

# Improving Memory and Cognition in Individuals with Down Syndrome

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**Abstract** Down syndrome (DS), often due to trisomy 21, is the most common genetic cause of intellectual disability (ID). In addition, virtually all individuals with DS develop the neuropathology of Alzheimer's disease (AD) by the age of 40 years and almost 60 % will manifest symptoms of AD dementia by the age of 65 years. Currently, there are no pharmacological treatments available for ID in individuals with DS and only limited symptomatic treatments for AD dementia. Advances in our understanding in both the molecular basis of ID and the pathogenesis of AD have created opportunities to study potential therapeutic targets. Recent studies in animal models of DS continue to provide a rational basis for translating specific compounds into human clinical trials. However, target and compound selection are only initial steps in the drug development pathway. Other necessary considerations include appropriate study designs to assess efficacy in the DS population, as well as operational aspects specifically tailored to assess cognition in this population. We discuss recent progress in the development of compounds for both ID and AD in individuals with DS, as well as concepts for the design and conduct of clinical trials with such compounds.

## Key Points

Recent advances in understanding of the molecular basis of intellectual disability (ID) and Alzheimer's disease (AD) in Down syndrome (DS) have created an array of potential therapeutic targets.

The two main therapeutic strategies that have been examined for DS in recent years include restoration of synaptic plasticity to treat ID and modification of the amyloid cascade for the treatment of AD.

Longitudinal studies of aging are ongoing to better understand biomarker changes across the lifespan in anticipation of disease-modifying clinical trials.

## 1 Introduction

In the USA, approximately 5000 children are born with Down syndrome (DS) (1/792 live births) per year [1] and at least 250,000 people are living with DS [2]. With improvements in medical care, the number of older adults with DS in the USA is increasing dramatically, just as in the general population.

People with DS display a variety of characteristic physical and behavioral features, as well as cognitive abilities that can vary from mild to profound in intellectual disability (ID) [3, 4]. In addition, with increasing age, people with DS are at increased risk of developing dementia. The cognitive phenotype of DS can therefore be characterized by two distinct conditions, one being a life-long ID that manifests during childhood and the second being the emergence of dementia due to Alzheimer's

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disease (AD) in older adulthood. Different therapeutic strategies will be required for each condition due to their differing etiologies. The two main strategies that have been examined in recent years include restoration of synaptic plasticity to treat ID and modification of the amyloid cascade for the treatment of AD.

## 2 Intellectual Disability in Down Syndrome

DS is associated with intellectual abilities that range from only slightly below average for the general population to profound disability [4, 5]. One key animal model for DS, the Ts65Dn mouse, has been extensively studied to better elucidate the neural mechanisms, including perturbations in neurotransmission thought to underlie the intellectual disabilities seen in individuals with DS [6].

Particularly well-characterized phenotypes in the Ts65Dn mouse are deficits in learning, memory, object recognition, and fear conditioning [7]. The ID in individuals with DS has been proposed to be linked to an imbalance in neurotransmitter function, specifically excess gamma-aminobutyric acid (GABA) activity and therefore enhanced synaptic inhibition. Electrophysiological abnormalities reflect concurrent alterations in GABA-ergic and glutamatergic transmission in the hippocampus [8]. A number of studies have demonstrated diminished synaptic long-term potentiation (LTP) and increased long-term depression (LTD) in the Ts65Dn mouse leading to an enhanced inhibitory tone within neuronal circuits in areas of the brain subserving memory, including the hippocampus [9–12].

Administration of chronically low doses of GABA<sub>A</sub> receptor antagonists to Ts65Dn mice resulted in normalization of learning and memory impairments as well as synaptic plasticity [13]. This and related work [14] set the stage for human studies to improve synaptic function in DS. One of the first such human studies to target the GABA-ergic imbalance in DS was the phase I multi-center, randomized, double-blind, placebo-controlled, multiple-dose study that investigated the safety and tolerability of the GABA<sub>A</sub>  $\alpha 5$  antagonist, RG1662 (NCT01436955). The study started in November of 2011 and was completed in September 2013. Participants ( $N = 35$ ) aged 18–30 years were treated for approximately 38 days with 16 weeks' safety follow-up. The primary outcome measure was safety and tolerability, that is, incidence of adverse events. The secondary outcome measures included pharmacokinetics and performance on a brief neurocognitive battery.

