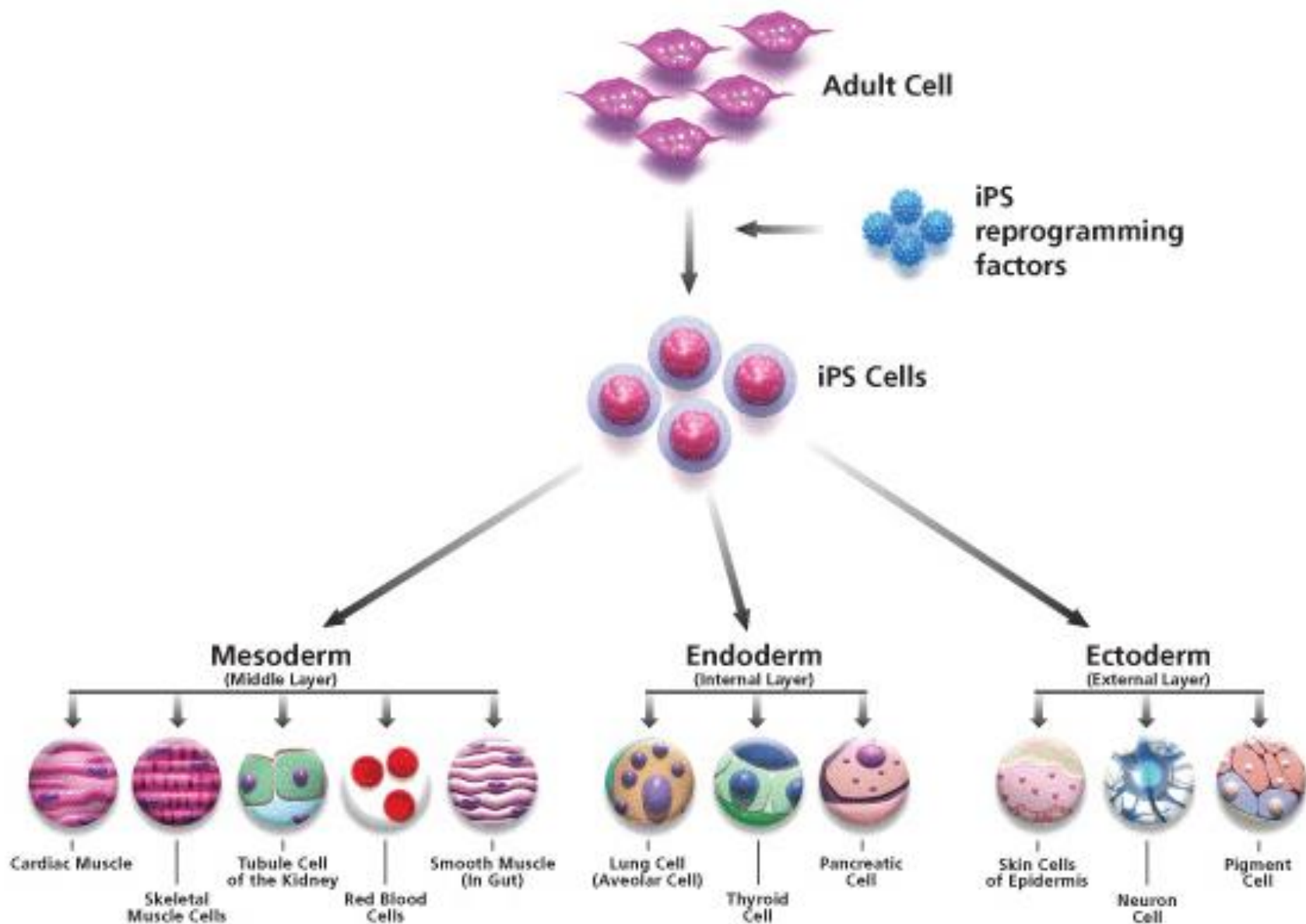
DOI 10.1016/j.cell.2007.11.019

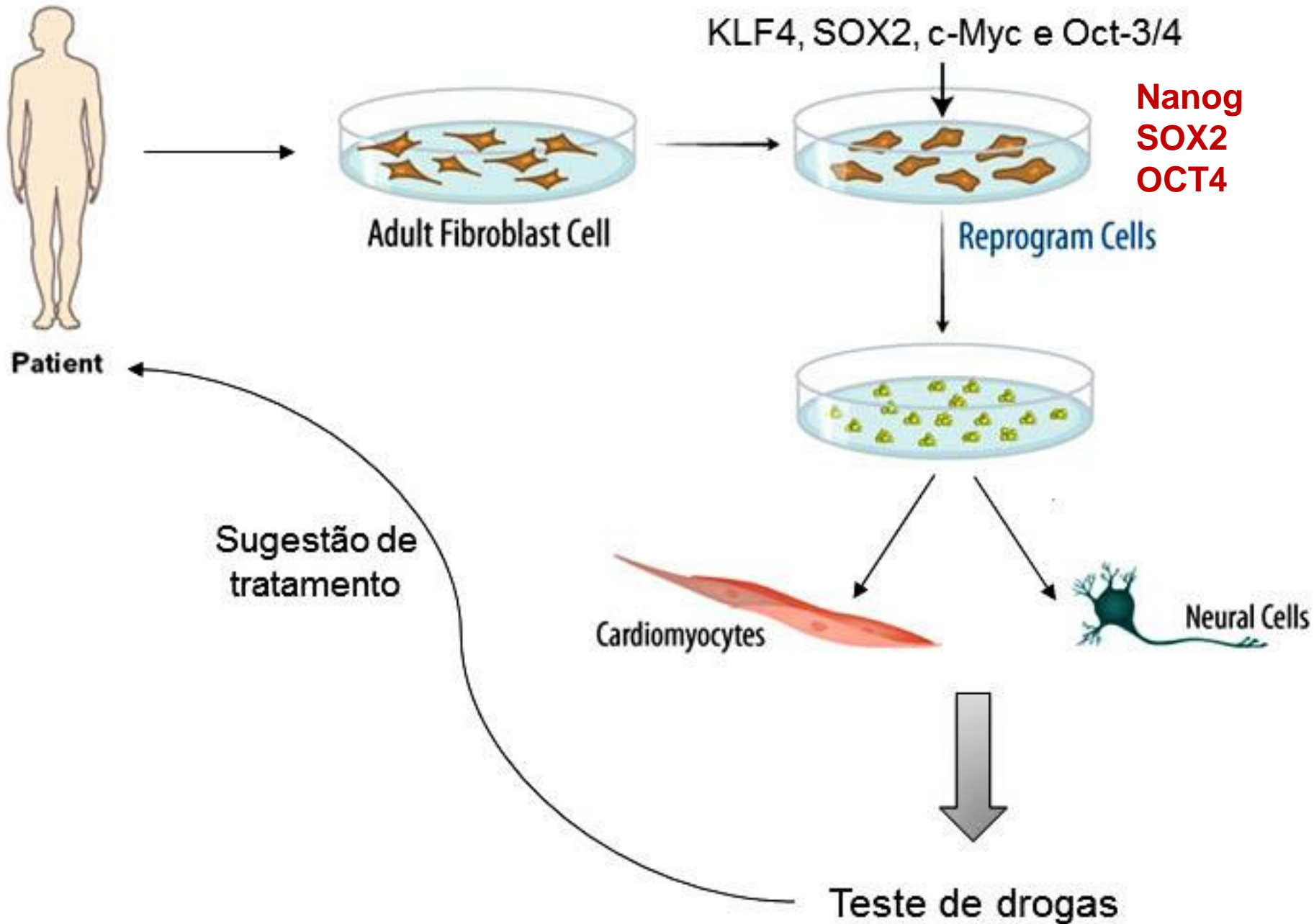
Induced pluripotent stem cells for therapy personalization



Tecnologia das iPSC

- **Reprogramação de células somáticas adultas em células que possuem propriedades de células-tronco embrionárias**
- **Modelagem do fenótipo molecular e fisiológico de doenças *in vitro***
- **Teste dos efeitos de medicamentos novos e existentes**
- **Estudo dos mecanismos moleculares de diferentes doenças**





iPSC humanas

- **Neurônios gabaérgicos**
- **Serotoninérgicos**
- **Dopaminérgicos**



Onde chegamos


- **Sistema totalmente automatizado para reprogramação celular, o que pode permitir a geração de um grande número de iPSC paciente-específicas**

