

# On the Promise of Pharmacotherapies Targeted at Cognitive and Neurodegenerative Components of Down Syndrome

Alberto C.S. Costa

Division of Clinical Pharmacology and Toxicology, Department of Medicine, University of Colorado Denver School of Medicine, and Neuroscience Training Program, University of Colorado Denver, and Intellectual and Developmental Disabilities Research Center, Aurora, Colo., USA

## Key Words

Trisomy 21 • Ts65Dn mice • Drug therapy • Memantine • N-methyl-D-aspartate receptors • Alzheimer's disease • Intellectual disability • Developmental disability

## Abstract

Down syndrome (DS) is the phenotypic consequence of trisomy 21 and is the most common genetically defined cause of intellectual disability. The most complete, widely available, and well-studied animal model of DS is the Ts65Dn mouse. Recent preclinical successes in rescuing learning and memory deficits in Ts65Dn mice are legitimate causes for optimism that pharmacotherapies for cognitive deficits in DS might be within reach. This article provides a snapshot of potential pharmacotherapies for DS, with emphasis on our recent results showing that the N-methyl-D-aspartate receptor antagonist memantine can reverse learning and memory deficits in Ts65Dn mice. Because memantine has already been approved for the therapy of Alzheimer's dementia, we have been able to very rapidly translate these results into human research and are currently conducting a 16-week, randomized, double-blind, placebo-controlled evaluation of the efficacy, tolerability and safety of memantine hydrochloride on enhancing the cognitive abilities of young adults with DS. The design and current status of this clinical trial will be discussed, which will be followed by some speculation on the potential impact of this and future clinical trials in the field of DS.

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## Introduction

Down syndrome (DS) is the most common genetically defined cause of intellectual disability. It has been 52 years since Dr. Jérôme Lejeune discovered that DS is the result of the trisomy of chromosome 21 [1]. Currently, the approximate rate of live births with DS in the United States is 1 in 732, or 5,429 births each year, and the total estimated number of people with DS in this country is roughly 300,000 [2]. Due to a documented increase in the life expectancy of people with DS, projections indicate that this figure is expected to continue increasing; at least in the near future [3]. This population trend certainly reflects improvements in the general health care of individuals with DS. However, until recently, we had not seen a parallel progress in the basic understanding of the pathogenesis of the neuropsychological and neurological components of DS, much less in the development of potential pharmacotherapies.

DS-associated phenotypes affecting the central nervous system include various degrees of intellectual disability (with moderate intellectual disability being the most common outcome), increased incidence of seizure disorder in relation to the general population, motor dysfunction, and a neuropathology indistinguishable from Alzheimer's disease (AD) [4–10]. Over the last 10–20 years, the availability of postnatal viable aneuploid mouse models of DS, our progressively more sophisticated knowledge of the human and mouse genomes, and the

identification of specific neuropsychological traits associated with DS have provided investigators in the field with a realistic opportunity to start bridging the gap between basic and clinical research in DS.

This report will start with a short discussion on the use of the mouse model Ts65Dn as a discovery tool in both basic and translational DS research. Recent results by our research group showing that the AD drug memantine can reverse learning and memory deficits in Ts65Dn mice will be highlighted. This will be followed by a description of our current efforts to translate these findings into a double-blind, placebo-controlled clinical trial to evaluate the efficacy, tolerability, and safety of this drug on enhancing the cognitive abilities of young adults with DS. We will conclude with some thoughts on what one should realistically expect from this and future clinical trials.

### The Ts65Dn Mouse Model of DS

A key event in the DS research field was the generation of the Ts(17<sup>16</sup>)65Dn (more commonly known as Ts65Dn) segmental trisomy mouse model over two decades ago by Dr. Muriel Davison and her research team at The Jackson Laboratory [11]. At that time, the Ts65Dn mouse was the first postnatal viable aneuploid mouse model for DS. Since then, several other aneuploid mouse models for DS have been produced [12–15]. Nevertheless, the Ts65Dn mouse continues to be the most complete mouse model for DS that is widely available to the scientific community. In addition, more than 190 PubMed-indexed publications and more than a dozen book chapters and other non-PubMed-indexed publications featuring the use of this mouse make it, by far, the most extensively studied animal model for DS.

