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ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ieid20

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To cite this article: Theodora Markati, Jessica Duis & Laurent Servais (2021) Therapies in preclinical and clinical development for Angelman syndrome, Expert Opinion on Investigational Drugs, 30:7, 709-720, DOI: 10.1080/13543784.2021.1939674

To link to this article: https://doi.org/10.1080/13543784.2021.1939674

Published online: 28 Jun 2021.

EXPERT OPINION ON INVESTIGATIONAL DRUGS
2021, VOL. 30, NO. 7, 709–720
https://doi.org/10.1080/13543784.2021.1939674

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REVIEW

Therapies in preclinical and clinical development for Angelman syndrome

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ABSTRACT

Introduction: Angelman syndrome is a rare genetic neurodevelopmental disorder, caused by deficiency or abnormal function of the maternal ubiquitin protein-ligase E3A, known as UBE3A, in the central nervous system. There is no disease-modifying treatment available, but the therapeutic pipeline of Angelman syndrome includes at least 15 different approaches at preclinical or clinical development. In the coming years, several clinical trials will be enrolling patients, which prompted this comprehensive review.

Areas covered: We summarize and critically review the different therapeutic approaches. Some approaches attempt to restore the missing or nonfunctional UBE3A protein in the neurons via gene replacement or enzyme replacement therapies. Other therapies aim to induce expression of the normal paternal copy of the *UBE3A* gene by targeting a long non-coding RNA, the *UBE3A-ATS*, which interferes with its own expression. Another therapeutic category includes compounds that target molecular pathways and effector proteins known to be involved in Angelman syndrome pathophysiology.

Expert opinion: We believe that by 2022–2023, more than five disease-modifying treatments will be simultaneously at clinical testing. However, the are several challenges with regards to safety and efficacy, which need to be addressed. Additionally, there is still a significant unmet need for clinical trial readiness.

ARTICLE HISTORY
Received 19 March 2021
Accepted 3 June 2021

KEYWORDS
Adeno-associated virus; angelman syndrome; antisense oligonucleotide; cell therapy; crispr-cas9; gene therapy; genomic imprinting; ube3a; ube3a-ats ; zinc fingers

1. Introduction

Angelman syndrome (AS), first characterized by Dr Harry Angelman in 1965, is a rare genetic neurodevelopmental disorder diagnosed in one in 12,000–20,000 live births (NORD, 2018 & OMIM 105830). AS patients present with global developmental delay, learning difficulties, and particularly severe expressive language delay. Behaviorally, patients have a characteristically happy demeanor, which is usually expressed as unprovoked laughter, a love for water, and maladaptive behavior. Patients have movement disorders, including gait ataxia, tremulousness of the limbs, and generalized hypotonia of the trunk. Commonly, patients also have seizure activity and sleep disturbance [1–8]. Treatment is supportive with a focus on seizures, sleep, and behavior, as no disease-modifying or AS-specific treatments are currently available.

The cause of AS is deficiency or abnormal function of the ubiquitin-protein ligase E3A, known as UBE3A, which is expressed from the maternal *UBE3A* allele, located on chromosome 15 in humans [9,10]. Loss of expression of the maternal *UBE3A* occurs via several molecular mechanisms. Most commonly, it occurs from *de novo* deletion of the maternal 15q11.2-q13 critical area on chromosome 15 (approximately 75% of cases) [11]. Other causes include frameshift, nonsense, or missense mutations in *UBE3A*, paternal uniparental disomy, and imprinting defects [5,10]. *UBE3A* is imprinted in the central nervous system (CNS), wherein the paternal copy is silenced by a long non-coding antisense transcript, the *UBE3A-ATS*. In both humans and mice, the antisense transcript silences the production of the paternal *UBE3A* gene [12,13].

UBE3A catalyzes ubiquitination, a process by which proteins are tagged for degradation in the proteasome [14,15]. Several candidate UBE3A substrates have been identified, including the calcium ($Ca2^{+}$)-activated small conductance potassium channel SK2, ephexin-5, p53, and p27 [16,17]. Network analysis of UBE3A suggests that several molecular pathways could potentially contribute to AS pathophysiology [18]. UBE3A plays a critical role in activity-dependent synaptic plasticity during development [19]. Mouse models of AS present morphological abnormalities in the dendritic spine, impaired long-term potentiation (LTP) [20–22], and an ataxic phenotype [23].

Three strategies are being pursued in preclinical and clinical development for the treatment of AS. One strategy aims to restore the missing or nonfunctional UBE3A protein in the neurons via gene replacement or enzyme replacement therapies. The goal of a second approach is to 'unsilence' the paternal copy of the *UBE3A* gene. The third approach involves compounds that target molecular pathways and effector proteins known to be involved in AS pathophysiology. The wide range of mechanistic approaches and the rapidly accelerating

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Article highlights

- Angelman syndrome (AS) is a rare genetic neurodevelopmental disorder, which is caused by deficiency or abnormal function of the maternal, ubiquitin protein-ligase E3A, known as UBE3A protein in the central nervous system.
- Several molecular mechanisms, including deletions and mutations, can affect the maternal *UBE3A* gene on chromosome 15 and subsequently expression of a normal protein. The paternal copy of the gene is silenced in neurons by genomic imprinting. A long non-coding RNA, the *UBE3A-ATS* is believed to hinder expression of the normal paternal *UBE3A* gene.
- Among the therapeutic strategies in the AS pipeline are those approaches that aim to restore the missing or non-functional UBE3A protein in the neurons via gene replacement or enzyme replacement therapies. An adeno-associated virus-mediated gene replacement therapy is in late preclinical development, close to clinical testing.
- Another promising category of therapeutic approaches for AS is targeting the *UBE3A-ATS* transcript intending to 'unsilence' the paternal *UBE3A* gene. This category includes, among other, antisense oligonucleotides, topoisomerase inhibitors, and genome engineering approaches. Two antisense oligonucleotides are currently in clinical trials with a third one following.
- Other therapeutic approaches are targeting downstream molecular pathways, known to be involved in AS pathophysiology.
- More than 15 therapeutic approaches with the potential to treat AS are currently at preclinical and clinical development stages. There is still no available disease-modifying treatment for AS. However, we believe that in the next few years several candidates will be in clinical development simultaneously for AS.

This box summarizes key points contained in the article.

pace of discovery render the understanding of the current pipeline challenging. An additional issue for physicians in contact with families is that there are data in public domains, such as social networks or websites of patient advocacy groups that have not been published in peer-reviewed medical literature. This can make it difficult to provide sound and current advice to patients and to manage their expectations. The aim of this review is to summarize the candidate treatments and therapies at clinical and late preclinical stages by describing not only peer-reviewed publications but also all publicly available data.

2. Materials and methods

We performed a comprehensive review of publications on PubMed and Cochrane using the keywords 'Angelman Syndrome' or 'Angelman' and 'therapy/-ies', 'treatment/-s', or 'therapeutic/-s'. We also searched all the ongoing clinical trials and studies registered with ClinicalTrials.gov by using the key word 'Angelman Syndrome'. All publicly available information from the annual Foundation for Angelman Syndrome Therapeutics annual Summit and GALA (mentioned in the text as FAST Summit) available on the link:

https://www.youtube.com/channel/UCuAoKMiWQXb_OcBnZppzQrQ was used.

Additionally, we reviewed publicly available information from official websites of the Foundation for Angelman Syndrome Therapeutics, the Angelman Syndrome Foundation. [As a consequence, data reported below are collated not only from peer-reviewed publications, but also from press releases, or public presentations given at various conferences by the primary investigators, or industry representatives. Non-peer reviewed sources are listed as 'additional sources' to clearly indicate they have not been peer-reviewed.]

3. Results

3.1. Gene/enzyme replacement therapies

Several drugs in development for AS aim to restore the missing or nonfunctional UBE3A protein in the neurons via gene replacement or enzyme replacement. The compounds, companies or institutions involved, and stages in development are listed in Table 1.

3.1.1. Adeno-associated virus-mediated gene replacement

Daily *et al*. [24] provided the first proof of concept that AS can be treated by exogenously supplying a copy of the *UBE3A* gene that codes for the homonymous protein to neurons. In the reported experiments, mice deficient in maternal Ube3a due to a null mutation received direct hippocampal injections of an adeno-associated virus (AAV) serotype 9, AAV-9, which had been transformed to carry a copy of the murine *Ube3a* gene [21,24]. Mice given gene replacement therapy showed significant associative learning and memory improvement as compared to controls, which were injected with an AAV-9 vector carrying the transgene encoding a green fluorescent protein. However, in contrast to the memory, LTP was not completely rescued, as revealed via electrophysiology. Additionally, there was not adequate transduction of the transgene into the cerebellum, and, therefore, motor deficits, believed to be associated with this part of the brain, were not improved [24,25].