Produção de organoides

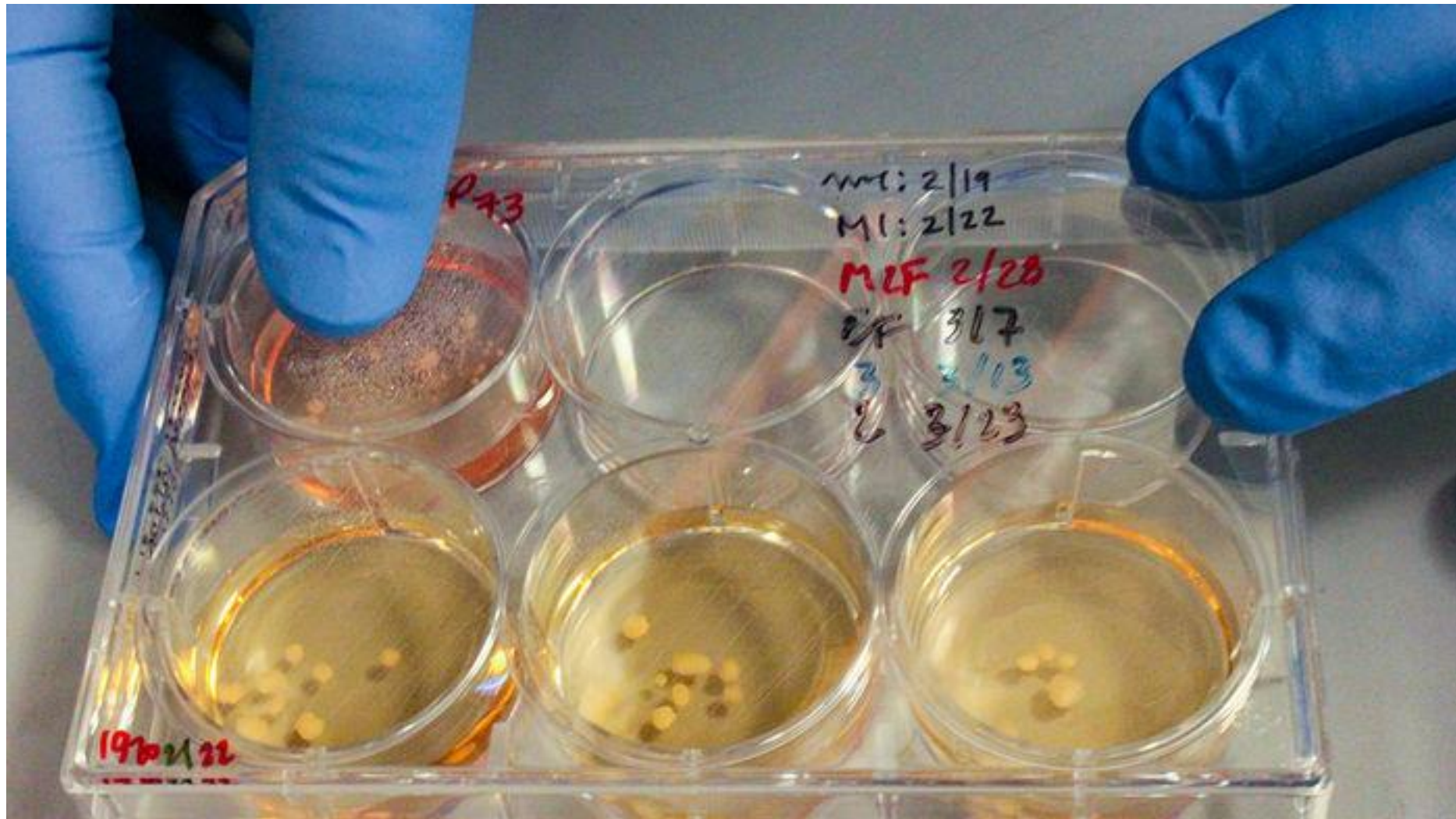
Letter | Published: 03 July 2013

Vascularized and functional human liver from an iPSC-derived organ bud transplant

Takanori Takebe , Keisuke Sekine, Masahiro Enomura, Hiroyuki Koike, Masaki Kimura, Takunori Ogaeri, Ran-Ran Zhang, Yasuharu Ueno, Yun-Wen Zheng, Naoto Koike, Shinsuke Aoyama, Yasuhisa Adachi & Hideki Taniguchi 

Nature **499**, 481–484 (25 July 2013) | [Download Citation](#) 