Ts65Dn mice carry a segmental trisomy of the distal portion of mouse chromosome 16 (MMU16) in which many of the genes in human chromosome 21 (HSA21) are conserved [11, 16, 17]. The Ts65Dn trisomic chromosome segment contains approximately 55% of the mouse orthologous protein-coding genes to HSA21 [17]. Although Ts65Dn mice do not present all the features associated with DS (for example, the age-related AD-type pathology), these animals display a remarkably diverse array of DS-like phenotypes. These include significant learning deficits in specific behavioral tasks, craniofacial dysmorphogenesis, motor dysfunction, congenital vascular and intracardiac defects, and age-dependent loss of cholinergic markers in basal forebrain cholinergic neurons (reviewed in Patterson and Costa [16], Rachidi and Lopes

[18], Davidsson and Costa [19] and Seregaza et al. [20]; congenital vascular and intracardiac defects described in Williams et al. [21]).

In addition to recapitulating many features of DS, the Ts65Dn mouse model has also had a significant predictive value. For example, it was the finding of deficits on putatively hippocampus-dependent tasks in Ts65Dn mice that led to the inclusion of hippocampus-dependent measures on a comprehensive neuropsychological battery applied to individuals with DS [22]. This pivotal work revealed a disproportionately large performance deficit in hippocampus-dependent measures by persons with DS compared to typically developing mental age (MA)-matched individuals, which now form the basis for the design of our clinical trial (see below), as well as a similar battery of tests developed by Dr. Lynn Nadel's research team [23] at the University of Arizona. Another interesting example of the predictive power of the Ts65Dn mouse has been the finding of a reduced density of cerebellar granule cells in these mice, which has led to the finding of an analogous pathology in individuals with DS [24].

Because mice share many biochemical and physiological characteristics with us, they can serve as our surrogates for experiments not practically or ethically permissible in human beings [25]. Their small size, short gestation and life span, and ease of genetic manipulation make them an ideal experimental system. Therefore, given the complexity and variability of phenotypes displayed by persons with DS, which poses enormous difficulties for the planning and execution of well-controlled clinical studies, mouse models in DS research are slowly evolving into essential tools for testing potential new therapies in a preclinical setting. However, one should not forget the obvious and not-so-obvious shortcomings of using mouse brain cells, brain tissues, and central nervous system to model their human counterparts. For example, 161 protein-coding genes have been identified in HSA21 [26]. In contrast, there are 101 HSA21 mouse orthologous protein-coding genes located in MMU16. In mouse chromosomes 10 (MMU10) and 17 (MMU17), there are 37 and 19 orthologous genes, respectively. (Note that although a recently published analysis [26] identified 552 putative non-keratin-associated proteins, nonpseudogenes, genes in the long arm of HSA21, we are narrowing the discussion to GenBank RefSeq protein-coding genes for simplicity and because the numbers are less likely to change with time.) This means that creating a mouse that contains 3 copies of all HSA21 orthologous protein-coding genes involves the considerable effort of breeding all 3 chromosome-engineered mice trisomic for each 3 ho-

mologous mouse chromosome segments that were created recently by Yu et al. [27]; the combined mouse is currently named Dp(10)1Yey/+;Dp(16)1Yey/+;Dp(17)1Yey/+. In addition, 3 genes in MMU16 are mouse specific [26], and 4 HSA21 genes are human specific and not found in MMU (which explains why the sum of orthologous genes in the mouse does not add to the total of protein-coding genes in HSA21). This means that even Dp(10)1Yey/+;Dp(16)1Yey/+;Dp(17)1Yey/+ mice still lack some human genes and carry some genes present only in mice. Although more studies are needed to further characterize this new mouse model, the initial work by Yu et al. [27] has shown many phenotypes similar to those described on Ts65Dn mice and no additional, significant DS-like phenotype was found in these animals.

### **First Attempts on Preclinical Research Using the Ts65Dn Mouse**

For many years, research on Ts65Dn mice and other mouse models has focused on validating these animals as models for DS. Recently, however, there has been a steady move toward using these animals in pharmacological rescuing studies aimed at testing preclinically potential therapeutic agents [28–42]. Although a thorough review of such attempts and others involving transgenic models [43] would be beyond the scope of the present study, three studies will be briefly described here due to our research team's direct or indirect involvement in the discovery process and their potential for translation into clinical trials. These studies involve (1) the antidepressant fluoxetine, (2) different GABA<sub>A</sub> receptor antagonists, and (3) the AD drug memantine.