In principle, AAV vectors, transformed to carry a copy of the gene that requires replacement, are recognized by cell surface receptors of the target cells and they get internalized via endocytosis. They are then trafficked intracellularly in endosomal vesicles and, after entering the nucleus through the nuclear pore complex, their genome gets released (uncoating). The single-stranded DNA undergoes second strand synthesis using the host polymerase, as a double-strand is required for transcription; some AAVs are 'self-complementary'. Following this, the genome is usually stabilized as circular episomes, which can then be transcribed to mRNA and translated to protein by the cellular machinery. AAV genome can also integrate into the host at low frequency (Figure 1) [26,27]. Researchers are focusing on engineering AAV vectors for CNS diseases with better bioavailability potential and neuronal transduction capability. Additionally, many factors can affect the efficacy of a gene replacement therapy for AS, including the use of different promoters, regulatory areas, or *UBE3A* transgenes. More specifically, *UBE3A* codes for the three UBE3A isoforms, which can occur via alternative splicing; it is yet unknown if some isoforms are more critical in the pathophysiology of AS [28]. Based on presentations from the annual FAST Summit (additional sources 1–5), several institutions and companies are working toward an efficacious gene

Table 1. Gene/protein replacement therapies.

Compound	Institution or Company	Mechanism of action	Clinical phase	Method of administration	Reference
Gene replacement via AVV-9 (e.g, GT-AS)	USF/PTC Therapeutics, UPenn,Sarepta/ StrideBio, UNC/AskBio, Bamboo/Pfizer	Gene replacement in neurons	Preclinical	Intrathecal/ intraventricular	Daily *et al.*, 2011
Cell therapy	UC Davis	Cell therapy	Preclinical	Intravenous	Additional sources: 6–7
Enzyme Replacement Therapy (ERT)	UC Davis	Protein replacement in neurons	Discovery	Intrathecal/ intravenous	Additional source: 8

Abbreviations: AAV: adeno-associated virus, USF: University of South Florida, UPenn: University of Pennsylvania, UNC: University of North Carolina – Chapel Hill, AskBio: Asklepios Biopharmaceutical, UC Davis: University of California Davis

replacement therapy for AS. A gene therapy approach, using a modified AAV vector (USF-AAV), GT-AS (previously known as AGIL-AS), is in late preclinical development.

3.1.2. Cell therapy

A gene therapy, based on autologous hemopoietic stem cell (HSC) transplantation after *ex vivo* lentiviral-mediated insertion of the *UBE3A* gene, is currently in late preclinical development (Figure 1) [29]. This type of approach is being investigated for several genetic disorders including immune deficiency disorders [30], and other neurogenetic diseases like metachromatic leukodystrophy [31].

According to presentations at the FAST Summit (additional sources 6–7), this approach will initially require the collection of peripheral blood stem cells from patients. *Ex vivo*, a normal copy of the *UBE3A* gene will be inserted into the genome of the HSCs using a lentiviral-mediated approach, and then the cells will be re-infused intravenously back into the patient. This approach requires chemotherapy to allow adequate bone marrow occupation of the programmed HSCs. Following successful autologous transplantation, HSCs will differentiate into physiologically occurring cell lines, including immune cells, that are able to cross the blood-brain barrier. In the CNS, the UBE3A protein will be secreted from the successfully engrafted cells and will be received by the deficient neurons in a process named *cross-correction*. This type of therapy has two main advantages. Firstly, the autologous transplantation of cells edited *ex vivo* increases the chances of success, as compared

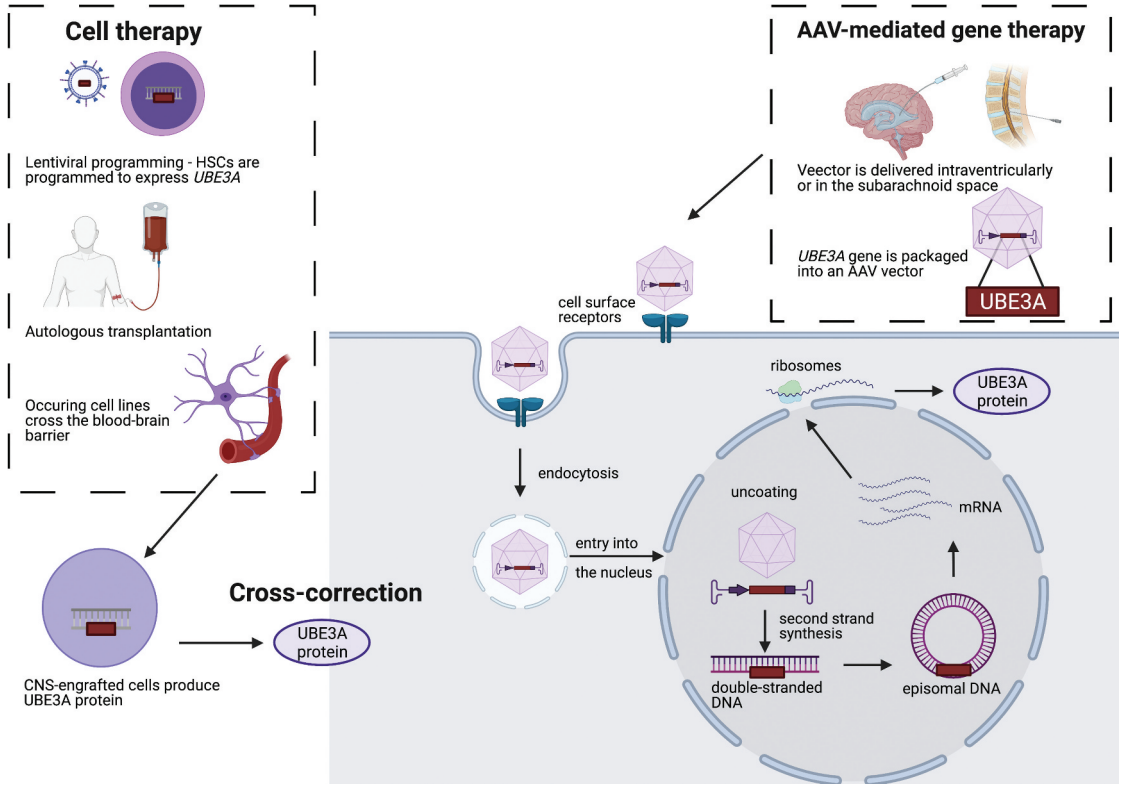

Figure 1. *In vivo* gene therapy and *ex vivo* gene therapy (cell therapy). In AAV-mediated gene therapy, AAV vectors, transformed to carry a copy of the gene that requires replacement, are recognized by cell surface receptors of the target cells and they get internalized via endocytosis. They are then trafficked intracellularly in endosomal vesicles and, after entering the nucleus through the nuclear pore complex, their genome gets released (uncoating). The single-stranded DNA undergoes second strand synthesis using the host polymerase, as a double-strand is required for transcription; some AAVs are "self-complementary". Following this, the genome is usually stabilized as circular episomes, which can then be transcribed to mRNA and translated to protein by the cellular machinery. AAV genome can also integrate into the host at low frequency. In cell therapy, HSCs are isolated from the patient. *Ex vivo* lentiviral programming of these HSCs leads to integration of the UBE3A gene into the genome. After autologous transplantation, the HSCs carrying the normal copy of the gene differentiate into cells that have the ability to cross the blood-brain barrier. Once successfully engrafted in the CNS, cells produce UBE3A protein and supply the deficient neurons via *cross-correction*.

to allogeneic transplantation against which an immune reaction is more probable. Secondly, the strategy will likely provide a permanent treatment, as *UBE3A* will theoretically be integrated into the genome of the HSCs and will, therefore, continue to be present after cell divisions.

3.1.3. Enzyme replacement therapies

Enzyme replacement therapies (ERTs) are broadly used for the treatment of metabolic diseases associated with a single enzyme deficiency or abnormal function, like Gaucher's disease and Pompe disease [32,33]. An ERT is currently in preclinical development for AS. ERT aims to deliver a purified form of the missing or nonfunctional UBE3A protein into neurons, both in the intracellular and extracellular space. In a recent animal study, researchers found that UBE3A is not only excreted but maintains the enzymatic ubiquitinating activity outside neurons [34]. ERT is still at the discovery level. Cell-based and animal studies are underway to prove the concept and assess the safety of such therapy (additional source 8).

3.2. 'Unsilencing' of the paternal allele

In both humans and mouse models, the transcription of the long non-coding RNA transcript, the *UBE3A-ATS* and *Ube3a-ATS* respectively, is regulated from areas at or upstream the *Prader-Willi syndrome imprinting center (PWS-IC)*. The *Ube3a-ATS* runs through the *Snurf/Snrpn, Snord116, Ipw, Snord115* and to the *Ube3a* coding region in antisense orientation [35]. On the paternal chromosome 15, the transcription of the *UBE3A-ATS* results in 'silencing' of the *UBE3A* gene (Figure 2a) [12].