This was followed by a multi-center, randomized, double-blind, three-arm, parallel-group, 26-week phase II trial of RG1662 that started in May 2014 (NCT02024789). This is an international trial with approximately 180 participants

aged 12–30 years; enrollment was completed in early 2016. Primary outcomes are cognition as assessed by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) sub-tests, adaptive behavior as assessed by the Vineland Adaptive Behavior Scales-II (VABS-II) standard scores, and Clinical global impression as assessed by Clinician Rated Global Impression Scale-Improvement (CGI-I). A second study with the same compound is being conducted in approximately 46 children with DS, aged 6–11 years; estimated study completion date is June 2017.

Also targeting the GABA-ergic system is a phase II trial using the GABA<sub>A</sub> receptor antagonist pentylentetrazole, BTD-001. This is a randomized, double-blind, placebo-controlled, parallel-group study being conducted in Australia with approximately 90 subjects with DS aged 13–35 years, with a 12-week treatment period and a 4-week follow-up.

Postsynaptic targets of locus coeruleus (LC) axons, such as in the hippocampus, are rich in  $\beta 1$ -adrenergic receptors and have been shown to be markedly reduced in both DS and AD [15, 16]. Substantial defects in the noradrenergic system have also been reported in the Ts65Dn mouse, including the hippocampus [17]. A significant improvement in contextual fear conditioning was found after treatment of Ts65Dn mice with droxidopa (1-threo-3,4-dihydroxyphenylserine; L-DOPS), a norepinephrine precursor [18]. In addition, treatment with the  $\beta 1$ -adrenergic receptor agonist xamoterol rescued some of the learning deficits in Ts65Dn mice [19]. These results suggest that behavioral deficits observed in the Ts65Dn mouse may be mediated by an imbalance in the noradrenergic system and be potentially reversible. Human trials are being considered.

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), has been shown in animal models to stimulate learning and memory, promote hippocampal neurogenesis and synaptogenesis, and reduce levels of GABA-ergic inhibition [20–23]. Chronic treatment with fluoxetine administered in drinking water to adult Ts65Dn mice normalized GABA release and promoted recovery of spatial memory abilities and hippocampal synaptic plasticity [24]. The defects in dendritic hypotrophy and neuronal connectivity, in addition to neurogenesis, were further studied by Guidi et al. [25]. Here, pregnant Ts65Dn females were treated with fluoxetine from embryonic day 10 until delivery. As compared with untreated Ts65Dn mice, which exhibit a severe reduction in neurogenesis throughout the developing brain, the embryonically treated Ts65Dn mice had neurogenesis nearly fully restored throughout all brain regions. Moreover, treatment during the embryonic stage restored brain volume in the Ts65Dn mice to a volume similar to that of normal controls.

A pilot clinical study is being planned at the University of Texas Southwestern Medical Center; 21 pregnant mothers carrying a fetus with confirmed DS will be randomized 2:1 to fluoxetine:placebo beginning at 13–24 weeks' gestation. Fluoxetine (or placebo) will be taken orally by the mother and initiated at 20 mg/day and increased weekly as tolerated to 80 mg/day and continued throughout pregnancy. The primary outcomes of the study will assess feasibility and safety. Efficacy will be assessed at 6 months, 1 and 2 years, and will be the composite score of the Bayley Scales of Infant Development—third edition.

The *DYRK1A* gene, located on the 21st chromosome is thought to play an important role in the pathogenesis of the ID associated with DS by virtue of its involvement in neuronal differentiation [26]. Its overexpression, as studied in transgenic mice (TgDyrk1A) is thought to lead to cognitive impairment and deficient synaptic architecture [27]. Epigallocatechin gallate (EGCG) is a flavonoid and natural antioxidant found in green tea and has been shown to be a potent DYRK1A inhibitor [28, 29]. A phase II clinical trial involving 31 participants with DS aged 14–29 years enrolled in a 6-month, placebo-controlled study of oral EGCG (9 mg/kg/day) found significant effects on episodic memory function after 3 months of treatment as compared with placebo [30]. These participants had moderate ID (IQ ~45) at baseline and were enrolled from a large cohort of outpatients in the “Fundació Catalana Síndrome de Down” program in Barcelona, Spain. The primary outcome measures were pattern recognition memory (PRM), Fuld object memory evaluation, and paired-associate learning (PAL) as well as plasma homocysteine, NAD(P)H: quinone oxidoreductase activity and DYRK1A gene expression in lymphocytes. Overall, EGCG was well tolerated. Two participants experienced side effects: one in the EGCG group presented increased excitability that required withdrawal of treatment and one in the placebo-treated group required dose reduction due to abdominal pain.

