ERN ITHACA

Webinar 2025

Therapeutic approaches in Angelman syndrome

Tuesday 25 November, 17:30 – 18:00 (CET time)

Chaired by Ellen Koekoeckx, FAST & Samantha Eisenhauer FAST France

Speakers: Prof. Michaela Semeraro; Dr. Stefano D'Arrigo; Dr. Claudia Ciaccio

European Commission

EUROPEAN REFERENCE NETWORKS

Helping patients with rare or low-prevalence complex diseases

Project Manager Anne Hugon

2025

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Welcome – Technical points

- We are please to be numerous 161 registrations online 78
- Webinar being recorded

- Thank you for
  - Turn off your microphone and disconnect your camera
  - Raise your hand at the time of the questions and discussions
  - We will answer the questions sent in the registration form
  - A satisfaction survey will be sent to you :

- Webinars # will be available on ITHACA's Website
- https://ern-ithaca.eu/webinars/

- Anne Hugon Project Manager ERN ITHACA - anne.hugon@aphp.fr

Welcome and Introduction

- **Public:** Clinicians, researchers and AS caregivers

- This webinar is the third of a webinar series dedicated to Angelman syndrome, a neurogenetic disorder. In this session leading experts will break down the therapeutic strategies that are being developed to treat Angelman syndrome. Our goal is to provide a clear overview of the therapeutic pillars currently being investigated — from approaches aimed at fixing the maternal gene, to unsilencing the paternal gene, to targeting downstream pathways that may improve cellular function.

- Chaired by **Ellen Koekoeckx & Samantha Eisenhauer** on behalf of **FAST** (Foundation of Angelman Syndrome Therapeutics)

- Invited expert speakers:
- **Prof. Michaela Semeraro**: Pediatrician at Hôpital Necker-Enfants malades in Paris, France
- **Dr. Stefano D'Arrigo:** Pediatric Neurologist, Fondazione IRCCS Instituto Carlo Besta in Milan, Italy
- **Dr. Claudia Ciaccio**: Pediatric geneticist, Fondazione IRCCS Instituto Carlo Besta in Milan, Italy

Agenda

Our expert speakers will provide insights on the following topics:

- Introduction, Ellen Koekoeckx

- Overview of therapeutic pillars, Prof Michaela Semeraro
- Pillar 1: Replace mom's UBE3A, Prof Michaela Semeraro
- Pillar 2: Turn on Dad's UBE3A, Dr. Stefano D'Arrigo, Dr. Claudia Ciaccio
- Pillar 3: Downstream targets, Dr. Stefano D'Arrigo, Dr. Claudia Ciaccio
- Future outlook, Prof Semeraro

- Q&A and discussion - all

Overview of therapeutic pillars

Michaela Semeraro MD PhD

Centre d'Investigation Clinique Mère Enfant , Unité de Recherche Clinique
Hôpital Necker enfants malades APHP-CENTRE -Université Paris Cité

PARIS, FRANCE

2025

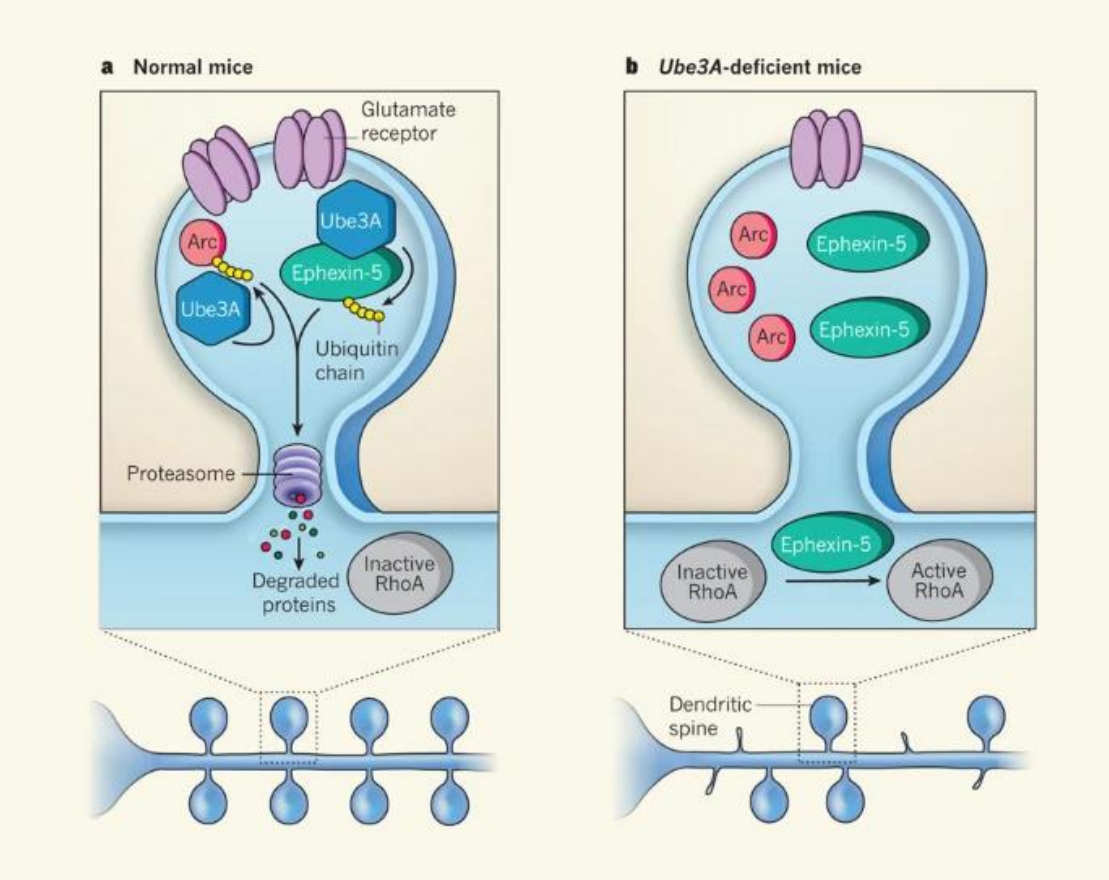
UBE3A is critical to maintaining the balance between the synthesis and degradation of several proteins in neurons

In normal neurons

- The protein UBE3A adds ubiquitin tags to two other proteins: Arc and Ephexin-5.
- Tagging them causes their degradation, keeping their levels low.
- Low levels of Arc and Ephexin-5 allow healthy dendritic-spine growth, maturation, and normal synaptic function.

Ube3A-deficient neurons

- Without UBE3A, both proteins build up, leading to immature dendritic spines and reduced synaptic strength.

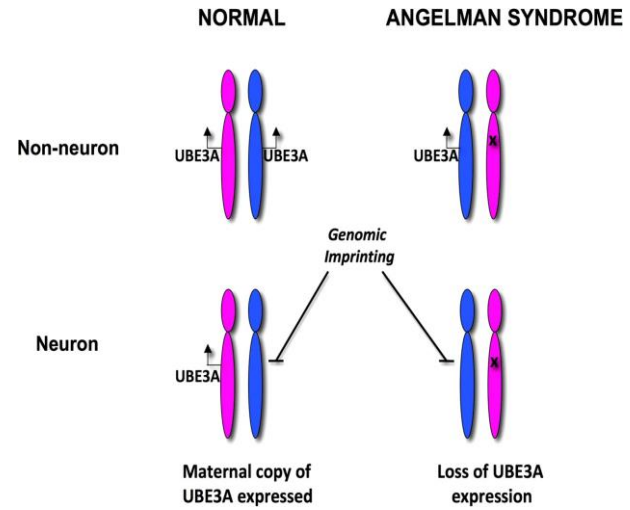


Scheiffele, P., Beg, A. Angelman syndrome connections. *Nature* **468**, 907–908 (2010).

Genetics of Angelman Syndrome

Majority of cases arise from deletions in the chromosome 15 q11-q13 region on the MATERNAL inherited chromosome.

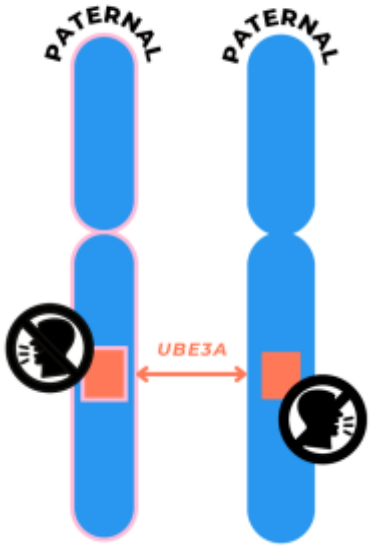
- E3 ubiquitin protein ligase (UBE3A) gene deletion is the most common cause of Angelman syndrome



Stormy J. et al Journal of Neuroscience 28 July 2010

Minority of cases are from paternal uniparental disomy.

- Both copies of chromosome 15 are inherited from the dad (instead of one from mum and one from dad), this means that there is no working copy of the mother's UBE3A gene.