In mice, decreased *Ube3a-ATS* levels, due to deletion of its promoter area, lead to increased expression of paternal *Ube3a* [12,36]. Mice with a poly(A) cassette between the *Snord115* and *Ube3a* areas on the paternal chromosome, which results in premature termination of the *Ube3a-ATS* transcript, have decreased *Ube3a-ATS* levels and twice the amount of *Ube3a* mRNA, as compared to control mice. Mice deficient in maternal Ube3a due to a null mutation [21], which received the poly(A) cassette on the paternal side, had increased Ube3a levels in different regions of the brain. These mice exhibited improved phenotypical characteristics, including improved motor coordination and LTP enhancement [13]. This was the first proof of concept that selective inhibition of *UBE3A-ATS* transcription can lead to the 'unsilencing' of the paternal *UBE3A* allele and triggered several therapeutic approaches. The compounds, companies or institutions involved, and stages in development are listed in Table 2.

3.2.1. Antisense oligonucleotides

The use of antisense oligonucleotides (ASOs) complementary to the distal part of the *Ube3a-ATS* increases paternal *Ube3a* expression, likely by recruitment of RNase H, which degrades

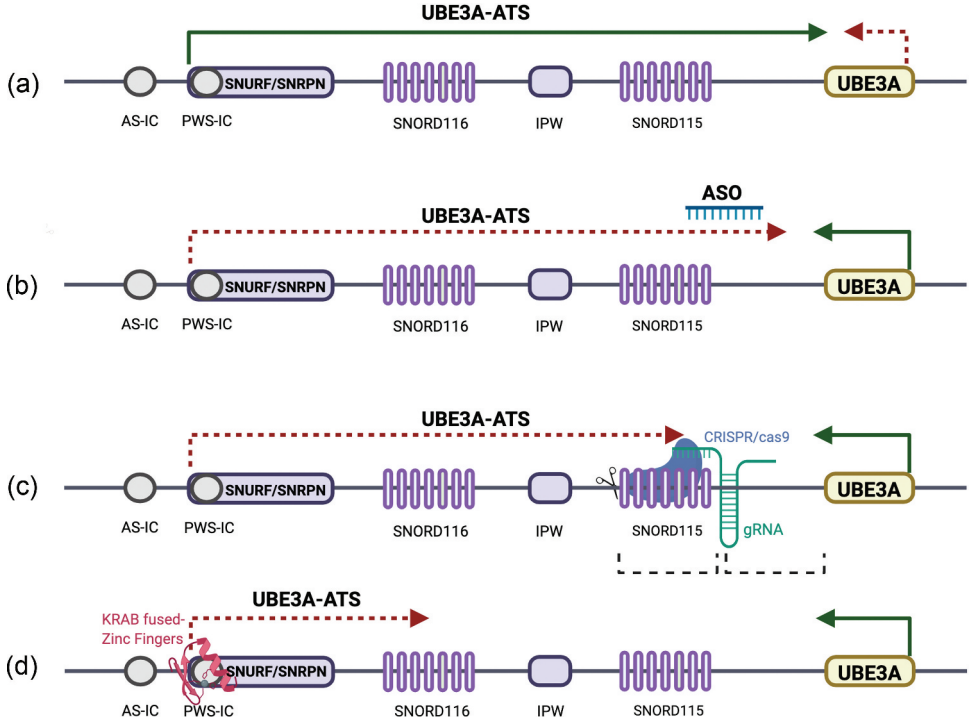

Figure 2. Therapeutic strategies for "unsilencing" of the paternal *UBE3A*. (a) The normal paternal copy of the *UBE3A* gene on chromosome 15 is "silenced" due to genomic imprinting. In both humans and mouse models, the transcription of the long non-coding RNA, the *UBE3A-ATS* and *Ube3a-ATS* respectively, is regulated from areas at or upstream the PWS-IC. The *Ube3a-ATS* runs through the Snurf/Snrpn, Snord116, Ipw, Snord115 and to the *Ube3a* coding region in antisense orientation. On the paternal chromosome 15, the transcription of the *UBE3A-ATS* results in "silencing" of the *UBE3A* gene. (b) ASOs complementary to the distal part of *UBE3A-ATS* can lead to RNase H-mediated cleavage of the ASO/RNA hybrid and to premature termination of *UBE3A-ATS* transcription. In the absence of *UBE3A-ATS* transcription, the paternal *UBE3A* is expressed. ASOs are currently in clinical development. (c) CRISPR/Cas9-mediated mutagenesis in the genomic areas that code for the *UBE3A-ATS* can potentially lead to the "unsilencing" of the paternal *UBE3A* likely by early cessation of the *UBE3A-ATS* transcription. Brackets indicate the areas which lead to "unsilencing" of the paternal *UBE3A* when targeted in animal studies. This approach is still in preclinical development. (d) KRAB fused-zinc finger proteins that bind to the promoter of *UBE3A-ATS* can potentially suppress *UBE3A-ATS* transcription and "unsilence" the paternal *UBE3A*. This approach is still in preclinical development.

Table 2. Unsilencing of paternal copy.

Compound	Institution or Company	Mechanism of action	Clinical phase	Method of administration	Reference
GTX-102	GeneTx Biotherapeutics/ Ultragenyx Pharmaceutical	ASO against the distal part of *UBE3A-ATS*	Phase 1/2 NCT04259281(KIK-AS)	Intrathecal	Meng *et al*., 2015
RO7248824 (RG6091)	Hoffmann La Roche	ASO against the distal part of *UBE3A-ATS*	Phase 1 NCT04428281 (TANGELO)	Intrathecal	Meng *et al*., 2015
ION582	Ionis/Biogen	ASO against the distal part of *UBE3A-ATS*	Preclinical	Intrathecal	Meng *et al*., 2015
Topoisomerase inhibitors type I and II (e.g. topotecan, indotecan)	UNC	Inhibits *UBE3A-ATS* expression potentially via R-loop stabilization over *SNORD116*	Preclinical (for AS)	Oral	Huang *et al*., 2012 and Powell *et al*., 2013
CRISPR/Cas9	UNC/AskBio, UPenn, UC Davis	Mutagenesis of *UBE3A-ATS* coding area	Preclinical	Intrathecal/ intraventricular	Wolter *et al*., 2020 and Schmid *et al*., 2021
Zinc Finger-based ATFs	UC Davis	*UBE3A-ATS* regulation/ expression reduction	Preclinical	Likely systematic	Bailus *et al*., 2016
shRNAs (e.g. TSHA-106, OV882)	Taysha Gene Therapies/UT Southwestern Medical Center, UConn/Ovid Therapeutics	*UBE3A-ATS* regulation/ expression reduction	Discovery	-	Additional sources: 13–14
miRNAs	UPenn	*UBE3A-ATS* regulation/ expression reduction	Discovery	-	Additional source: 15
Small molecules	UNC/Pfizer	-	Discovery	-	Additional source: 16

Abbreviations: ASO: antisense oligonucleotide, UNC: University of North Carolina, Chapel Hill, AskBio: Asklepios Biopharmaceutical, UPenn: University of Pennsylvania, UC Davis: University of California Davis, ATFs: artificial transcription factors, shRNAs: small hairin RNAs, UT Southwestern Medical Center: University of Texas Southwestern Medical Center, UConn: University of Connecticut, miRNAs: microRNAs

the ASO/RNA hybrid. *Ube3a-ATS* and the critical for Prader-Willi syndrome (PWS), *Snord116*, are processed from the same precursor RNA. Interestingly, the production of mature *Snord116* is not affected, probably due to the fast rate of splicing compared to the time required for transcription between the *Snord116* and the ASO binding site (Figure 2b) [37].

Currently, two ASOs are actively in human trials, named GTX-102 and RO7248824. A third, ION582, is in preclinical development. ASOs can differ both in their sequences and structures [38]. For example, locked nucleic acids (LNAs) are a specific type of ASOs with an unnatural backbone that results in higher affinity, increased metabolic stability, and lower toxicity. LNAs have a biradicle bridge between C2 and C4 carbons of the ribose [39].

The first phase 1/2 clinical trial (KIK-AS, NCT04259281) of the ASO, GTX-102 showed promising results. According to the press release (additional source 9) five patients have been treated with the GTX-102. The drug was administered intrathecally with an ascending five-dose scheme. Participants presented with clinical improvement that lasted at least three to five months. All patients showed improvement at least in three domains of the AS-adjusted Clinical Global Impressions (CGI) Scale. After treatment, patients had improved scores in the domains of receptive and expressive communication on the Bayley Scales of Infant and Toddler Development-4 and three of them on the Observer Reporter Communication Ability (ORCA) communication tool. However, all five participants presented the serious adverse event of lower limb weakness at the highest doses tested, which was associated with inflammation of the meninges and the nerve roots in the region of the intrathecal administration. The lower limb weakness resolved for all participants, and the observed clinical benefits of the treatment lasted far longer than the duration of the adverse event, approximately three to five months after the last dose.