Produção de organoides

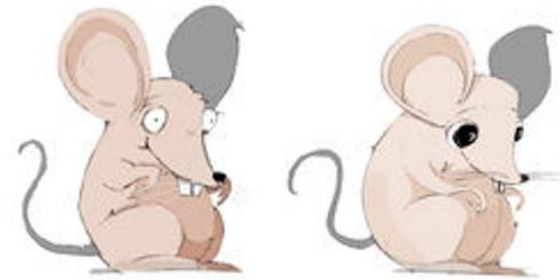


Biotecnologia *in vivo*

- **Vias de sinalização envolvida em uma doença**
- **Descoberta de novos fármacos**



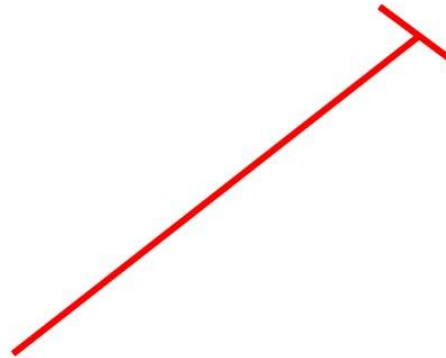
Estresse agudo de restrição



Comportamentos tipo-depressivo e tipo-ansioso



Composto orgânico contendo selênio



Biotecnologia *in silico*

Alvo

Ligante

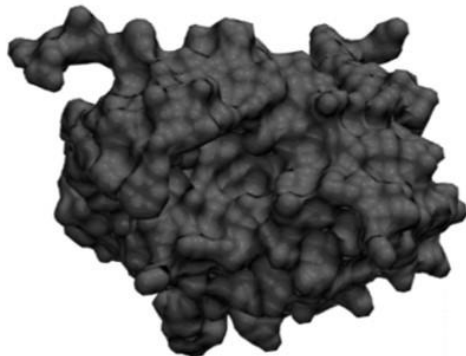
Complexo



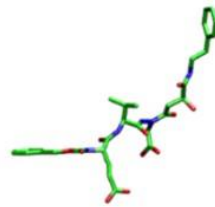
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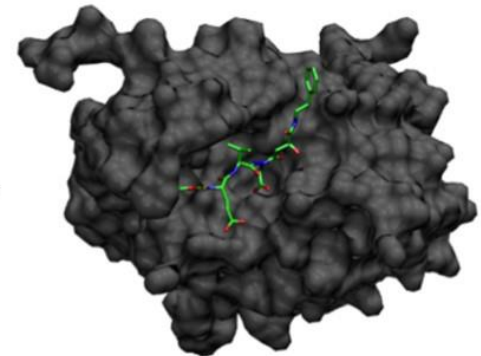
docking



+



docking



Depressão

CAUSAS

- . Problemas de saúde
- . Questões familiares e financeiras
- . Eventos estressantes/ traumas
- . Fatores físicos
- . Envelhecimento
- . Uso de álcool e drogas



SINAIS

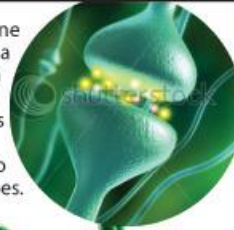
- . Pensamentos ruins persistentes
- . Falta de interesse e de prazer em realizar suas atividades
- . Alteração de peso não intencional
- . Dormir muito ou dificuldade para dormir
- . Dificuldade de concentração
- . Falta de energia
- . Culpa excessiva
- . Pensamentos suicidas.

NO ALVO

Conheça como foi a experiência

O QUE SE SABIA

Conhecia-se o papel do gene p11 no desenvolvimento da depressão. Ele produz uma proteína, a p11, que ajuda a serotonina a penetrar nos neurônios. A substância é fundamental para o correto processamento das emoções.



Mas se o gene p11 estiver defeituoso, a proteína não é fabricada e a serotonina não consegue ser absorvida pelas células nervosas cerebrais. Há risco de surgimento da doença

Sabia-se já há alguns anos que a depressão tem um forte componente genético – ou seja, que erros no modo de atuação de determinados genes podem levar ao seu desencadeamento.

Os antidepressivos, junto com a psicoterapia, são atualmente os instrumentos mais utilizados.