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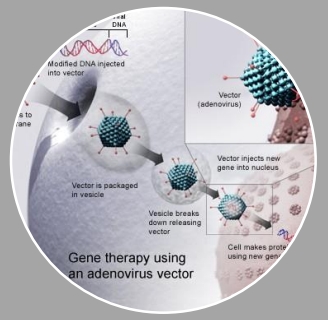
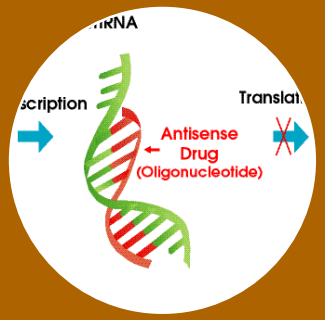
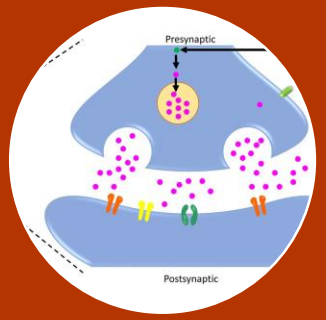
Therapeutic pillars in Angelman syndrome



Replace Mom's UBE3A



Turn on Dad's UBE3A



Downstream targets

RESTORING NEURONAL UBE3A FUNCTION

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Pillar 1: Replace Mom's UBE3A

Prof Semeraro

Available therapeutic approaches to replace the UBE3A gene

Adeno-associated virus gene therapy (AAV-GT)

Hematopoietic stem cell gene therapy (HSC-GT)

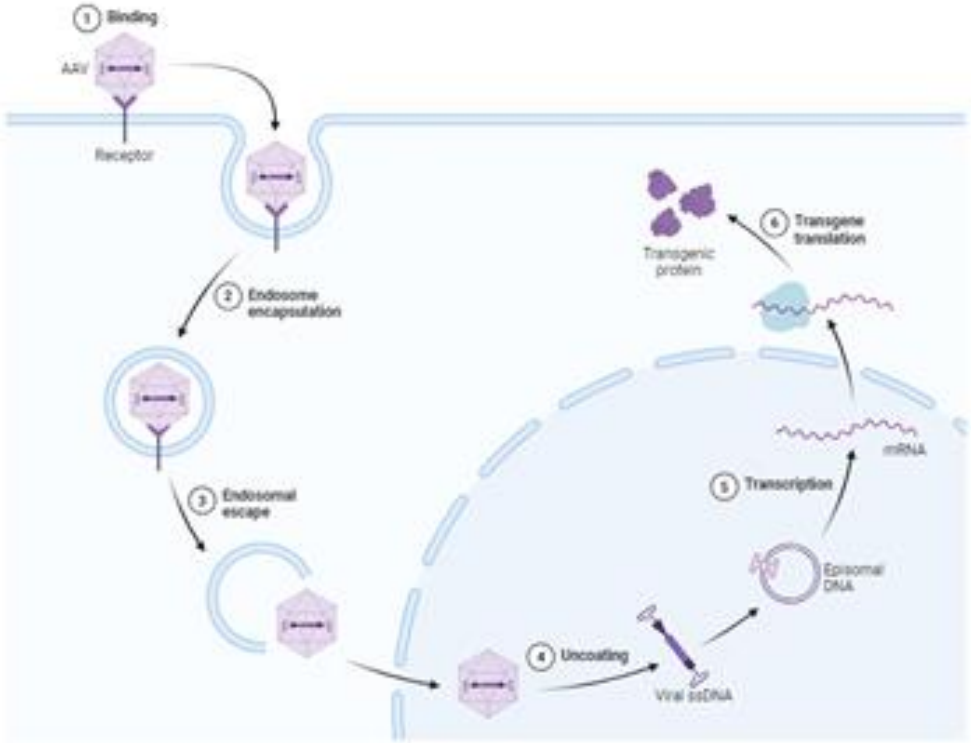
Experimental strategies related to **enzyme replacement therapy (ERT)**

Aim: increasing UBE3A levels

AAV strategy

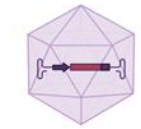
GENE ADDITION STRATEGY

Adeno-associated virus

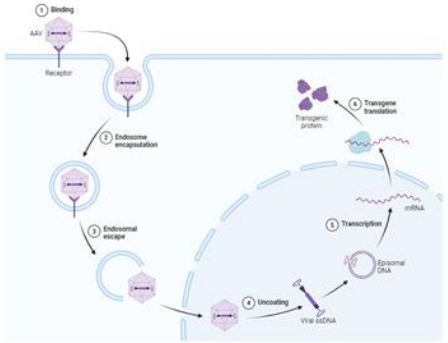


Deliver a functional copy of the missing or mutated gene to neurons using an AAV vector.

AAV strategy

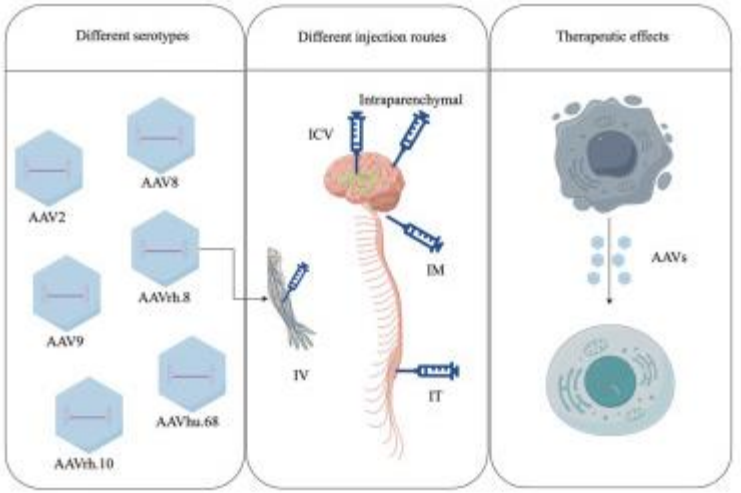


Adeno-associated virus



Gene addition strategy for neurodevelopmental diseases

The virus enters brain cells and restores the gene's function long-term
AAV vectors are the predominant platform due to their favorable safety profile and ability to transduce neurons and glia.



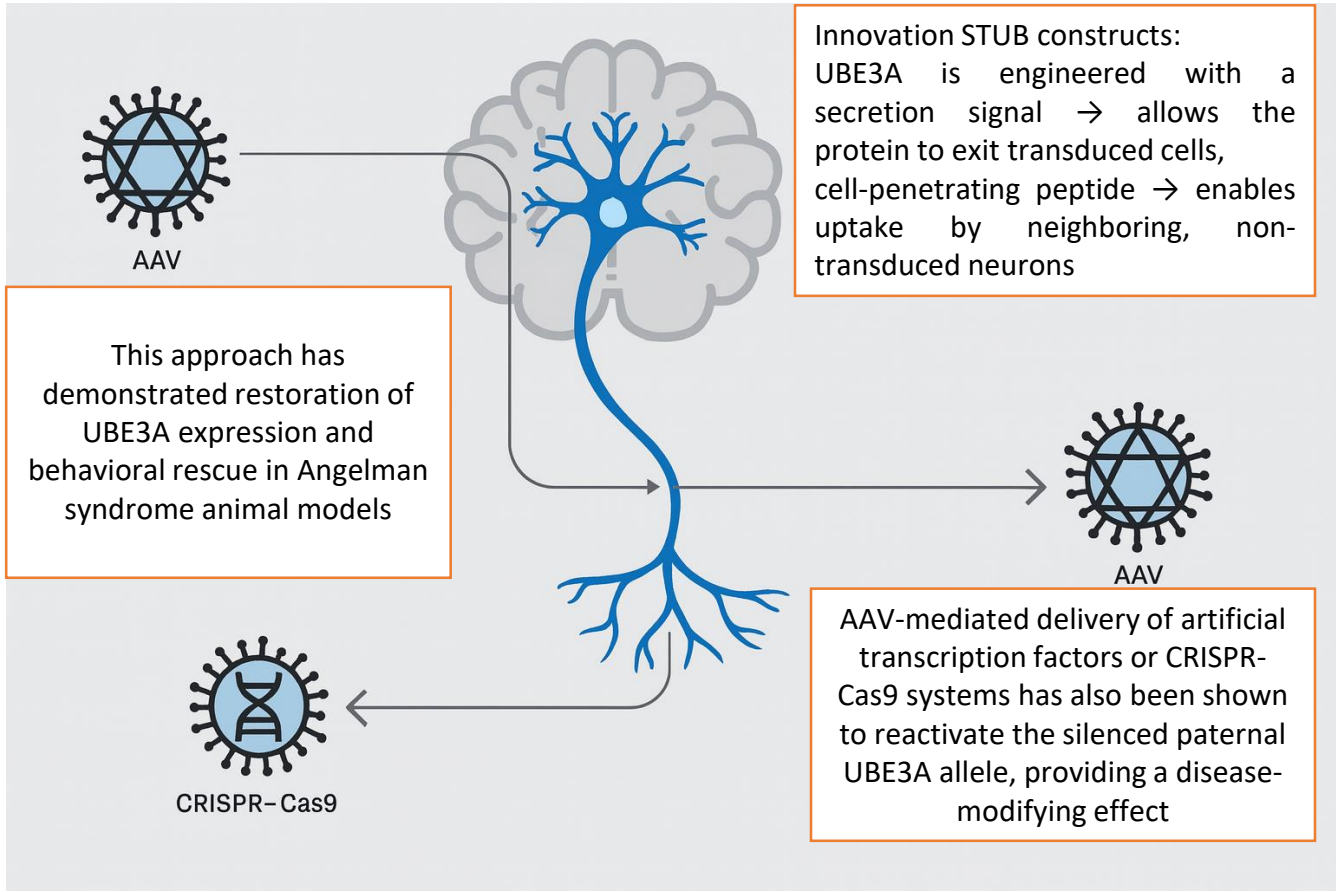
Livia Zhou et al Mol Therapy 2024

Examples of AAV trials for neurodevelopmental diseases

- **Rett syndrome (MECP2)** Multiple early-phase clinical trials are ongoing, leveraging AAV vectors to deliver MECP2 gene supplementation
- **Aromatic L-amino acid decarboxylase (AADC deficiency):** AAV-based gene therapy (Upstaza) is approved in some regions and has shown clinical benefit in motor and cognitive function in affected children
- **Dravet syndrome:** ongoing, primarily targeting SCN1A upregulation or gene replacement in GABAergic interneurons, with ETX101

AAV strategy for Angelman

Utilizes adeno-associated viral vectors to deliver a functional copy of the **UBE3A gene directly to neurons**.