RO7248824 (or RG6091) is currently at phase 1 clinical trial (TANGELO, NCT04428281) (additional source 10). ION582 is in preclinical development (additional source 11).

3.2.2. Topoisomerase inhibitors

During a screening process of small molecules with the potential to 'unsilence' the paternal *UBE3A* allele, 16 topoisomerase type I and II inhibitors showed promising results. The topoisomerase inhibitor I, topotecan, which is approved by the US Food and Drug Administration (FDA) for use as a chemotherapeutic agent, showed promising results both *in vitro* and *in vivo* [40]. Mice carrying a fusion gene between the paternal *Ube3a* and a coding gene for a yellow fluorescent protein were used for the screening [20]. *In vitro*, topotecan administration resulted in restoration of functional Ube3a to wild type levels in cultures of primary cortical neurons from mice deficient in maternal Ube3a due to a null mutation [21]. When these mice were treated with topotecan via intracerebroventricular administration, Ube3a levels were increased in the hippocampus, striatum, cerebral cortex, and cerebellum in a dose-dependent manner [40]. When administered intrathecally, topotecan increased paternal *Ube3a* expression primarily in spinal cord neurons and the results remained 12 weeks after the last dose [40]. Mechanistically, topotecan suppresses

Ube3a-ATS transcription via the stabilization of R-loops over the paternal *Snord116* cluster, which are known to create genomic instability and transcription cessation [41,42]. Since the bioavailability of topotecan in the CNS is limited, other topoisomerase I inhibitors were investigated, and indotecan was shown to have a better pharmacological profile [43].

However, despite the favorable effect, topotecan led to nonspecific reduction in expression of other genomic areas [40]. Additionally, impairment of topoisomerase activity represses the expression of several long genes linked to autism *in vitro* [44]. For such reasons, the 'off-target' effects of using topoisomerase inhibitors require careful consideration.

3.2.3. CRISPR/Cas9

CRISPR/Cas9 has been successfully used in preclinical studies to mutate the region encoding the *Ube3a-ATS* transcript, block its expression and 'unsilence' the paternal *Ube3a* allele. Researchers screened a library of different guide RNAs (gRNAs), which target regulatory areas close to or within the *Ube3a-ATS* coding area of the genome. Of those, the gRNAs targeting the *Snord116* and *Snord115* clusters resulted in the most efficient 'unsilencing' the paternal copy of *Ube3a* when transduced to cortical neurons of mice that carry a fusion between the paternal *Ube3a* allele and a coding gene for a yellow fluorescent protein [20,45]. A gRNA, Spjw33, that simultaneously targets 76 areas within *Snord115* was selected for further experiments (Figure 2c). Spjw33 selectively reduced the transcription of targeted *Ube3a-ATS* areas, in contrast to the controls treated with topotecan [45].

When mice lacking the maternal *Ube3a* allele were intracerebroventricularly injected with an AAV carrying a *Staphylococcus aureus* Cas9 and a gRNA that targets a region similar to Spjw33 there was a significant increase in paternal *Ube3a* expression throughout the brain including the cortical neurons, the hippocampus, and the spinal cord (but not the cerebellum). The effects persisted for 17 months after a single injection, as confirmed by histological analysis of cortical neurons. Mice injected twice (during the embryonic and early postnatal period) had improved anatomical and behavioral features. This approach also resulted in increased biallelic *Ube3a* expression in primary human neural progenitor-derived neurons transduced with gRNAs targeting the *Snord115* cluster area. Researchers observed the integration of the vector into the host genome in the targeted areas [45].

A recent study showed that CRISPR/Cas9-mediated indel formation in the genomic area between *Snord115* and the paternal *Ube3a* can 'unsilence' the paternal allele and restore the motor and behavioral phenotype in mice lacking maternal *Ube3a* (Figure 2c). Neonatal mice were injected intracerebroventricularly with an AAV vector carrying the *S. aureus* Cas9 and the gRNA (ATS-GE) under control of the *synapsin* promoter to drive neuronal expression. Sequencing analysis showed that approximately 20% of neurons underwent gene editing, suggesting that gene editing in a subset of neurons is adequate to alter phenotypical characteristics. The researchers suggested a pause of the *Ube3a-ATS* transcription at the indel insertion sites, allowing *Ube3a* transcription [46]. Differences between the murine and the human genome will not allow use of the same gRNA sequences for human applications.

Researchers are also looking into the potential of using CRISPR/Cas13 to target directly the *UBE3A-ATS* RNA, rather than its coding DNA area (additional source 12).

3.2.4. Artificial transcription factors

Artificial transcription factors (ATFs) are binary systems that consist of a DNA-binding region and an effector that can regulate expression levels of the targeted gene [47]. Zinc-finger based ATFs successfully cross the blood-brain barrier when injected subcutaneously or intraperitoneally and suppress expression of *Ube3a-ATS* in mouse models of AS [21,48]. Systemic administration of an ATF composed of a zinc finger domain fused with the Krüppel associated box (KRAB) transcription repressor, the human immunodeficiency virus (HIV) cell-penetrating protein TAT (to facilitate endocytosis), and a nuclear signal resulted in distribution throughout the brain, as confirmed by *in vivo* fluorescence and immunochemistry. The ATF suppressed the production of the *Ube3a-ATS* by binding to the *Snurf/Snrpn* promoter area (Figure 2d). This led to restoration of Ube3a to levels intermediate between the AS mice and the wild type mice, as confirmed by both immunochemistry of the hippocampus and cerebellum, as well as western blotting. Different dosing regimens or a combination of zinc fingers targeting different upstream promoter areas of *UBE3A-ATS* could increase the efficacy of this type of treatment [48]. Behavioral experiments have not been performed to assess the effect on the phenotypes of the treated mice.

3.2.5. Short hairpin RNAs and microRNAs

Short hairpin RNAs (shRNAs) and microRNAs (miRNAs) can be used to target the *UBE3A-ATS* transcript for degradation through the RNA interference process. Viral vectors (e.g. AAVs) or DNA plasmids can be used to induce production of shRNAs in neurons. The stability of shRNAs in cellular environment makes this type of agent a promising candidate for use in treatments with infrequent dosing. Therapies for AS utilizing shRNAs (e.g. TSHA-106, OV882) and miRNAs are at the discovery level (additional source 13–15).

3.2.6. Small molecules

Three small-molecule compounds were shown to be efficacious in 'unsilencing' the paternal *UBE3A* (additional source 16). These compounds appear to have better safety profiles than topoisomerase inhibitors. These compounds are at the discovery stage of development. Further studies to assess the efficacy, bioavailability, and safety profile of these molecules are required.

3.3. Downstream treatments

The types of therapeutic agents discussed above aim to provide definitive treatment for AS via restoration of UBE3A function in neurons. An alternative is to target molecular pathways and effector proteins known to be involved in AS pathophysiology. The goals of these downstream treatments are to restore inhibitory transmission and to improve synaptic function and plasticity. Some other downstream treatments target

Table 3. Downstream treatments.

Compound	Institution or Company	Mechanism of action	Clinical phase	Method of administration	Reference
Gaboxadol (OV101)	Ovid Therapeutics	Tonic inhibition restoration	Did not meet primary endpoint in phase 3 NCT04106557 (NEPTUNE)	Oral	Egawa *et al*., 2012
IGF-2 R ligands	NYU	Improves synaptic growth and maintenance	Preclinical	Subcutaneous	Cruz *et al*., 2021
Cyclic glycine-proline analog (NNZ-2591)	Neuren Therapeutics	Improves synaptic growth and maintenance	Phase 1 NCT04379869 in healthy volunteers	Oral	Additional sources: 19–20
PP2A inhibitor (LB-100)	UC Davis, Lixte	Inhibition of PP2A: improves synaptic function, enhances synaptic plasticity	Phase 1 NCT01837667 as a treatment for adults with solid tumors. Currently assessed for ability to cross blood-brain barrier in NCT03027388	Oral	Wang *et al*., 2019
NSI-189 phosphate	Seneca Biopharma	Improves synaptic function, enhances synaptic plasticity	Phase 2 NCT02695472 as a treatment for major depressive disorder	Oral	Liu *et al*., 2019
SAGE-324	Sage Therapeutics	Improves GABAergic transmission	Phase 2 NCT04305275 as a treatment for essential tremor	Oral	Additional source: 21
Ketone esters (exogenous supplementation)	University of Colorado/ Trumacro Nutrition (Disruptive Enterprises)	Seizures	Phase 2 NCT03644693	Oral	Ciarlone *et al*., 2016 and Herber *et al*., 2020

Abbreviations: IGF-2 R: insulin-like growth factor-2 receptor, NYU: New York University, PP2A: protein phosphatase 2A, UC Davis: University of California Davis, GABA: gamma-aminobutyric acid

pathways associated with specific symptoms such as epilepsy or non-epileptic myoclonus. The compounds, companies or institutions involved, and stages in development are listed in Table 3.