The first study to be discussed is the one performed a few years ago by my research group in collaboration with Dr. Paul Yarowsky at the University of Maryland [33]. In this study, we showed that the chronic use of the antidepressant fluoxetine can pharmacologically rescue the deficiency in basal levels of hippocampal neurogenesis in adult Ts65Dn mice. Such findings are of interest because of the putative relationship between major depression and decreased adult neurogenesis [44] and of reports indicating that depression is the most common psychiatric disorder in adults with DS [45, 46]. Therefore, if the deficit in adult neurogenesis in the Ts65Dn mouse proves to be a reliable surrogate marker for mood disorders in persons with DS, our study could provide some insight into the biological mechanisms underlying the high incidence of depression in individuals with DS. Obviously, more studies will

be needed before we can reach such conclusions, but this is definitely an area of inquiry in DS research worth focusing. More recently, Bianchi et al. [34] have reported that the treatment of neonate (3 days old) Ts65Dn mice for 12 days with fluoxetine restored the expression of 5-HT<sub>1A</sub> receptors and BDNF in the hippocampus, and increased the number of cells with a neuronal phenotype, proliferating precursors, and surviving granule cells in the dentate gyrus of treated Ts65Dn mice versus untreated animals. Our research team had previously showed that Ts65Dn mice display impaired performance in a contextual fear conditioning task [36], which is a behavioral task generally believed to be dependent on the functional integrity of the hippocampus and amygdala [47]. This created the opportunity for Bianchi and colleagues to test fluoxetine-treated Ts65Dn mice on contextual fear conditioning, which they did 1 month after the end of the treatment, and found that the treatment produced a virtually complete rescue of their performance deficit in this behavioral task.

The second example of a successful pharmacological rescuing experiment with Ts65Dn mice involved a collaborative work between Drs. Craig Garner and Robert Malenka's research teams at Stanford University [32]. These authors based their investigations on previous electrophysiological studies of the neuroplasticity of hippocampal slices obtained from Ts65Dn mice. These electrophysiological studies had shown deficits in long-term potentiation in both the dentate gyrus [48] and the CA1 region of the hippocampus proper [49], which could be reversed by acute superfusion of the brain slice with the GABA<sub>A</sub> receptor antagonist picrotoxin. Additionally, these same findings had also led to the hypothesis that such synaptic plasticity deficits were the result of excessive GABA-mediated inhibitory tone. Fernandez et al. [32] then took a further step in proposing that hippocampal-dependent learning and memory deficits seen in Ts65Dn mice might also be explained by a similar mechanism. To test this hypothesis, these investigators designed experiments in which they administered chronically low doses of noncompetitive GABA<sub>A</sub> receptor antagonists to Ts65Dn mice. This treatment elicited a long-lasting (months after treatment) normalization of memory and learning in these mice (as assessed by the animal performance on an object recognition test) and normalization of synaptic plasticity (as assessed by electrophysiological recording of long-term potentiation in the dentate gyrus in Ts65Dn mouse brain slices). Although these results were quite exciting, and may eventually have clinical implications, the need for a chronic administration regimen (instead of the acute drug administration used in the previously men-

tioned electrophysiological studies) stands in stark contrast with the simple hypothesis that the excessive GABA-mediated inhibitory tone is the culprit for the observed behavioral performance deficits. Therefore, these authors have proposed that synaptic function normalization in Ts65Dn mice chronically treated with GABA<sub>A</sub> receptor antagonists may be the consequence of the triggering of neuroadaptive changes in hippocampal circuits, ‘which stably but modestly reset the excitatory/inhibitory balance in the brain’ [50].