Clinical research

Recruiting

A Phase 1/2 Study of the Safety and Efficacy of MVX-220 in Angelman Syndrome (ASCEND-AS)

ClinicalTrials.gov ID NCT07181837

Sponsor MavriX Bio, LLC

Information provided by MavriX Bio, LLC (Responsible Party)

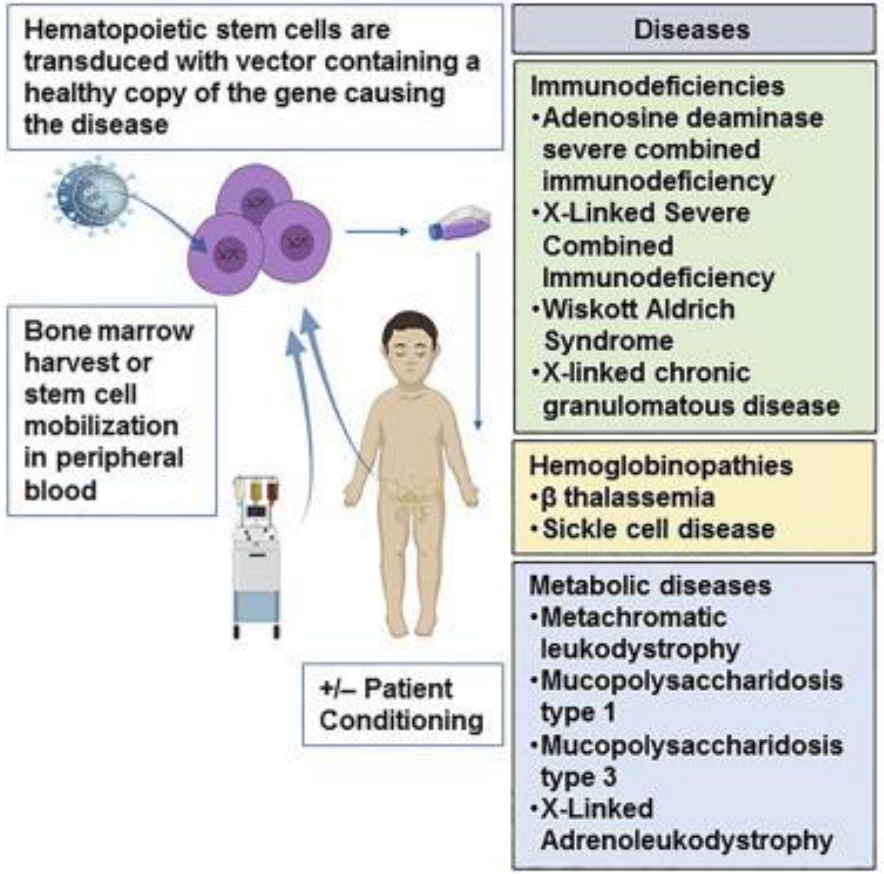
Last Update Posted 2025-11-13

Challenges

- Immune responses
- Vector dose-dependent toxicity (including hepatotoxicity, neurotoxicity, and thrombotic microangiopathy)
- Optimized capsid engineering to enhance safety and specificity. The safety profile is generally favorable, with most adverse events being mild to moderate and related to immune activation; however, rare severe events have been reported at high vector doses.

Hematopoietic stem cell gene therapy (HSC-GT)

EX VIVO GENETIC MODIFICATION OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS USING LENTIVIRAL VECTORS TO EXPRESS UBE3A



Systematic reviews of HSC-GT for monogenic disorders report high rates of stable hematopoietic reconstitution

Francesca Tucci et al Molecular Therapy, Volume 29, Issue 2, 2021

2025

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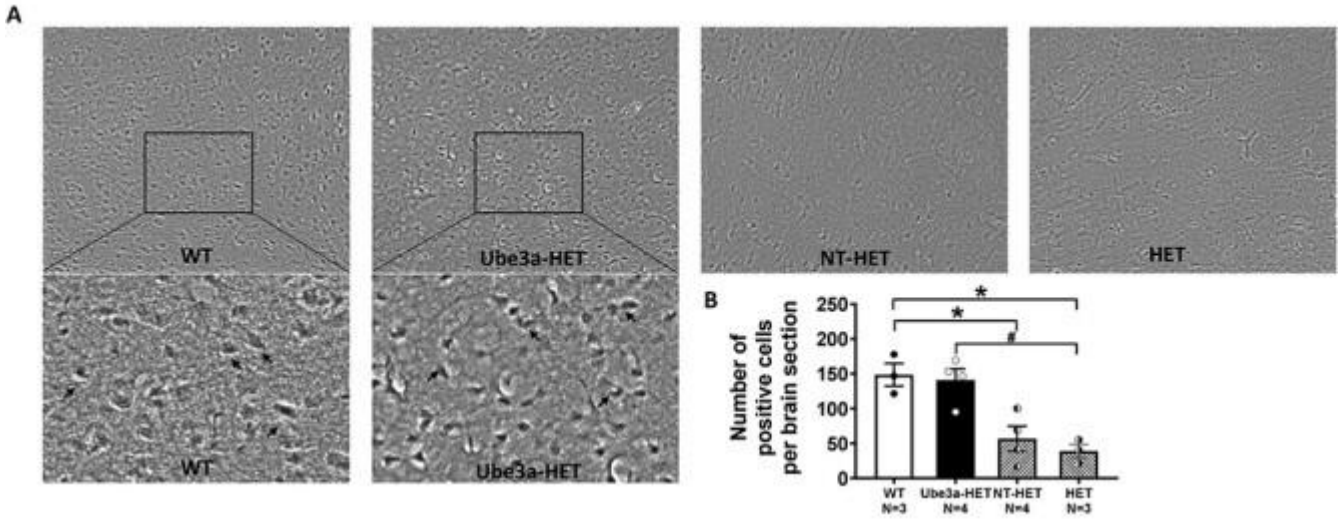
Hematopoietic stem cell gene therapy (HSC-GT)

EX VIVO GENETIC MODIFICATION OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS USING LENTIVIRAL VECTORS TO EXPRESS UBE3A

engraftment within the brain and functional rescue of Angelman syndrome phenotypes in **preclinical models**, with evidence of UBE3A expression in neural tissue and improvement in motor and cognitive outcomes.
Advantages: durable, systemic delivery of the therapeutic gene



Adhikari A, et al Hum Mol Genet. 2021 Jun 9

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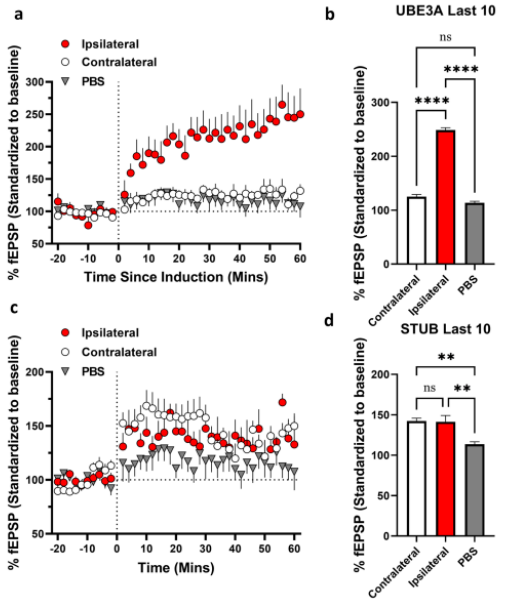
Enzyme replacement therapy (ERT)

AIM => supplement UBE3A protein directly (purified form of the missing or nonfunctional UBE3A protein into neurons)

UBE3A is an intracellular enzyme and not amenable to conventional enzyme replacement strategies

In a recent animal study[1], researchers found that UBE3A is not only excreted but maintains the enzymatic ubiquitinating activity outside neurons

Modified **gene therapy** constructs that secrete UBE3A protein (as in the **STUB** approach[2]) mimic aspects of ERT by enabling uptake of the enzyme by neighboring cells, thus broadening therapeutic reach



1 Autism Research 14: 645–655, 2021
2 Nenninger et al., Neurotherapeutics, May 2022

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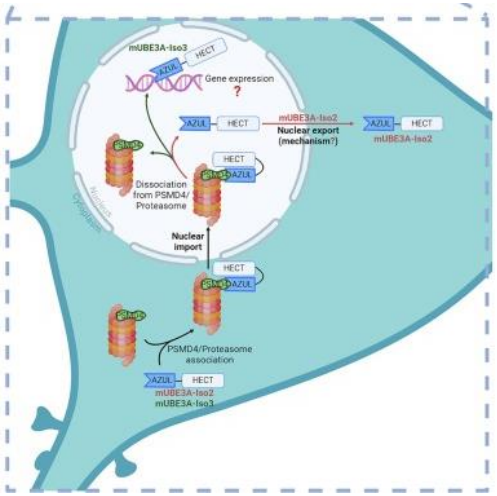
Enzyme replacement therapy (ERT)