### 3 Alzheimer's Disease in Down Syndrome

The triplication of chromosome 21 results in a life-long overproduction of brain amyloid [31]. Overproduction of  $\beta$  amyloid invariably leads to the early development of AD-like pathology in DS [32]. Because of this overproduction of  $A\beta$ , by the age of 40 years, virtually all people with DS show the same neuropathological changes as seen in AD [33]. Moreover, the cholinergic losses seen in brains of individuals with DS are identical to those observed in AD [34]. However, in contrast to sporadic AD, amyloid plaques and neurofibrillary tangles in DS can start developing as early as in the teen years [35]. AD dementia has been

reported to affect at least 60 % of individuals with DS over the age of 65 years [36].

Recent data also indicate AD biomarker changes in DS are similar to those observed in familial and sporadic AD [37]. Results of amyloid positron emission tomography (PET) imaging from individuals with DS are also consistent with those seen in non-DS individuals with AD [38]. Furthermore, as in familial and sporadic AD, presence of the Apo  $\epsilon 4$  allele is associated with greater accumulation of  $A\beta$  protein in the brains of adults with DS [39], and greater risk of an earlier age of onset of dementia [40]. The link between triplication of the *APP* gene and subsequent overproduction of  $A\beta$  leading to AD dementia in DS is further supported by the case of an individual with DS with partial trisomy of chromosome 21 who was disomic for the *APP* gene and who did not develop dementia or any AD pathology [41].

As individuals with DS represent a population at high risk for AD, the launch of longitudinal studies of AD biomarkers in DS, including the DSBI (Down Syndrome Biomarker Initiative) and the NIAD (Neurodegeneration in Aging Down Syndrome), will be important in understanding the natural history study of AD from the pre-symptomatic through dementia stages and will help inform the design of future clinical trials in this, and perhaps the sporadic AD population.

Elevated levels of myo-inositol are also thought to contribute to the cognitive impairment in DS [42]. ELND005 (scyllo-inositol), an endogenous myo-inositol isomer, is hypothesized to reduce amyloid toxicity and regulate myo-inositol levels to improve cognitive function in patients with DS. ELND005 (scyllo-inositol; cyclohexane-1,2,3,4,5,6-hexol) has also been evaluated as a potential disease-modifying treatment of AD. In preclinical studies, ELND005 has shown amyloid anti-aggregation effects in vitro, protective effects on oligomer-induced neuronal toxicity, and positive effects on learning in animal models of AD [43–45]. ELND005 has shown both amyloid- and myo-inositol-lowering effects in cerebrospinal fluid (CSF) and brain, respectively, in patients with AD [46]. At a dose with acceptable long-term safety, ELND005 showed beneficial trends on cognition in mild AD [46]. A phase II randomized, double-blind, placebo-controlled study of oral ELND005 in 26 individuals with DS (mean age 28 years) without dementia was recently completed [47]. The compound was well-tolerated, and results are expected to be published later this year.

ACI-24 is a new-generation liposomal vaccine that is designed to elicit an antibody response only against aggregated  $A\beta$  peptides without concomitant pro-inflammatory T-cell activation [48, 49]. ACI-24 is based on the truncated  $A\beta 1-15$  sequence, which is devoid of T-cell epitopes located closer to the peptide's C-terminus. The

peptide sequence is anchored into the surface of liposomes in such a way that peptides adopt an aggregated  $\beta$ -sheet structure, forming a conformational epitope.

Previous active vaccines (e.g., AN-1792) elicited a T-cell response, which led to an increased risk of meningoencephalitis [50]. In the view of potentially adverse inflammatory responses caused by a T-cell response, new-generation vaccines have been designed in which the epitopes for T-cell activation have been removed such that there is only a B-cell-mediated or humoral response, with generation of A $\beta$ -specific antibodies only. In addition to ACI-24, these new-generation active vaccines include ACC-001 [51] and CAD-106 [52]. Phase I trials for these approaches showed positive antibody responses with no signs of adverse inflammation [53, 54].