The third and last example to be discussed of a successful pharmacological experiment using Ts65Dn mice involves the use of the drug memantine by our research team to rescue learning and memory deficits in Ts65Dn mice [36]. Memantine is an uncompetitive, moderate-affinity N-methyl-D-aspartate (NMDA) receptor antagonist that has been approved in the United States, Canada, Europe, and several other markets for moderate to severe dementia [51]. NMDA receptors are receptor-ion channel complexes that have several features that make them ideally suitable for mediating plastic changes in the brain, such as those occurring during learning [52–54]. Our study originated from the hypothesis that the trisomy of HSA21 (or HSA21 orthologs in the Ts65Dn segment) may alter the functioning of NMDA receptors. This hypothesis had its origins on previous work showing that the increased expression of certain HSA21 gene products (such as RCAN1 and DIRK1A) or simply increased amounts of reactive oxygen species known to exist in the brains of persons with DS may interfere with the activity of the protein phosphatase calcineurin [55]. Calcineurin itself has been shown to modulate NMDA receptor activation kinetics by decreasing channel mean open time and opening probability [56]. In addition, previous studies had shown that conditional calcineurin null-mutant mice display increased responses to the locomotor-stimulating effects of the high-affinity noncompetitive NMDA receptor antagonist MK-801 [57]. Accordingly, in our study, we also noted that Ts65Dn mice displayed an increased sensitivity to the psychotomimetic effects of MK-801. Because MK-801 blocks NMDA receptor by binding to this ion channel in its open state, this observation made us hypothesize that in Ts65Dn mice (and possibly in persons with DS) NMDA receptors may be hyperactive, i.e. that such chromosomal aneuploidies may lead the NMDA receptor/channel to exist in a state of augmented mean open time and/or opening probability in relation to the receptor/channel in the euploid brain. This then led us to reason that, theoretically, a low affinity antagonist, such as the drug memantine, might be able to

normalize the function of NMDA receptors and, perhaps, behavioral performance on specific tests of learning and memory. To test this hypothesis, we chose a contextual fear conditioning task, because this task had previously been shown to be dependent on NMDA receptor activation [58]. In this classical conditioning experiment, mice are exposed to a novel environment (context serving as a conditioning stimulus) for a few minutes and then receive an electrical shock of moderate intensity (unconditioned stimulus). On the following day, mice are re-exposed to the same context. The typical response is that mice will spend a large percentage of their time ‘freezing’, defined as a species-specific defensive reaction, associated with a crouching posture and characterized by lack of movement other than respiration and heartbeat. Such freezing response is considered to be an indicator of learning for rodents [59]. These experiments led us to discover that Ts65Dn mice indeed display impaired performance in this behavioral task, which has since been confirmed by other research teams [34, 37]. Additionally, we found that a single injection of the drug memantine before the first exposure to the conditioning context, and a second memantine injection before the re-exposure to the same environment, increased the percentage of time adult Ts65Dn mice spend freezing during testing to levels statistically indistinguishable from those observed in control mice. To our knowledge, this was the first and only time that a compound injected acutely was capable of producing memory-enhancing effects in Ts65Dn mice. Recently, two different research teams have shown that during chronic administration regimens memantine maintains its memory-enhancing effects on Ts65Dn mice [39, 42]. One of these groups has demonstrated improved spatial learning in the Morris water maze task, reduced brain amyloid- $\beta$  protein precursor levels, and increased hippocampal vesicular glutamate transporter 1 levels [39]. The second research team has shown that memantine treatment rescued novel object recognition in Ts65Dn mice following both chronic and acute delivery [40]. Although we have used molecular information to hypothesize a memory and learning enhancing effect of memantine on Ts65Dn mice, and although our findings and those of these two different groups are consistent with our original hypothesis, with the data available so far, one cannot exclude alternative pathophysiologies or mechanisms of drug action for memantine.

One important message that can be drawn from all three studies mentioned above is that some of the cognitive components of DS might not be immutable and egregiously complex to approach therapeutically. Indeed, in

the case of our study involving the drug memantine, we can even say that some of the cognitive components of DS may carry some of the hallmarks of a more straightforward condition involving NMDA receptor dysfunction. In fact, because of memantine's status as a US FDA-approved drug with very few known side effects, we have already been able to build on our and other preclinical studies and translate them into a pilot clinical trial to study the tolerability and efficacy of memantine in young adults with DS. In the next sections, the rationale for the design of this clinical trial will be discussed.