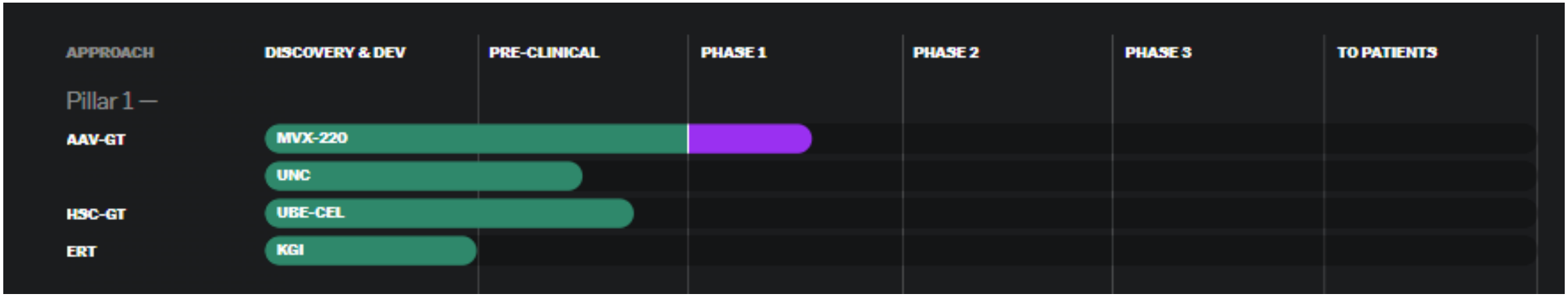
1. Protein stability
2. Delivery
3. Sustained expression in the brain



Adapted from: Neurobiology of Disease 201 (2024) 106669

Currently no clinical trials or established protocols for direct UBE3A protein supplementation in humans

Pipeline


APPROACH
DISCOVERY & DEV
PRE-CLINICAL
PHASE 1
PHASE 2
PHASE 3
TO PATIENTS
Pillar 1 —
AAV-GT
MVX-220
UNC
HSC-GT
UBE-CEL
ERT
KGI

Pillar 2: Turn on Dad's UBE3A

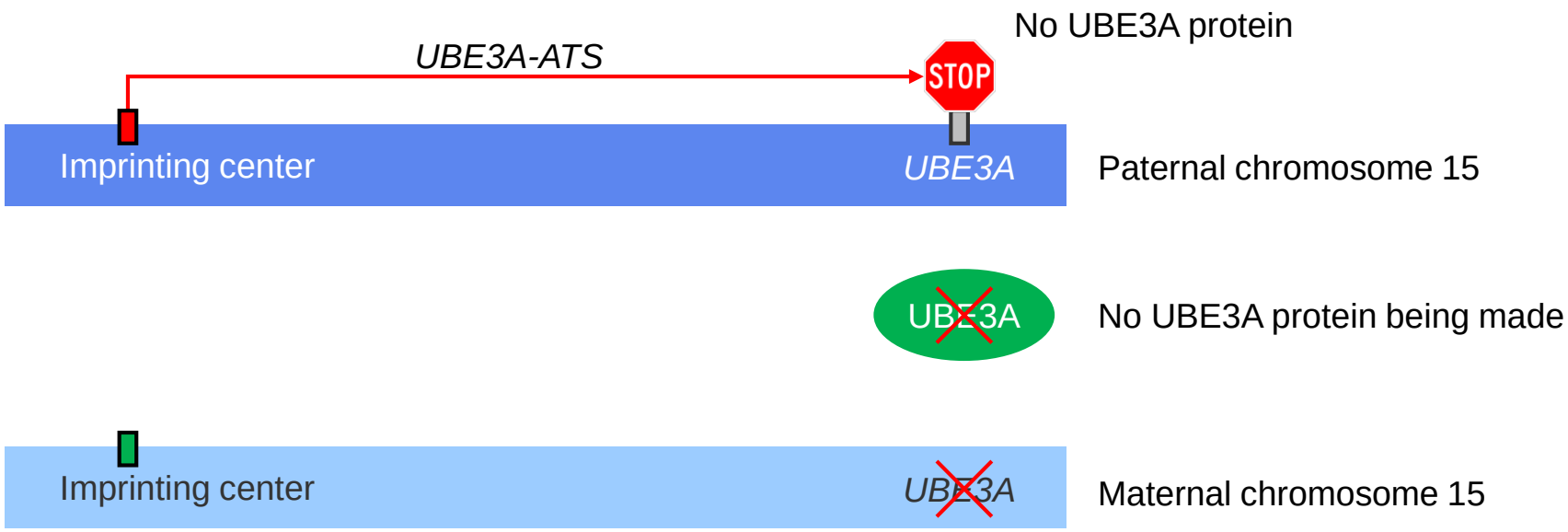
Dr. Stefano D'Arrigo, Dr. Claudia Ciaccio

Pillar 2: Turn on Dad's UBE3A

- ASOs: Antisense Oligonucleotides
- ATF / ZF: Artificial Transcription factors / Zinc Fingers
- shRNA/miRNA: Short hairpin RNA / Micro-RNA
- CRISPR gene editing