O QUE FOI FEITO

1

Os cientistas criaram cobaias com gene p11 defeituoso. Elas apresentavam sintomas da enfermidade

2

Depois, selecionaram um vírus inofensivo

3

Dentro dele, colocaram o gene p11 sem defeito

O QUE ACONTECEU

1

O vírus foi injetado no cérebro. Ao entrar nos neurônios, depositou neles seu próprio material genético

2

Seu genoma, com o gene p11 correto, misturou-se ao dos neurônios


3

Eles, então, passaram a produzir a proteína

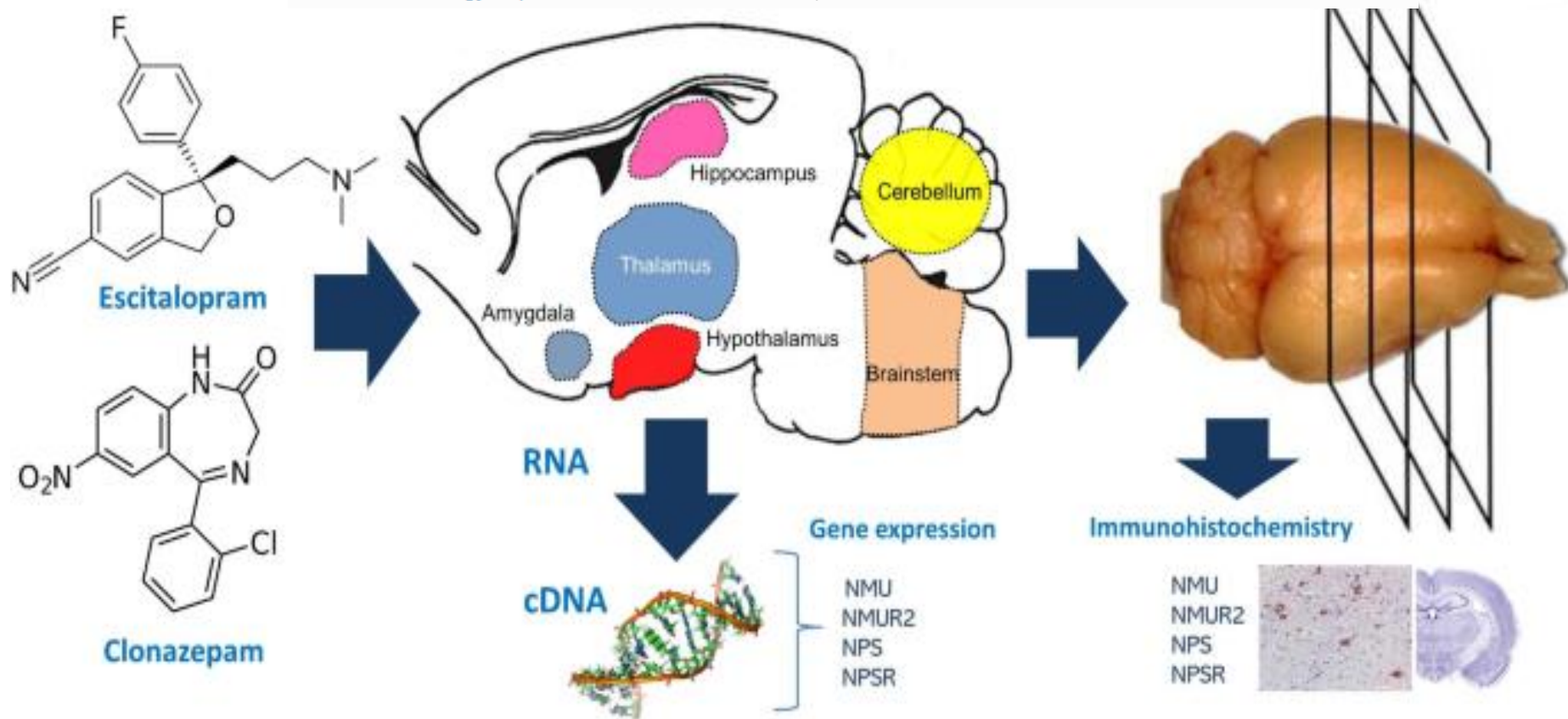
RESULTADOS

Os sinais da doença desapareceram

Modulatory effect of long-term treatment with escitalopram and clonazepam on the expression of anxiety-related neuropeptides: neuromedin U, neuropeptide S and their receptors in the rat brain

Aneta Piwowarczyk-Nowak, Artur Pałasz , Katarzyna Bogus, Marek Krzystanek, Iwona Błaszczyk, John J. Worthington & Anieli Grajoszek

Molecular Biology Reports 49, 9041–9049 (2022) | [Cite this article](#)





Obrigado

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