### **Neurodevelopmental and Neurodegenerative Components of DS**

DS has both neurodevelopmental and neurodegenerative components. The neurodevelopmental components are exemplified by the early onset of intellectual disabilities, a documented early peak of high incidence of seizure disorders, and an apparent decline in cognitive skills affecting individuals with DS in their first years of life [5, 60, 61]. Evidence for late-onset neurodegenerative processes come from the observed loss of cholinergic markers in basal forebrain cholinergic neurons in adults with DS [62, 63] and the universal presence of a neuropathology indistinguishable from AD in the brains of individuals with DS by their fourth decade of life [64, 65]. The high prevalence of early-onset dementia in this population is almost certainly the clinical manifestation of these neuropathologic findings [66]. It was this unique increased risk of AD-like pathology and AD-like dementia in people with DS that first led to the hypothesis that a gene on HSA21 must be involved in AD [67] and to the subsequent demonstration that mutations in a gene that encodes the amyloid precursor protein, *APP*, cause early-onset AD [68]. Presently, however, increased dosage of *APP* is thought to be necessary, but not sufficient for the development of AD-like pathology and dementia in persons with DS [93].

The best data available report the mean intellectual quotient (IQ) of school age children with DS to be in the low to mid 40s [69–71]. Individuals with DS display clear deficits in expressive language, syntactic/morphosyntactic processing, verbal working memory, and digit span [72–76]. Until a decade ago, however, the neuropsychological profile of individuals with DS was thought to faithfully reflect much of the individual's overall level of intellectual disability. The aforementioned work by Pennington et al. [22] has considerably added to this picture. These authors used a comprehensive battery of 18 neuro-

psychological measures and reported particular weakness in hippocampus-dependent function in persons with DS. The hippocampus-dependent measures (all of which require long-term memory) used by these authors were the NEPSY List Learning, the virtual Morris water maze, the CANTAB Pattern Recognition and Paired Associates Learning, and the Ecological Memory Questionnaire. Findings from parallel benchmark measures of verbal and spatial function showed that this work was in general agreement with the DS literature.

The work of Pennington et al. [22] has widely been recognized as groundbreaking to the field of DS. The main criticism to it has been their choice of an MA comparison group in their work. Such experimental design is quite common in the field of intellectual disabilities and is typically put in place to prevent floor effects in the affected group and ceiling effects in the control group due to large differences in test performance between persons with substantial intellectual disability and typically developing peers of similar chronological ages (CAs). It has been argued, however, that this strategy 'implicitly accepts developmental rather than difference theories of intellectual disability' [77, 78]. For example, the average CA of the participants with DS in the work by Pennington et al. was 15 years, compared to approximately 5 years of age for the control group of typically developing MA-matched participants. These groups are obviously qualitatively different in many aspects such as physical development, life experiences, and so forth. Therefore, in recent years, there has been a movement toward the choice of comparison groups comprised of individuals of similar CA and with intellectual disability of different etiologies.

From the point of view of researchers working with animal models, the choice of an MA comparison group also creates considerable confusion in terms of translating the human research results, because, for the casual reader, it obfuscates the global nature of the intellectual disability seen in persons with DS. Different from many neuropsychologists working with persons with DS, researchers working with animal models of DS typically choose comparison groups consisting of CA-matched euploid mice, preferably littermate control animals. This is done because of the somewhat simplistic nature of most behavioral assessments performed in rodents, and the fairly narrow band of possible performance levels in most of such assessments. In turn, such choice of comparison group by mouse researchers masks the fact that, in comparison to the human disorder it is designed to model, Ts65Dn mice and other mouse models of DS have a milder and more selective behavioral phenotype.