3.3.1. Restoration of tonic inhibition: gaboxadol (OV101)

Preclinical studies in mice deficient in maternal Ube3a showed that the likely cause of motor dysfunction lies in the functional disruption of the cerebellar cortex due to impaired tonic inhibition [49,50]. Electrophysiology of granule cells of cerebellar slices revealed that the γ-aminobutyric acid receptor type A ($GABA_A$)-associated current was significantly decreased into adulthood. In AS mice, levels of the γ-aminobutyric acid transporter GAT1, which is believed to be ubiquitinated by UBE3A, are high, and this results in excessive downregulation of $GABA_A$ receptors [49]. *In vivo* restoration of GABA levels by administration of the compound 4,5,6,7-tetrahydroisothiazole-[5,4-c]-pyridine-3-ol, an extrasynaptic $GABA_A$ agonist resulted in phenotypic rescue [49].

A delta (δ)-GABA receptor positive allosteric modulator, gaboxadol (OV101), was developed with the aim of restoring tonic inhibition for AS patients. In a phase 2 clinical trial (STARS, NCT02996305), gaboxadol was found to be overall safe with only mild to moderate adverse effects [51]. After 12 weeks of treatment, participants who were treated orally with 15 mg of gaboxadol in the evening showed significant overall improvement on the CGI Scale, specifically adapted for AS, as compared to placebo-treated controls. However, there was no significant improvement for participants treated with the higher daily dose of 25 mg of gaboxadol administered in two doses of 10 mg and 15 mg. The researchers suggested that this could be the effect of developed tolerance [51].

A phase 3 clinical trial (NEPTUNE, NCT04106557) was conducted in order to assess the efficacy of oral gaboxadol administered once daily. This was a randomized, double-blind, placebo-control trial using a revised AS-specific CGI, as a primary endpoint. A total of 97 AS patients participated. In December 2020, it was announced that the primary endpoint was not met and that no significant changes were observed in the secondary outcome measures (press release, additional source 17).

3.3.2. Agents to improve synapse growth, maintenance, and function

3.3.2.1. Insulin-like growth factors. Insulin-like growth factors IGF-1 and IGF-2 are important for the development, growth, and maintenance of synapses in the CNS [52,53]. In preclinical studies, an IGF-1 analogue (NNZ-2566) was ineffective (additional source 18). However, it was recently shown that subcutaneous administration of mannose-6-phosphate (M6P) and IGF-2, the ligands for the IGF-2 receptor (IGF2R), can significantly improve motor dysfunction, cognitive impairment, and memory in mice deficient in maternal Ube3a due to a null mutation [21]. Additionally, mice treated with IGF-2 showed a decrease in acoustically induced seizures [54].

3.3.2.2. Cyclic glycine-proline (NNZ-2591). Cyclic glycine-proline (cGP) is a naturally occurring metabolite of IGF-1 that regulates the bioavailability of IGF-1 [55,56]. NNZ-2591 is a synthetic analogue of cGP that has a longer half-life and improved bioavailability [57]. According to presentations at the FAST Summit (additional source 19), the 6-week treatment of AS mice with NNZ-2591 resulted in significant improvement in motor and cognitive deficits and decreased their seizure activity. Phase 1 clinical trial (NCT04379869) data showed no safety concerns in healthy volunteers in Australia (press release, additional source 20). A phase 2 clinical trial for efficacy in AS, Phelan-McDermid syndrome, and Pitt Hopkins syndrome patients is planned.

3.3.2.3. Protein phosphatase 2A inhibitor (LB-100).

Phosphotyrosyl phosphatase activator (PTPA), an activator of the protein phosphatase 2 (PP2A), is a substrate of UBE3A, and, therefore, some AS patients have abnormally high PP2A activity. The UBE3A-PTPA-PP2A signaling pathway is crucial during development for both the morphogenesis of the dendritic spine and the function of excitatory synapses [58]. Both the genetic decrease of the PTPA and the pharmacological inhibition of the PP2A restored the dendritic spine morphology in AS mouse models. Evaluation of brain slices from AS mice, which were treated with the PP2A inhibitor LB-100, showed enhanced synaptic transmission in the primary motor cortex compared to untreated mice [21,58]. Additionally, intraperitoneal injections of LB-100 into these mice led to significant improvement in muscle strength, motor coordination, and learning after 14 days. LB-100 was found to be safe in a phase 1 clinical trial (NCT01837667) as a treatment for adults with solid tumors [59]. This small molecule is currently being tested for its ability to cross the blood-brain barrier in patients with brain tumors (NCT03027388). LB-100 is currently in preclinical development for AS.

3.3.2.4. NSI-189 phosphate.

NSI-189 phosphate, a benzylpiperazine-aminopyridine, is a neuroprotective agent which was also shown to stimulate neurogenesis both *in vitro* in human hippocampus-derived neural stem cells and *in vivo* in murine hippocampus [60,61]. In a phase 2 clinical trial for the treatment of major depression disorder (NCT02695472), NSI-189 had both antidepressant and procognitive effects [62,63]. The therapeutic potential of NSI-189 for AS has been tested in preclinical studies. Electrophysiology of hippocampal slices from AS mice [22], which were treated with NSI-189, showed improved theta burst stimulation-induced LTP at the CA1 region [22,64]. Further, those treated for 16 days demonstrated improved learning and memory functions, as assessed with fear conditioning. Within 5 days of treatment with the NSI-189-treated AS mice had improved motor function and their performance on treatment even exceeded that of the wild type mice at the highest doses. With a few days of treatment, the effects persisted for more than 3 weeks, even though the half-life of the compound is about 2 hours in mice. Mechanistically, these changes are believed to be mediated by the TrkB-Akt pathway, which is known to be involved in synaptic plasticity. Changes likely require gene transcription, which probably accounts for the time-dependence of the effects [64].

3.3.3. Treatment of symptoms

3.3.3.1. SAGE-324.

SAGE-324 is a positive allosteric modulator of the GABA receptor with a long half-life, which has the potential to improve disrupted GABAergic transmission and to treat symptoms of AS, such as epilepsy and non-epileptic myoclonus. Its efficacy is being tested for a broad spectrum of neurological conditions presenting with essential tremor, like Parkinson's disease (additional source 21). The compound is currently in phase 2 clinical trial, being administered orally to participants with essential tremor (NCT04305275). Participants are being assessed by The Essential Tremor Rating Assessment Scale, a validated rating method for essential tremor.

3.3.3.2. Ketone esters.

The increase of ketones by restriction of carbohydrates to less than 10 grams per day has been successfully used for intractable epilepsy, including AS patients [65]. A low glycemic index treatment, which focuses more on the glycemic indices of consumed carbohydrates, showed that restriction of low glycemic carbohydrates to 40–60 grams per day for AS patients was beneficial for the management of seizures [66]. A sustainable alternative to a ketogenic diet, with better-expected compliance, is the supplementation with ketone esters. In preclinical studies, this has significantly improved the seizure burden, behavioral phenotype, and hippocampal synaptic plasticity in AS mice [67]. A formulation for exogenous supplementation with the ketone ester beta-hydroxybutyrate was assessed in a phase 2 clinical trial [68].

4. Conclusion

At least 15 therapeutic approaches with potential to treat AS are currently at preclinical and clinical development stages. Among them, two ASOs and five downstream treatment approaches are in early clinical development. Gene replacement approaches and cell therapy are currently in late preclinical development. Recently, a compound aiming to restore tonic inhibition failed to meet the primary endpoint in a phase 3 clinical trial. There is still no available disease-modifying treatment for AS. However, we believe that in the next few years, several candidates will be in clinical development simultaneously for AS.

5. Expert opinion

The number of preclinical and clinical developments for AS is impressive. More than five disease-modifying treatments will be in clinical development in 2022–2023. In comparison, three clinical trials were underway in 2016 for spinal muscular atrophy (SMA), which is considered to be the paradigm of a rare disease with multiple simultaneous therapeutic developments [69,70]. Taking into consideration the rarity of the disease, it is expected that patients' availability will become an obstacle for later or less promising clinical trials.

AS is a monogenic disorder for which genetic therapies, such as ASOs or viral-mediated gene replacement therapies, have the potential to be disease-modifying. The potential impact of the first ASO in clinical development for AS, GTX-102, appears promising. However, participants experienced serious adverse events at the highest doses tested, which were associated with inflammation of the meninges and the nerve roots in the region of the intrathecal administration, but were ultimately resolved (press release, additional source 9). Intrathecal administration of ASOs has been demonstrated to be safe in the cases of SMA and amyotrophic lateral sclerosis [71–73]. With regards to safety, the main advantage of ASOs is their high specificity by which 'off-target' effects can be avoided. In contrast, topoisomerase inhibitors and genome engineering approaches might be efficient in 'unsilencing'

the paternal copy but are potentially less safe from this perspective.