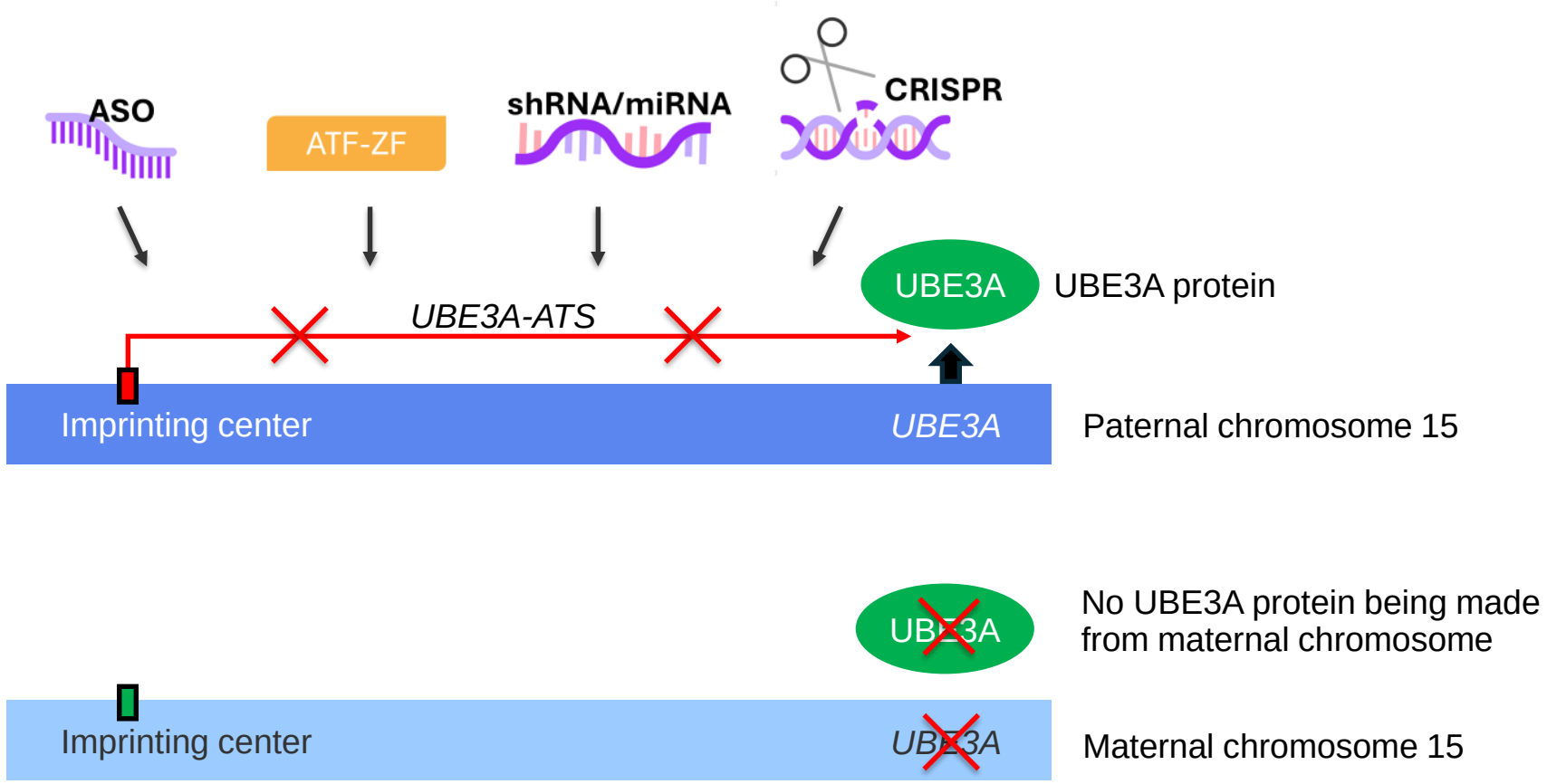
Pillar 2: Turn on Dad's UBE3A

How it works



Pillar 2: Turn on Dad's UBE3A

How we want it to work



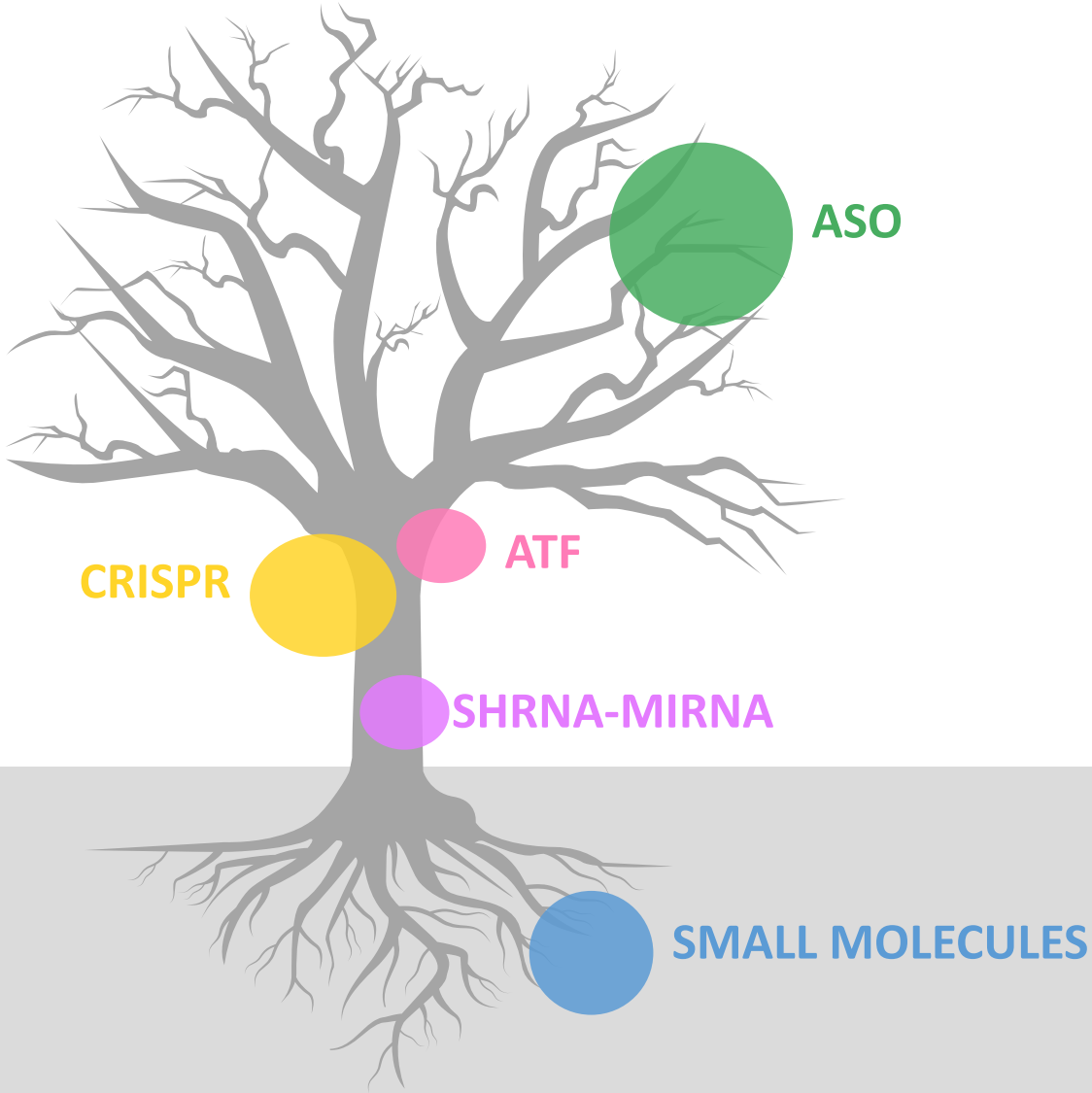
Pillar 2: Turn on Dad's UBE3A

Where are we now?

Clinical

Pre-Clinical

Drug development

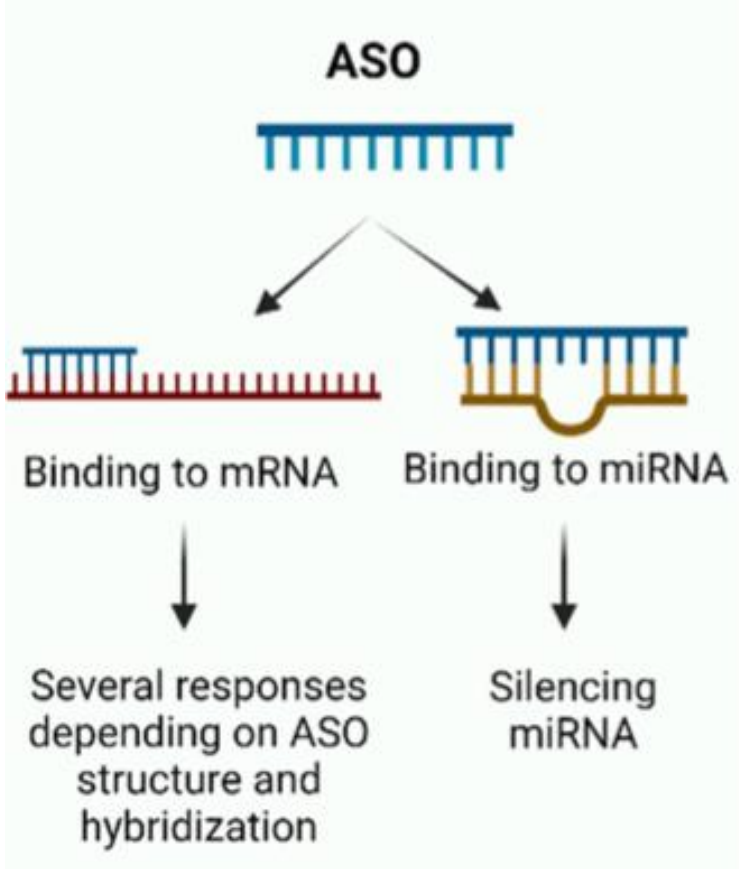


Pillar 2: Turn on Dad's UBE3A - ASOs

ASOs: Antisense Oligonucleotides

DNA fragments designed to bind to and block a specific mRNA

Anti-Sense: single strand of DNA that binds to the target RNA (sense) and induces its degradation



Targets:

Coding mRNA: block the translation from mRNA to protein in an altered gene > inhibit the production of the defective protein

Non-coding RNA (miRNA, regulatory RNA): increase the level of functional protein by modifying gene regulation

Pillar 2: Turn on Dad's UBE3A - ASOs

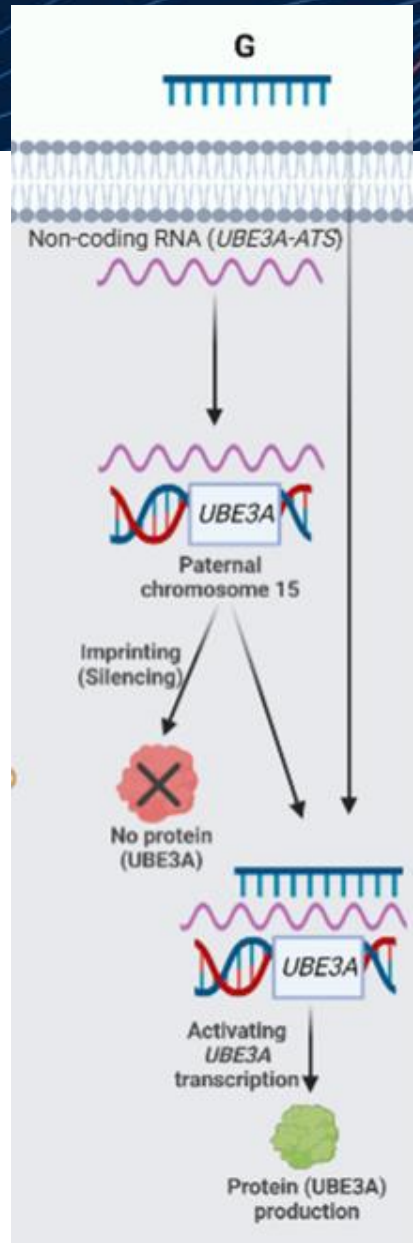
→ **Maternal UBE3A** is **not expressed in AS**

→ **Paternal UBE3A** is **normally silenced by an ncRNA** that is an antisense transcript of its mRNA (**UBE3A-ATS**)

Purpose: to reactivate the expression of UBE3A on the paternal allele, where it is present but silenced (imprinting)

ASOs bind and induce the degradation of UBE3A-ATS, thus allowing the expression of UBE3A on the paternal allele

They do not integrate in the genome, but work on the post-transcription phase and modulate gene expression



Pillar 2: Turn on Dad's UBE3A - ASOs

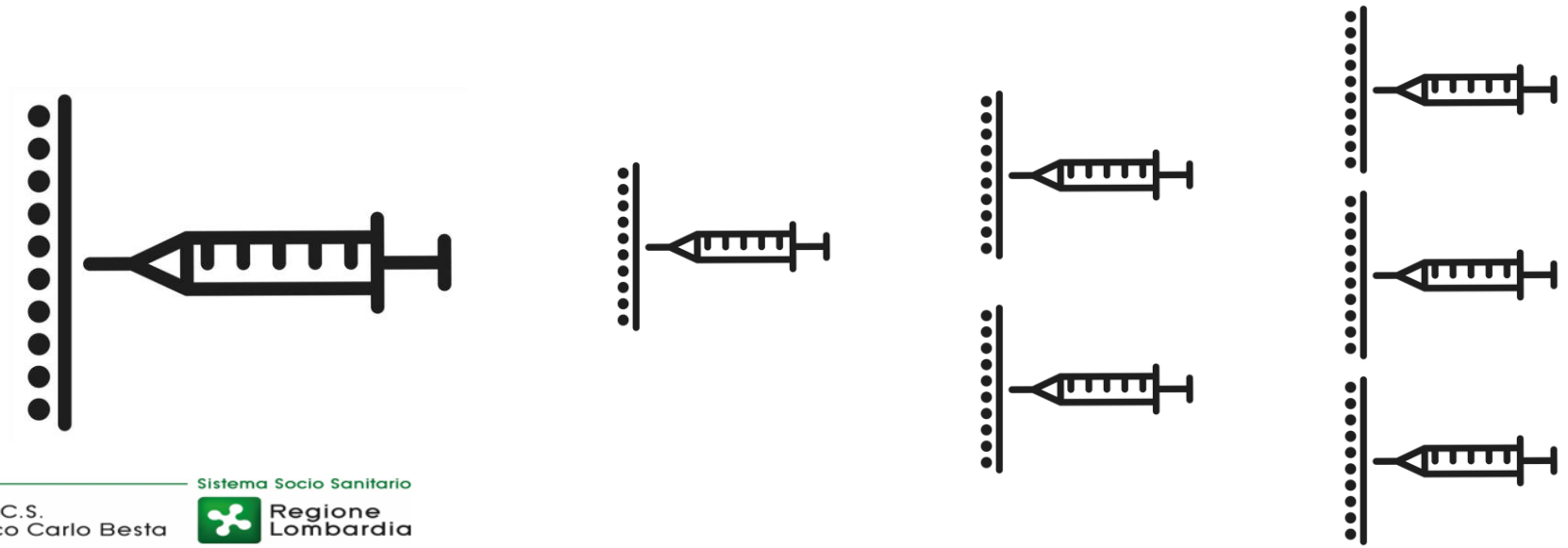
Current drug development and experimentation