## Memantine and the First Translational Clinical Trial in DS

As aforementioned, memantine is an uncompetitive, moderate-affinity NMDA receptor antagonist. At therapeutic doses, this drug is thought to inhibit the pathologic effect of NMDA receptor activation while leaving unaffected NMDA receptor-mediated physiological processes involved in learning and memory [52, 79]. Memantine is predicted to provide both neuroprotection and cognitive improvement by one and the same mechanism, i.e. by restoring the fidelity of synaptic transmission and synaptic plasticity [52]. The bioavailability of memantine is 100%, time to reach peak serum concentration is 3–7 h, its half-life is 60–100 h, it is not metabolized by the liver, and it is completely excreted by the kidneys [79]. In several clinical trials, memantine was found safe and well tolerated [80]. During the European Union approval process, and postmarketing safety experience from Germany, where memantine had been available for more than two decades, more than 100 million daily doses of memantine had been sold (<http://www.emea.europa.eu/humandocs/PDFs/EPAR/axura/094802en6.pdf>). Merz Pharmaceuticals GmbH received spontaneous reports of 73 adverse events in 48 patients. Of those, only the following events were reported in more than 1 patient: nervousness ( $n = 6$ ), convulsions ( $n = 4$ ), tremor ( $n = 3$ ), aggressive reaction ( $n = 3$ ), circulatory failure ( $n = 2$ ), hypertension ( $n = 2$ ), dizziness ( $n = 2$ ), dyskinesia ( $n = 2$ ), nausea ( $n = 2$ ), menstrual disorder ( $n = 2$ ), bullous eruption ( $n = 2$ ), and pruritus ( $n = 2$ ). Recent open-label studies suggest that memantine may be clinically useful and well tolerated in young individuals with other conditions that produce cognitive disabilities, such as autism and attention deficit hyperactivity disorder [81–83].

Because of our preclinical findings and the safety profile of memantine, which is superior to the anticholinesterase drug donepezil that had already been used in persons with DS [84, 85], and the possibility that memantine might indeed delay the onset of AD-type pathology in young adults with DS, we were able to assemble a group of physicians and psychologists who all agreed that a small-scale randomized controlled clinical trial was warranted at present. The main goal of this clinical trial is to assess whether a fairly short drug regimen of memantine can be efficacious in improving at least one subdomain of cognition (i.e. hippocampus-dependent tasks) in adults with DS. We reasoned that, once we prove the principle that DS may indeed be amenable to pharmacological interventions, this might open the doors for several other

types of clinical trials. Such trials could involve, for example, expanding the use of memantine to younger cohorts of participants with DS or a decade-long trial to assess efficacy at the neurodegenerative component of DS. It should be noted that a similar approach has been taken by investigators in other fields of genetically defined entities leading to intellectual disability. For example, findings from a small clinical trial in fragile X syndrome of the Novartis metabotropic glutamate receptor antagonist, AFQ056, have recently been published [86]. Although no significant effects of treatment on the primary outcome measure were found, post hoc analysis of the data showed that a subgroup of 7 of the patients with full fragile X mental retardation gene 1 (*FMR1*) promoter methylation and no detectable *FMR1* mRNA in blood cells exhibited statistically significant improvement on several measures after AFQ056 treatment when compared to placebo.

I am the principal investigator of the study, Drs. Richard Boada, Timothy Benke, and Edward Goldson (University of Colorado) are the co-principal investigators, and Dr. Bruce Pennington (University of Denver) has served as a consultant. Our research protocol has been funded as an Investigator-Initiated Trial by the Forest Research Institute and is titled: ‘A Sixteen-Week, Randomized, Double-Blind, Placebo-Controlled Evaluation of the Efficacy, Tolerability and Safety of Memantine Hydrochloride on Enhancing the Cognitive Abilities of Young Adults with DS.’ A total of 42 persons with DS of both genders and between the ages of 18 and 32 have been recruited locally. It should be noted that, by necessity, the trial participants recruited had to be verbal and capable of understanding the instructions of the trial’s neuropsychological tests, i.e. the participants of this trial function in the moderate and mild ranges of intellectual disability and, hence, do not represent the full range of cognitive functioning displayed by persons with DS.

In this study, we hypothesize that memantine may improve test scores of young adults with DS on hippocampus-dependent measures. Although in a recent work, my research team has demonstrated that we cannot completely exclude a contribution of the amygdala in the production of the deficit in fear conditioning tasks seen in Ts65Dn mice [87], we decided to focus on the hippocampus in this clinical trial because: (1) the amount of contextual fear conditioning deficit in Ts65Dn mice is much greater than the deficit in sound-cued fear conditioning in these animals; (2) disproportional deficit in tasks thought to be dependent on the functional integrity of the hippocampus have been demonstrated in persons with

DS (see below), and (3) the two aforementioned follow-up studies also identified deficits in putatively hippocampus-dependent tasks in Ts65Dn mice.