A viral-mediated *ex vivo* gene therapy (cell therapy) using AAV is presently at late preclinical development, close to clinical testing. This will be the first time for a therapeutic approach of its kind to be tested for AS. A major challenge when translating results of viral-mediated gene therapies from mice and non-human primates to humans is dose scaling; this is particularly challenging for AS, as UBE3A is required throughout the brain and the threshold of expression needed for phenotypic rescue remains unknown. The most straightforward routes of administration for such therapies in the case of AS are those directed into the subarachnoid space, intrathecally via lumbar puncture or via intra-cisterna magna injection, and intracerebroventricularly. Certainly, improved bioavailability in the CNS can be achieved using these routes; however, they are highly interventional, especially considering the likelihood that redosing will be required. Nevertheless, AAVs have a good transduction capability with neurons. Additionally, their genome usually remains as extrachromosomal episomes in transduced cells and does not incorporate into the host genome [74]. This is reassuring from a safety perspective but raises concerns for the durability of expression. In contrast, the upcoming *ex vivo* gene therapy presents the advantage of being a permanent treatment: by using lentiviral programming, the *UBE3A* gene is integrated into the chromosomes of the MSCs and therefore is copied with cell divisions.

Major challenges related to host immune response, inflammation, and subsequent cytotoxicity are expected not only in the clinical development of both ASOs and gene replacement therapies. Even though AAVs are considered to have a better immunogenicity profile compared to other viral vectors (such as adenoviruses), their safety needs to be determined. So far, the only approved gene replacement therapy for a pediatric neurological disorder is onasemnogene abeparvovec for SMA. In an animal study of non-human primates and piglets, using the same AAV serotype and gene therapy construct as onasemnogene abeparvovec, it was demonstrated that its administration led to the degeneration of the dorsal root ganglia cell bodies and their axons [75]. Following this, the FDA placed a hold on the clinical trial of the intrathecal form of onasemnogene abeparvovec (press release, additional source 22). The addition of miR183 targets in the vectors could help reduce transgene expression and, therefore toxicity, in the dorsal root ganglia [76]. This approach has the potential to achieve better transduction in the brain without the rate-limiting step of dorsal root ganglia toxicity.

Preclinical studies have been conducted in animal models with mutations or deletions of the maternal gene. Patients with genotypes other than maternal mutation or deletion of *UBE3A* must be carefully enrolled in clinical trials, by taking into consideration the mechanism of action of the therapy under testing. For example, in cases of paternal uniparental disomy, strategies that aim to 'unsilence' the paternal copy could theoretically lead to expression of UBE3A protein to toxic levels from both gene copies. The main advantage of downstream treatments is that they are theoretically active on all genotypes with the same toxicology package. Although none of these downstream treatments will provide a definite therapy for AS, we expect that they will improve symptoms and quality of life in combination with the upstream treatment approaches.

Currently, the identification of appropriate outcome measures with the potential to serve as endpoints in clinical trials remains one of the main unmet needs for AS. In order to prepare for such clinical trials, the AS community established the AS Biomarker and Outcome Measure Consortium (ABOM) to ensure that progress is made with this requirement. Furthermore, natural history studies have been initiated to collect longitudinal baseline data from AS patients, as well as create an easily accessible environment for clinical trial execution: one study led by Boston Children's Hospital of Harvard University (USA) has been running for more than 8 years; another is presently being initiated at the University of Oxford (UK). Additionally, as a precursor to clinical trials, two non-interventional biomarker studies have been set up. The first (NCT04103333), which is focusing on the identification of cerebrospinal fluid biomarkers, is currently underway. The second (FREESIAS study), which aims to identify outcome measures with potential to become endpoints in upcoming clinical trials, is focusing on a variety of domains including sleep, seizures, independent self-care, and expressive communication. Measures under investigation include home-based electroencephalography (EEG) and sleep monitoring. Data analysis is currently underway for this fully enrolled and closed study.

Innovative and AS-specific outcomes are currently at validation stages. One example is the ORCA tool developed by Duke University to evaluate the communication domain for AS. EEG biomarkers, such as delta frequency, are also under investigation [77]. Furthermore, spontaneous movement measurements, captured using magneto-inertial technology, were demonstrated to be very precise and sensitive outcome measures in Duchenne muscular dystrophy and SMA populations [78,79]. Preliminary, and very encouraging, data were likewise obtained for AS patients (manuscript under review). These methods can provide clinical investigators with a platform of digital outcomes that could be used at different stages of clinical development.

The recent developments in SMA have demonstrated that drugs which bring minor but significant benefits in postsymptomatic patients can be transformative when administered before the onset of symptoms [80], prompting the addition of SMA to newborn screening programs in a number of countries [81]. A similar concept of a critical 'time-window' for intervention has been proposed for AS based on data from animal studies [80,82,83], at least for some physiological functions [37]. However, SMA is a neurodegenerative condition and the recent clinical data reported after GTX-102 treatment would support the conclusion that by contrast, in AS there is potential for meaningful changes in patients of varied ages, and therefore, the concept of a critical developmental 'time-window' must be considered individually (press release, additional source 9). Regardless, the development of newborn screening methods will be of crucial importance, as this will allow trials to be conducted in presymptomatic patients.

Acknowledgments

We would like to thank Dr Allyson C. Berent for her contribution in reviewing this paper. Figures are original and were created with BioRender.com under paid subscription.

Funding

This work was supported by the Foundation for Angelman Syndrome Therapeutics (FAST) UK and the Onassis Foundation, of which T Markati is a Scholar (Scholarship ID: F ZQ 040-1/2020-2021).