Same mechanism - Different drugs (developed by different companies)

Phase 2 / Phase 3 of clinical trials

Requires:

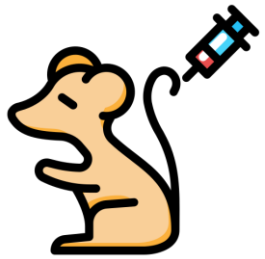
- **Intrathecal injections** in order to reach CNS
- **Multiple administrations** in order to maintain the effect



Pillar 2: Turn on Dad's UBE3A - ASOs

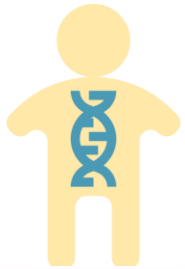
Preliminary outcomes

ASOs successfully reactivated the expression of paternal UBE3A allele in IPSC derived from AS patients and controls and differentiated into neurons



Improvement of two core symptoms: **sleep disorder** and **epilepsy**

- Correlation epilepsy / severity of symptoms:
  Can a better epilepsy control improve cognitive functions?
- Sleep regularization:
  Positive effect on the overall well-being of the patient and family



Proved safety and tolerability in different AS genotypes and ages

Pillar 2: Turn on Dad's UBE3A - ATF / ZF

ATF / ZF: Artificial Transcription factors / Zinc Fingers

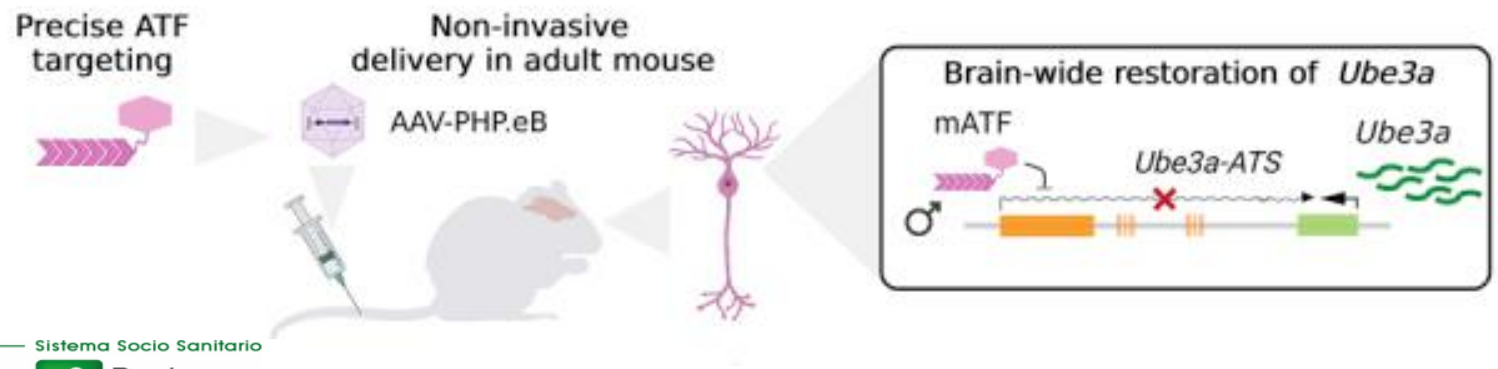
Zinc finger proteins are prevalent transcription factors in eukaryotic cells

They can be **re-programmed and engineered** to target specific sequences in the genome in order to **activate or repress gene transcription**

Consist of two domains linked together:

DNA-binding domain: targets a specific DNA sequence with high affinity

Regulatory domain: effector and functional domain



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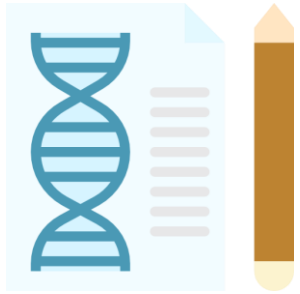
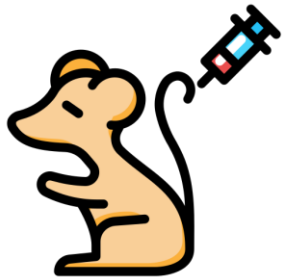
Pillar 2: Turn on Dad's UBE3A - ATF / ZF

Applications in AS



Purpose: to vehiculate the ATF drug in the brain in order to reactivate paternal UBE3A expression

Good results **in preclinical studies**



<u>Studies in mouse models</u>:

→ **Subcutaneous OR tail vein injection** of the artificial transcription factor ATF-S1K could lead to the **restoration of endogenous UBE3A in the brain**

→ Aims to be a **1-time administration**

Pillar 2: Turn on Dad's UBE3A - shRNA/miRNA

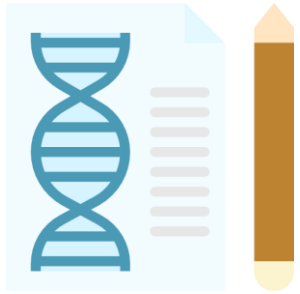
shRNA/miRNA: Short hairpin RNA / Micro-RNA

Small, non-coding RNA molecules that **regulate gene expression** by:
mRNA translation inhibition
MRNA degradation

Introduced in the brain using a **viral vector**
(ex. adeno-associated virus - AAV)

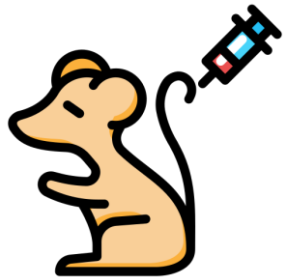
Pillar 2: Turn on Dad's UBE3A - shRNA/miRNA

Applications in AS



Bind to the UBE3A-ATS and suppress the transcription of UBE3A-ATS in order to **reactivate the paternal UBE3A expression**

Significant **progress in preclinical studies**



<u>Studies in mouse models</u>:

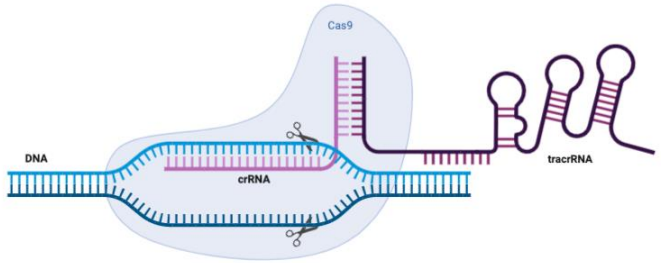
Increased UBE3A expression

Improvement of disease-related **symptoms**

- Requires **intrathecal injections** in order to reach CNS
- Aims to be a **1-time administration**

Pillar 2: Turn on Dad's UBE3A - CRISPR

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats



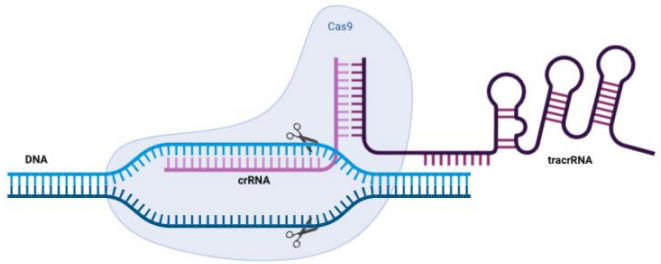
Family of **DNA sequences** found in the genomes of prokaryotics

Derived from a DNA fragment of a bacteriophage that had previously infected the prokaryote or one of its ancestors.

The sequences are used to detect and destroy DNA from similar bacteriophages during subsequent infections, playing a key role in the antiviral defense system of prokaryotes and providing **heritable immunity**

CRISPR gene editing

Cas9: CRISPR-associated protein 9



Enzyme that uses CRISPR sequences as a guide to **recognize and open up specific strands of DNA** that are complementary to the CRISPR sequence