A phase I/II trial of ACI-24 in Europe is ongoing and aims to address safety, immunogenicity, and efficacy. The study is enrolling up to 198 patients aged  $\geq 40$  with mild to moderate AD dementia who have a positive amyloid PET scan and are receiving stable cholinesterase-inhibitor therapy. This double-blind, randomized, placebo-controlled study compares three doses with placebo delivered subcutaneously. Participants are being treated for 1 year and followed-up for 2 more. Primary outcomes include safety and tolerability measures as well as serum titers of anti-A $\beta$ 42 immunoglobulin-G (IgG) antibodies and 1-year change from baseline in cognitive performance on the Neuropsychological Test Battery. As well as clinical endpoints, secondary outcomes include amyloid burden as assessed by PET and biomarker measures such as magnetic resonance imaging (MRI) volumetrics and tau, phospho-tau, and A $\beta$  levels in CSF.

A phase Ib placebo-controlled, multicenter study with ACI-24 for the treatment of AD in individuals with DS was launched in 2016. The study will enroll 24 adults with DS aged 35–45 years and treat them with ACI-24 for 1 year with an additional year of follow-up. Primary endpoints include measures of safety, tolerability, and immunogenicity. Secondary endpoints of this clinical trial are effects on biomarkers of AD pathology, including amyloid burden as measured by amyloid PET, as well as cognitive and clinical function.

#### 4 Designing Clinical Trials for Down Syndrome

The conduct of clinical trials in the DS population raises methodological challenges in studying cognitive functioning. Given the variability in baseline IDs and functioning, assessments in this population require a unique set of considerations. Specifically, demands on the memory, attention, and language ability of individuals with DS must be taken into account for successful and accurate cognitive

assessment and interpretation of results. Moreover, thoughtfully designed cognitive testing sessions using validated instruments with consistent examiners who can develop a familiarity with the study participant and vice versa will be critical.

A number of validated instruments currently exist for assessing cognition and functioning in individuals with DS across many ages. These include the Cambridge Neuropsychological Testing Automated Battery (CANTAB), the Arizona Cognitive Test Battery (ACTB), and the VABS-II. The CANTAB is a computerized assessment with touch-screen technology designed for individuals aged 4–90 years that is normed with alternate versions. Its memory tasks, such as the PAL, spatial recognition, and spatial span subtests, may be particularly well-suited for individuals with DS [55, 56]. The ACTB was developed specifically to assess the cognitive phenotype in DS and includes tests of general cognitive ability and prefrontal, hippocampal, and cerebellar function [57]. In a study of 74 participants with DS and 50 mental age-matched controls, the ACTB provided consistent results across contexts, including home versus clinic, cross-site, and among individuals with a wide range of socio-economic backgrounds and differences in ethnicity, and correlated well with benchmark clinical assessments. The VABS-II has also been used in a number of DS clinical trials [58]. It covers birth to age 90 years, is primarily informant based and takes approximately 60 minutes to administer and score.

An additional consideration that may aid in reducing variance associated with significant individual differences in DS is the double-crossover clinical trial design. The essential feature distinguishing a crossover trial from a conventional parallel-group trial is that each participant serves as his/her own control since they receive study drug and placebo for equal intervals during the course of the study [59]. The crossover design may thus help avoid problems of comparability with regard to confounding variables.

Finally, the delayed-start study design, also called the randomized-start design, may be particularly useful in assessing disease-modifying effects of potential treatments for the pre-symptomatic stage of AD. In delayed-start studies, patients are randomly assigned to start active treatment at the beginning of the study or are placed in a “delayed-start” group that receives a placebo for a period of time before receiving the active treatment. Comparison is then made between the two groups at a later pre-defined point in time [60]. If the active treatment is limited to reducing symptoms, both early-start and delayed-start participants experience the same benefit. If the active treatment is disease-modifying, both groups will benefit, but the delayed-start group will remain temporally separated. This design has been applied successfully in clinical

trials in AD [61] and other neurodegenerative diseases that develop slowly in their early stages [62].

## 5 Conclusions

Over the past decade, we have seen steady progress being made toward understanding the basis of ID and AD in individuals with DS. Animal models are providing deeper understanding of the cellular and molecular basis of ID in DS. With this knowledge comes an array of potential therapeutic targets. As new compounds enter clinical trials targeting these various mechanisms, study design and conduct will need to be further refined. This is also a significant time for AD research in general, and many of the ongoing secondary-prevention studies will provide results as to whether the amyloid hypothesis, postulating that A $\beta$  accumulation is one of the initial events in AD pathology, is correct. Ongoing longitudinal studies of aging will help us better understand AD biomarker changes and power disease-modifying therapy trials in people with DS.

### Compliance with Ethical Standards

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