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Williams CA, Driscoll DJ, Dagli AI. Clinical and genetic aspects of Angelman syndrome. Genet Med. 2010;12(7):385–395.
2. Thibert RL, Larson AM, Hsieh DT, et al. Neurologic manifestations of Angelman syndrome. Pediatr Neurol. 2013;48(4):271–279.
3. Larson AM, Shinnick JE, Shaaya EA, et al. Angelman syndrome in adulthood. American Journal of Medical Genetics Part A. 2015;167 (2):331–344.
4. Bird LM. Angelman syndrome: review of clinical and molecular aspects. The Application of Clinical Genetics. 2014;93. DOI:10.2147/TACG.S57386
5. Dagli A, Buiting K, Williams CA. Molecular and clinical aspects of Angelman syndrome. Mol Syndromol. 2012;2(3–5):100–112.
6. Kyllerman M. Angelman syndrome. In: Handbook of clinical neurology. 2013;111:287-290.
7. Sadhwani A, Wheeler A, Gwaltney A, et al. Developmental skills of individuals with angelman syndrome assessed using the bayley-III. J Autism Dev Disord. 2021. 10.1007/s10803-020-04861-1.
8. Wheeler AC, Sacco P, Cabo R. Unmet clinical needs and burden in Angelman syndrome: a review of the literature. Orphanet J Rare Dis. 2017;12(1). 10.1186/s13023-017-0716-z
9. Kishino T, Lalande M, Wagstaff J. UBE3A/E6-AP mutations cause Angelman syndrome. Nat Genet. 1997;15(1):70–73.
10. Matsuura T, Sutcliffe JS, Fang P, et al. De novo truncating mutations in E6-Ap ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nat Genet. 1997;15(1):74–77.
11. Buiting K, Williams C, Horsthemke B. Angelman syndrome — insights into a rare neurogenetic disorder. Nat Rev Neurol. 2016;12(10):584–593.
12. Meng L, Person RE, Beaudet AL. Beaudet AL. Ube3a-ATS is an atypical RNA polymerase II transcript that represses the paternal expression of Ube3a. Hum Mol Genet. 2012;21(13):3001–3012.
•*UBE3A-ATS* mediates the "silencing" of the paternal *UBE3A* copy
13. Meng L, Person RE, Huang W, et al., Truncation of Ube3a-ATS unsilences paternal Ube3a and ameliorates behavioral defects in the angelman syndrome mouse model. PLoS Genet. 2013; 9(12): e1004039.
•Targeting of the *UBE3A-ATS* has the potential to "unsilenced" the paternal *UBE3A* copy.
14. Lee SY, Ramirez J, Franco M, et al. Ube3a, the E3 ubiquitin ligase causing Angelman syndrome and linked to autism, regulates protein homeostasis through the proteasomal shuttle Rpn10. Cell Mol Life Sci. 2014;71(14):2747–2758.
15. Jiang YH, Beaudet AL. Human disorders of ubiquitination and proteasomal degradation. Curr Opin Pediatr. 2004;16(4):419–426.
16. Yang X. Towards an understanding of Angelman syndrome in mice studies. J Neurosci Res. 2020;98(6):1162–1173.
17. Sun J, Liu Y, Zhu G, et al. PKA and Ube3a regulate SK2 channel trafficking to promote synaptic plasticity in hippocampus: implications for angelman syndrome. Sci Rep. 2020;10:9824.
18. Martínez-Noël G, Luck K, Kühnle S, et al. Network Analysis of UBE3A/E6AP-associated proteins provides connections to several distinct cellular processes. J Mol Biol. 2018;430(7):1024–1050.
19. Yashiro K, Riday TT, Condon KH, et al. Ube3a is required for experience-dependent maturation of the neocortex. Nat Neurosci. 2009;12(6):777–783.
20. Dindot SV, Antalffy BA, Bhattacharjee MB, et al. The Angelman syndrome ubiquitin ligase localizes to the synapse and nucleus, and maternal deficiency results in abnormal dendritic spine morphology. Hum Mol Genet. 2008;17(1):111–118.
21. Jiang YH, Armstrong D, Albrecht U, et al. Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. Neuron. 1998;21(4):799–811.
22. Baudry M, Kramar E, Xu X, et al. Ampakines promote spine actin polymerization, long-term potentiation, and learning in a mouse model of Angelman syndrome. Neurobiol Dis. 2012;47(2):210–215.
23. Cheron G, Servais L, Wagstaff J, et al. Fast cerebellar oscillation associated with ataxia in a mouse model of angelman syndrome. Neuroscience. 2005;130(3):631–637.
24. Daily JL, Nash K, Jinwal U, et al., Adeno-associated virus-mediated rescue of the cognitive defects in a mouse model for Angelman syndrome. PLoS ONE. 2011; 6(12):e27221.
•• Proof of concept for gene replacement therapy.
25. Cheron G, Servais L, Dan B. Cerebellar network plasticity: from genes to fast oscillation. Neuroscience. 2008;153(1):1–19.
26. Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. Nat Rev Genet. 2020;21(4):255–272.
27. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov. 2019;18:358–378.
28. Sirois CL, Bloom JE, Fink JJ, et al. Abundance and localization of human UBE3A protein isoforms. Hum Mol Genet. 2020;48(4):271–279.
29. Adhikari A, Copping NA, Beegle J, et al. Functional rescue in an Angelman syndrome model following treatment with lentivector transduced hematopoietic stem cells. Hum Mol Genet. Internet]. 2021 [cited 2021 Apr 22]; Available from:https://pubmed.ncbi.nlm.nih.gov/33856035/.
•• Proof of concept for cell therapy.
30. Garcia-Perez L, Ordas A, Canté-Barrett K, et al. Preclinical development of autologous hematopoietic stem cell-based gene therapy for immune deficiencies: a journey from mouse cage to bed side. Pharmaceutics. 2020;12(6):549.
31. Biffi A, Montini E, Lorioli L, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science. 2013;341(6148):1233158.
32. Shemesh E, Deroma L, Bembi B, et al. Enzyme replacement and substrate reduction therapy for Gaucher disease. Cochrane Database Syst Rev. 2015. DOI:10.1002/14651858.CD010324.pub2.
33. Angelini C, Semplicini C. Enzyme replacement therapy for pompe disease. Curr Neurol Neurosci Rep. 2012;12(1):70–75.

34. Dodge A, Willman J, Willman M, et al. Identification of UBE3A Protein in CSF and extracellular space of the hippocampus suggest a potential novel function in synaptic plasticity. Autism Res. 2021;14(4):645–655.
35. Galiveti CR, Raabe CA, Konthur Z, et al. Differential regulation of non-protein coding RNAs from prader-willi syndrome locus. Sci Rep. 2014;4. 10.1038/srep06445.
36. Chamberlain SJ, Brannan CI. The Prader–willi syndrome imprinting center activates the paternally expressed murine Ube3a antisense transcript but represses paternal Ube3a. Genomics. 2001;73(3):316–322.
37. Meng L, Ward AJ, Chun S, et al., Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. Nature. 2015;518 (7539): 409–412.

•• **Proof of concept for the use of antisense oligonucleotides to "unsilence" the paternal *UBE3A* copy**

38. Rinaldi C, Wood MJA. Antisense oligonucleotides: the next frontier for treatment of neurological disorders. Nat Rev Neurol. 2018;14 (1):9–21.
39. Gr??nweller A, Hartmann RK. Locked nucleic acid oligonucleotides: the next generation of antisense agents? BioDrugs. 2007;21(4):235–243.
40. Huang HS, Allen JA, Mabb AM, et al. Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons. Nature. 2012;481(7380):185–189.
41. Powell WT, Coulson RL, Gonzales ML, et al. R-loop formation at Snord116 mediates topotecan inhibition of Ube3a-antisense and allele-specific chromatin decondensation. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(34):13938-13943.
42. Skourti-Stathaki K, Proudfoot NJ. A double-edged sword: r loops as threats to genome integrity and powerful regulators of gene expression. Genes Dev. 2014;28(13):1384–1396.
43. Lee HM, Clark EP, Kuijer MB, et al. Characterization and structure-activity relationships of indenoisoquinoline-derived topoisomerase i inhibitors in unsilencing the dormant Ube3a gene associated with Angelman syndrome. Mol Autism. 2018;9(1). 10.1186/s13229-018-0228-2.
44. King IF, Yandava CN, Mabb AM, et al. Topoisomerases facilitate transcription of long genes linked to autism. Nature. 2013;501 (7465):58–62.
45. Wolter JM, Mao H, Fragola G, et al., Cas9 gene therapy for Angelman syndrome traps Ube3a-ATS long non-coding RNA. Nature.2020; 587(7833): 281–284.

•• **Proof of concept for of CRISPR/Cas9 to "unsilence" the paternal *UBE3A* copy**

46. Schmid RS, Deng X, Panikker P, et al. CRISPR/Cas9 directed to the Ube3a antisense transcript improves Angelman syndrome phenotype in mice. J Clin Investig. 2021; 131(5). DOI: 10.1172/JCI142574

•• **Proof of concept for of CRISPR/Cas9 to "unsilence" the paternal *UBE3A* copy**