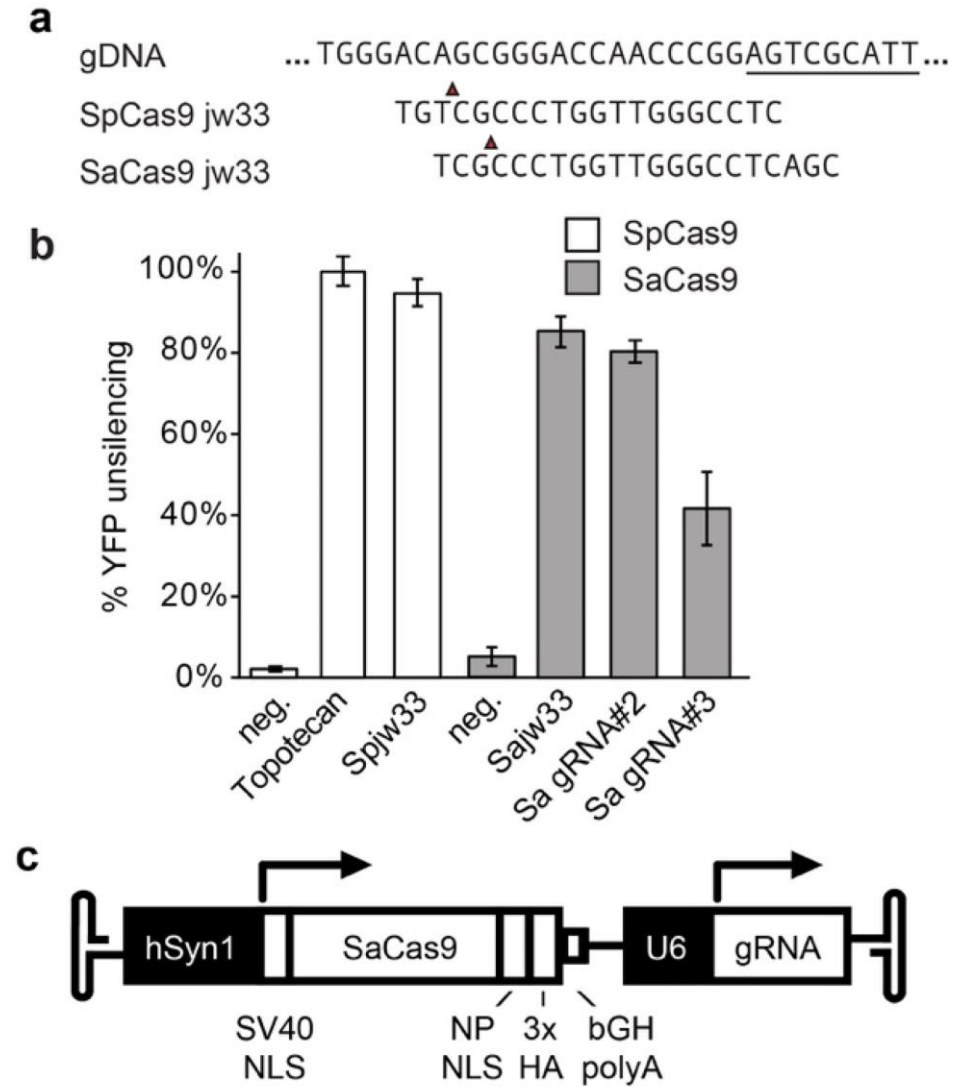
- **CRISPR-Cas9 editing process**

Pillar 2: Turn on Dad's UBE3A - CRISPR

Applications in AS

Cas9 targeted to Snord115 genes (small nucleolar RNAs clustered in the 3´ region of Ube3a-ATS) **vehiculated by an AAV**

The **genomic integration in the Cas9 target** site determine indels and therefore a **premature termination of Ube3a-ATS**, restoring paternal UBE3A



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Pillar 2: Turn on Dad's UBE3A - CRISPR

Applications in AS

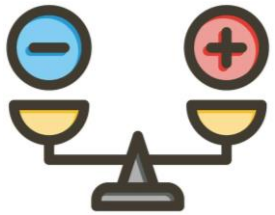
Proved effective in preclinical studies on **embryonic and early postnatal AS mice**

Good results in **reducing the transcription of targeted Ube3a-ATS**

Positive effects in motor and behavior phenotypes

- Requires **intrathecal injections** in order to reach CNS

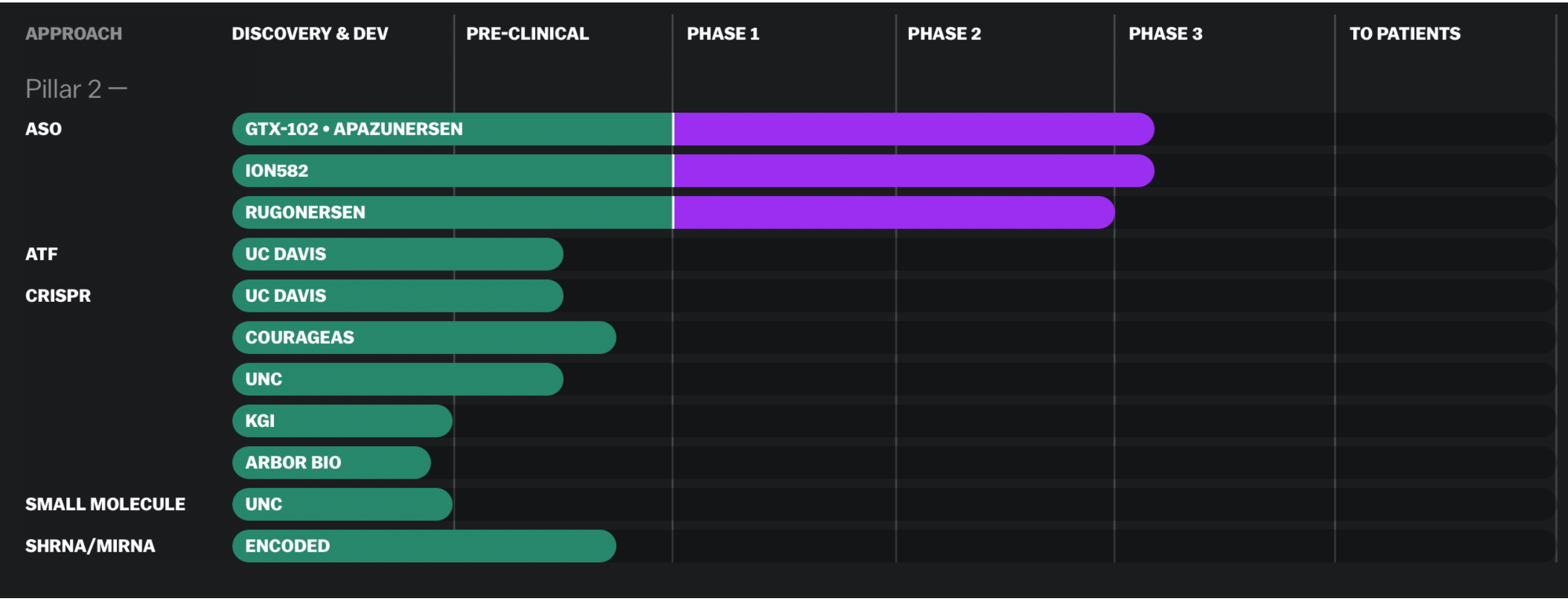
→ Aims to be a **1-time administration**



Pro & Cons

Permanent changes carrying the risk of unexpected outcomes
(large deletions, translocations, chromothripsis, integration of vector sequences in the genome, chromosomal rearrangements)

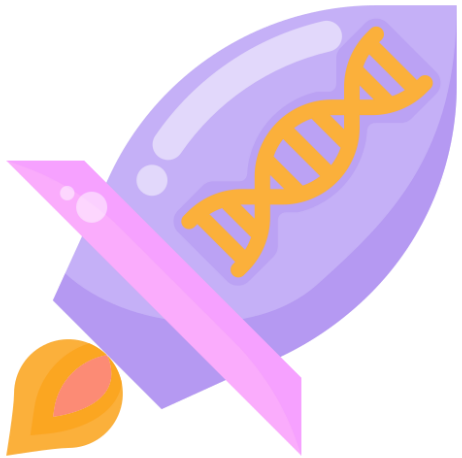
Pillar 2: Turn on Dad's UBE3A



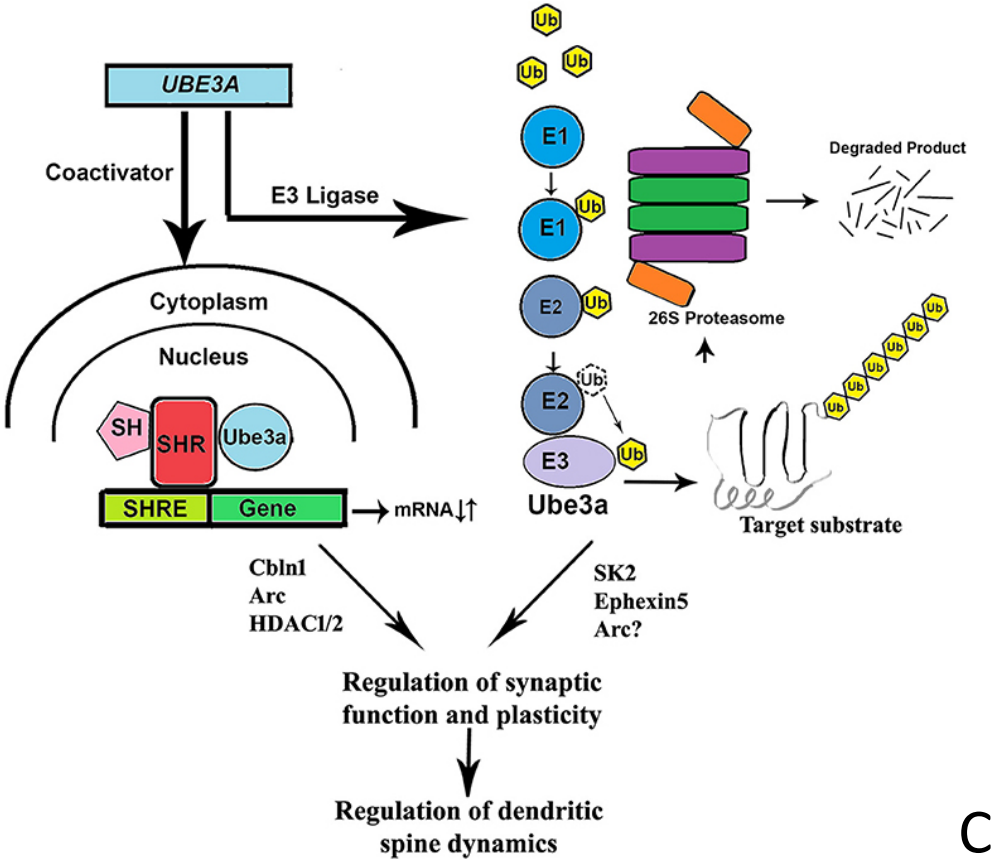
Pillar 3: Downstream targets

Dr. Stefano D’Arrigo, Dr. Claudia Ciaccio

Pillar 3: Downstream targets



Pillar 3: Downstream targets



UBE3A functions:

→ E3 ligase in the **ubiquitin proteasome pathway**

→ **Transcriptional coactivator**

UBE3A loss has an **impact on different effector proteins and pathways** regulated by Ube3a protein

Change of prospective and focus on what happens next

PMID: 30568575

Pillar 3: Downstream targets

IGF1 pathway

→ IGF-1 is a critical actor in **brain development and maintenance**

→ It reduces inflammation and restore a normal functioning of the glia

Preclinical studies:

→ Normalization of deficits in tests assessing anxiety, daily living, sociability, motor performance and cognition

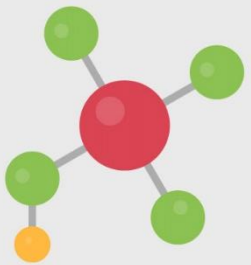
→ Obtained seizure free mice

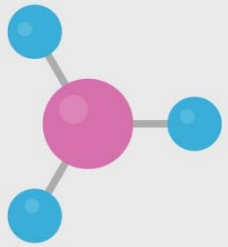
Phase II Clinical trial studies:

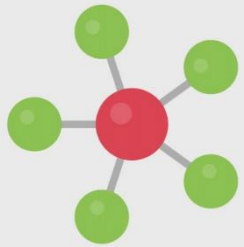
- **Oral solution**: no invasive intrathecal injection

→ Requires **multiple administrations**

Pillar 3: Downstream targets





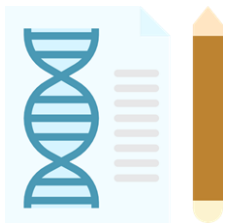


Small molecules

→ Low molecular weight compounds small enough to easily get into tissues, enter cells, and interact with specific biological targets

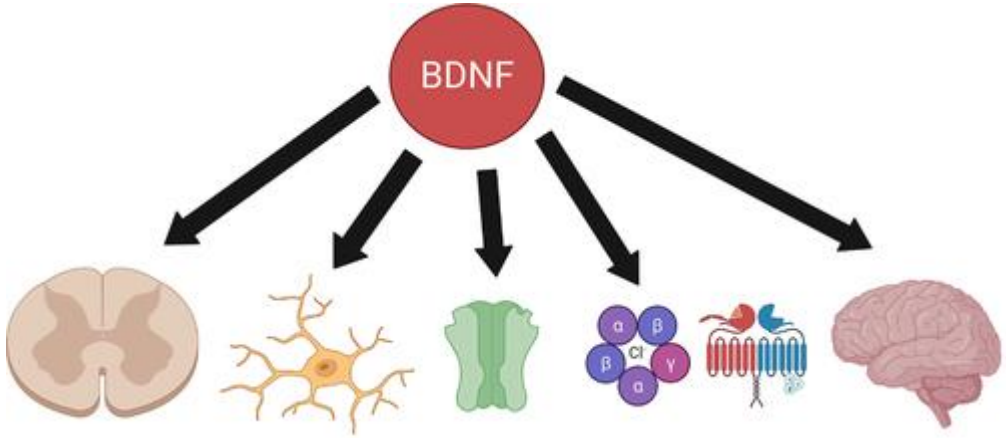
→ Aim to modulatw biochemical pathways, inhibiting or activating specific proteins, or altering cellular processes shown to be altered in AS neurons

→ Different types of molecules: proteins, DNA, RNA or ATS



Early- Preclinical studies

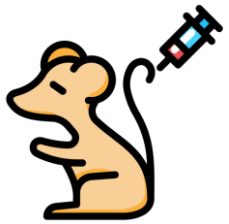
Pillar 3: Downstream targets



Brain-Derived Neurotrophic Factor (BDNF)

→ BDNF signaling (Brain-Derived Neurotropic Factor) has been proved to be altered in AS neurons and lead to synaptic dysfunction

→ Aims to **improve synapse functioning in Angelman patient brain**



Preclinical studies:

Reversed abnormalities of synaptic function in the hippocampus, restoring the number of synapses as well as the levels of synaptic proteins in mice

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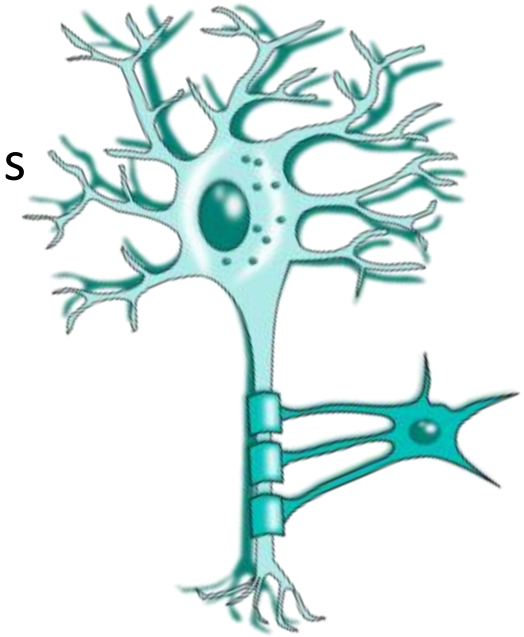
Pillar 3: Downstream targets

Oligodendrocyte Precursor Cells (OPC)



→ Oligodendrocytes are a subtype of neuroglia cells whose main function is to provide the myelin sheath to neuronal axons in CNS

→ They have been proved to have a role in the overall function of neurons in AS mouse models

→ Stimulate OPC precursors may improve AS symptoms

Preclinical studies
Stimulation of OPC showed an improvement of symptoms in AS mice

Pillar 3: Downstream targets

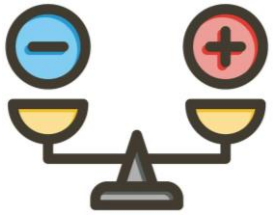
GABA pathway

→ UBE3A is responsible of an ubiquitin-mediated mechanism leading to degradation of gene products regulating GABA pathway

→ Large deletions may encompass genes encoding for GABA-A receptor subunits

First preclinical studies:

- improve tonic inhibitory deficits in slice preparations from AS mice
- improve deficits in motor coordination in mice



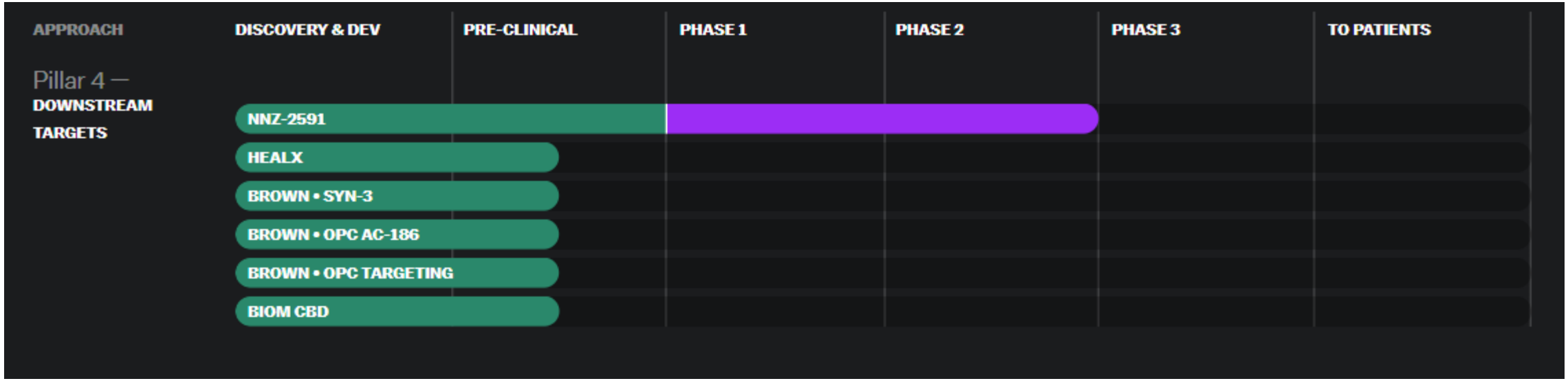
Phase II Clinical studies:

improvement of sleep, motor and communication abilities, challenging behavior, anxiety

Phase III Clinical studies:

failed in confirming efficacy vs placebo

Pillar 3: Downstream targets



Future outlook

Prof Semeraro

The future therapeutic approaches for the treatment of **Angelman syndrome** are centered on disease-modifying strategies that aim to restore neuronal UBE3A expression

- **AAV-GT** and **HSC-GT** are the leading gene replacement strategies for UBE3A, with ERT-like effects achievable through engineered gene therapy constructs, but not via traditional enzyme replacement therapy
- **Antisense oligonucleotides (ASOs)** targeting UBE3A-ATS, administered intrathecally, demonstrated an acceptable safety and tolerability profile. Additional ASOs are in clinical development, with intrathecal administration being the primary route
- **Efficacy signals** included dose-dependent partial normalization of the characteristic EEG delta power abnormality and improvements in developmental domains measured by the Bayley and Vineland scales. These changes exceeded expectations from natural history data, suggesting a disease-modifying effect.

Discussion time - Conclusion with speakers and moderator

All

Discussion & Conclusion

- Time for questions

- Satisfaction Survey :
  - https://forms.office.com/e/dA5BWDzyM4

Website :

- https://ern-ithaca.eu
- https://ern-ithaca.eu/webinars/

Thank you for your participation