47. Sera T. Zinc-finger-based artificial transcription factors and their applications. Adv Drug Deliv Rev. 2009;61(7–8):513–526.
48. Bailus BJ, Pyles B, Mcalister MM, et al. Protein delivery of an artificial transcription factor restores widespread Ube3a expression in an angelman syndrome mouse brain. Mol Ther. 2016;24(3):548–555.
49. Egawa K, Kitagawa K, Inoue K, et al. Decreased tonic inhibition in cerebellar granule cells causes motor dysfunction in a mouse model of angelman syndrome. Sci Transl Med. 2012;4 (163):163ra157–163ra157.
50. Miura K, Kishino T, Li E, et al. Neurobehavioral and electroencephalographic abnormalities in Ube3aMaternal-deficient mice. Neurobiol Dis. 2002;9(2):149–159.
51. Bird LM, Ochoa-Lubinoff C, Tan W-H, et al. The STARS Phase 2 Study: a randomized controlled trial of gaboxadol in angelman syndrome. Neurology 2020 10.1212/WNL.0000000000011409 10.1212/WNL.0000000000011409
52. Werner H, LeRoith D. Insulin and insulin-like growth factor receptors in the brain: physiological and pathological aspects. Eur Neuropsychopharmacol. 2014;24(12):1947–1953.
53. O'Kusky J, Ye P. Neurodevelopmental effects of insulin-like growth factor signaling. Front Neuroendocrinol. 2012;33(3):230-251.
54. Cruz E, Descalzi G, Steinmetz A, et al. CIM6P/IGF-2 receptor ligands reverse deficits in angelman syndrome model mice. Autism Res. 2021;14(1):29–45.
55. Guan J, Gluckman P, Yang P, et al. Cyclic glycine-proline regulates IGF-1 homeostasis by altering the binding of IGFBP-3 to IGF-1. Sci Rep. 2014;4:4388.
56. Guan J, Singh-Mallah G, Liu K, et al. The role for cyclic glycine-proline, a biological regulator of insulin-like growth factor-1 in pregnancy-related obesity and weight changes. J Biol Regul Homeost Agents. 2018;32(3):465–478.
57. Guan J, Zhang R, Dale-Gandar L, et al. NNZ-2591, a novel diketopiperazine, prevented scopolamine-induced acute memory impairment in the adult rat. Behav Brain Res. 2010;210(2):221–228.
58. Wang J, Sen LS, Wang T, et al. UBE3A-mediated PTPA ubiquitination and degradation regulate PP2A activity and dendritic spine morphology. Proceedings of the National Academy of Sciences of the United States of America. 2019;116 (25):12500-12505
59. Chung V, Mansfield AS, Braiteh F, et al. Safety, tolerability, and preliminary activity of LB-100, an inhibitor of protein phosphatase 2A, in patients with relapsed solid tumors: an open-label, dose escalation, first-in-human, phase I trial. Clin Cancer Res. 2017;23 (13):3277–3284.
60. Allen BD, Acharya MM, Lu C, et al. Remediation of radiation-induced cognitive dysfunction through oral administration of the neuroprotective compound NSI-189. Radiat Res. 2018;189(4):345.
61. McIntyre RS, Johe K, Rong C, et al. The neurogenic compound, NSI-189 phosphate: a novel multi-domain treatment capable of pro-cognitive and antidepressant effects. Expert Opin Investig Drugs. 2017;26(6):767–770.
62. Fava M, Johe K, Ereshefsky L, et al. A Phase 1B, randomized, double blind, placebo controlled, multiple-dose escalation study of NSI-189 phosphate, a neurogenic compound, in depressed patients. Mol Psychiatry. 2016;21(10):1483-1484.
63. Papakostas GI, Johe K, Hand H, et al. A phase 2, double-blind, placebo-controlled study of NSI-189 phosphate, a neurogenic compound, among outpatients with major depressive disorder. Mol Psychiatry. 2020;25(7):1569–1579.
64. Liu Y, Johe K, Sun J, et al. Enhancement of synaptic plasticity and reversal of impairments in motor and cognitive functions in a mouse model of Angelman Syndrome by a small neurogenic molecule, NSI-189. Neuropharmacology. 2019;144:337–344.
65. Evangeliou A, Doulioglou V, Haidopoulou K, et al. Ketogenic diet in a patient with Angelman syndrome. Pediatr Int. 2010;52(5):831–834.
66. Thibert RL, Pfeifer HH, Larson AM, et al. Low glycemic index treatment for seizures in Angelman syndrome. Epilepsia. 2012;53 (9):1498–1502.
67. Ciarlone SL, Grieco JC, D'Agostino DP, et al. Ketone ester supplementation attenuates seizure activity, and improves behavior and hippocampal synaptic plasticity in an Angelman syndrome mouse model. Neurobiol Dis. 2016;96:38–46.
68. Herber DL, Weeber EJ, D'Agostino DP, et al. Evaluation of the safety and tolerability of a nutritional Formulation in patients with ANgelman Syndrome (FANS): study protocol for a randomized controlled trial. Trials. 2020;21(1). DOI:10.1186/s13063-019-3996-x.
69. Ramdas S, Servais L. New treatments in spinal muscular atrophy: an overview of currently available data. Expert Opin Pharmacother. 2020;21(3):307–315.
70. Servais L, Baranello G, Scoto M, et al. Therapeutic interventions for spinal muscular atrophy: preclinical and early clinical development opportunities. Expert Opin Investig Drugs. 2021; Internet]. [cited 2021 Apr 22];1–9. Available from: https://pubmed.ncbi.nlm.nih.gov/33749510/.
71. Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus sham control in infantile-onset spinal muscular atrophy. N Engl J Med. 2017;377(18):1723–1732.

72. Mercuri E, Darras BT, Chiriboga CA, et al. Nusinersen versus sham control in later-onset spinal muscular atrophy. N Engl J Med. 2018;378(7):625–635.
73. Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1–2 trial of antisense oligonucleotide tofersen for SOD1 ALS. N Engl J Med. 2020;383(2):109–119.
74. Deyle DR, Russell DW. Adeno-associated virus vector integration. Curr Opin Mol Ther. 2009;11(4):442–447.
75. Hinderer C, Katz N, Buza EL, et al. Severe toxicity in nonhuman primates and piglets following high-dose intravenous administration of an adeno-associated virus vector expressing human SMN. Hum Gene Ther. 2018;29(3):285–298.
76. Hordeaux J, Buza EL, Jeffrey B, et al. MicroRNA-mediated inhibition of transgene expression reduces dorsal root ganglion toxicity by AAV vectors in primates. Sci Transl Med. 2020;12(569):eaba9188.
77. Sidorov MS, Deck GM, Dolatshahi M, et al. Delta rhythmicity is a reliable EEG biomarker in Angelman syndrome: a parallel mouse and human analysis. J Neurodev Disord. 2017;9:article number:17.
78. Lilien C, Gasnier E, Gidaro T, et al. Home-based monitor for gait and activity analysis. J Visualized Exp. 2019(150). doi:10.3791/59668.
79. Annoussamy M, Seferian AM, Daron A, et al. Natural history of Type 2 and 3 spinal muscular atrophy: 2-year NatHis-SMA study. Ann Clin Transl Neurol. 2020;8(2):359–373.
80. Dangouloff T, Servais L. Clinical evidence supporting early treatment of patients with spinal muscular atrophy: current perspectives. Ther Clin Risk Manag. 2019;15:1153–1161.
81. Dangouloff T, Burghes A, Tizzano EF, et al. 244th ENMC international workshop: newborn screening in spinal muscular atrophy May 10–12, 2019, Hoofdorp, The Netherlands. Neuromuscul Disord. 2020;30(1):93–103.
82. Silva-Santos S, Van Woerden GM, Bruinsma CF, et al. Ube3a reinstatement identifies distinct developmental windows in a murine Angelman syndrome model. J Clin Investig. 2015;125(5):2069–2076.
83. Sonzogni M, Hakonen J, Bernabé Kleijn M, et al. Delayed loss of UBE3A reduces the expression of Angelman syndrome-associated phenotypes. Mol Autism. 2019;10(1). DOI:10.1186/s13229-019-0277-1.

Additional sources

1.https://www.youtube.com/watch?v=EdZGTeoCILY (Gene Therapy – presentation at FAST Summit 2018)
2.https://www.youtube.com/watch?v=RL1lxU1VfU8 (Gene Therapy – presentation at FAST Summit 2018)
3.https://www.youtube.com/watch?v=cSVxoe2XhaQ (Gene Therapy – presentation at FAST Summit 2019)
4.https://www.youtube.com/watch?v=ePkGrb8UeTA (Gene Therapy – presentation at FAST Summit 2020)
5.https://www.youtube.com/watch?v=3qbqRfrlUdE (Gene Therapy – presentation at FAST Summit 2020)
6.https://www.youtube.com/watch?v=OxEVjlG4oGQ (Cell Therapy – presentation at FAST Summit 2019)
7.https://www.youtube.com/watch?v=xy5jnaL-cYY (Cell Therapy – presentation at FAST Summit 2020)
8.https://cureangelman.org/pilot-feasibility-of-an-enzyme-replacement-therapy-for-as (ERT)
9.https://ir.ultragenyx.com/news-releases/news-release-details/genetx-and-ultragenyx-announce-presentation-phase-12-data (press release for GTX-102)
10.https://forpatients.roche.com/en/trials/neurodevelopmental-disorder/angelman-syndrome/a-study-to-investigate-the-safety–tolerability–pharma-19556.html (RO7248824 or RG6091)
11.https://angelmansyndromenews.com/news-posts/2021/04/14/ionis-plans-to-launch-clinical-trial-this-year-ion582-angelman-syndrome/ (ION582)
12.https://cureangelman.org/the-difference-between-cas13-and-cas9-in-angelman-syndrome-research (CRISPR/Cas13)
13.https://tayshagtx.com/pipeline/ (shRNA)
14.https://www.angelman.org/for-parents/angelman-therapies/ (shRNA)
15.https://cureangelman.org/fast-introduces-research-program-for-development-of-potential-mirna-therapeutic (miRNA)
16.https://www.angelman.org/research/pilot-study-to-validate-three-novel-classes-of-small-molecules-to-unsilence-paternal-UBE3A-allele/ (small molecules)
17.https://www.globenewswire.com/news-release/2020/12/01/2137913/0/en/Ovid-Therapeutics-Announces-Phase-3-NEPTUNE-Clinical-Trial-of-OV101-for-the-Treatment-of-Angelman-Syndrome-Did-Not-Meet-Primary-Endpoint.html (press release for OV101/gaboxadol)
18.https://cureangelman.org/toward-therapeutics-the-fire-team-series-part-iv (IGF-1 analog)
19.https://www.youtube.com/watch?v=_AV-SKR6nJw (NNZ-2591 – presentation at FAST Summit 2019)
20.https://www.neurenpharma.com/irm/PDF/29bba6b7-7f35-4107-9c8b-27833803e8dc/SuccessfulPhase1trialforNeuren39sNNZ2591 (press release for NNZ-2591)
21.https://investor.sagerx.com/news-releases/news-release-details/sage-therapeutics-announces-clinical-updates-and-progress-across (SAGE-324)
22.https://www.novartis.com/news/media-releases/novartis-announces-avxs-101-intrathecal-study-update (press release for onasemnogene abeparvove)