A blinded randomization protocol is being used. Subjects have been paired according to age and gender. At the baseline visit, subjects are assigned to one of the two treatment regimens (memantine or placebo). The random code assigns subjects to treatments in a 1:1 ratio. At the time of submitting this study, we have concluded the recruitment phase of the trial, 98% of the participants have completed the entire protocol, and we are waiting until the last participant who is currently taking the study medication has finished the protocol to unseal the randomization codes. The primary and secondary measures of the study as well as the safety and tolerability assessments are briefly described below (see NCT01112683 at <http://www.clinicaltrials.gov> for more information).

The primary efficacy measures are aimed at assessing long-term memory, with an emphasis on hippocampus-dependent measures. The choice of appropriate measures for individuals with DS was based on the work by Pennington et al. [22]. We hypothesize that treatment with memantine will produce significant improvements in the following measures: (1) pattern recognition memory (part of the CANTAB battery); (2) paired associates task (also part of the CANTAB); (3) California Verbal Learning Test – Children’s Version, and (4) Rivermead Behavioral Memory Test – Children’s version. These measures are all dependent on temporal lobe (hippocampal formation) function. Improvement in performance in these measures is expected to be correlated to improvements in the individuals’ ability to acquire skills requiring the use of declarative memory. (The main idea being that continuous, long-term administration of memantine may eventually lead to a measurable improvement in the quality of life of persons with DS).

The secondary efficacy measures and benchmark measures are the receptive vocabulary on the Peabody Picture Vocabulary Test-III; the Test for the Reception of Grammar; verbal fluency (from the Developmental Neuropsychological Assessment); recall of digits (Differential Ability Scales); spatial working memory (also part of the CANTAB), and Scales of Independent Behavior Revised.

Safety and tolerability are being monitored by physical examinations, electrocardiograms, comprehensive clinical laboratory tests, and incidence of adverse event recording.

Because of the double-blind nature of the study, we will not be able to analyze efficacy outcomes until all participants have completed the entire protocol. So far, how-

ever, compliance has been outstanding (over 95% for all participants), and the study medication has been well tolerated.

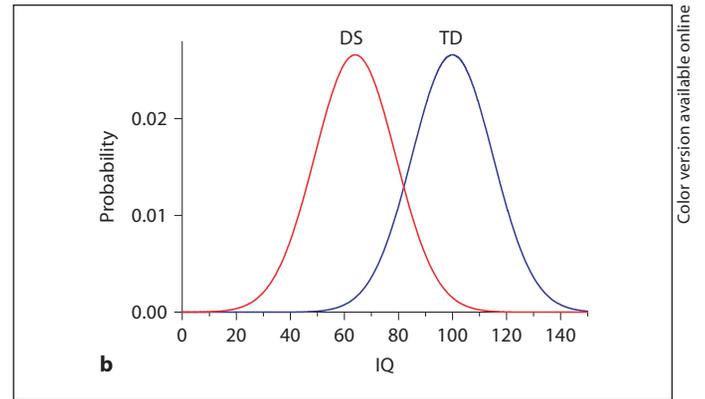
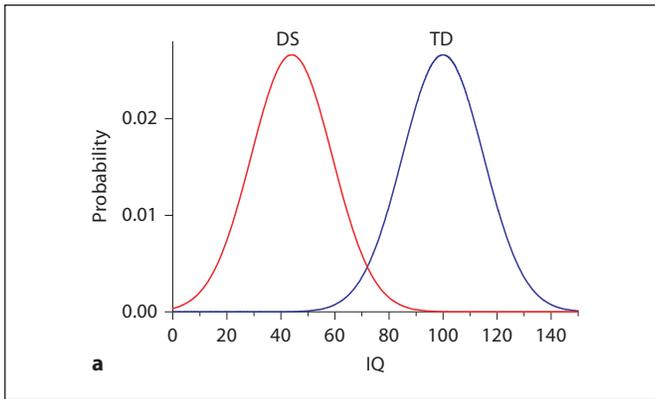
### **What Is the Potential Impact of Pharmacotherapies for DS?**

#### *The Simpleminded Version*

The scenario represented graphically in figure 1 was designed as a mental exercise to illustrate the potential impact of a hypothetical therapy that ultimately resulted in an average 20-point gain in IQ (which is an arbitrary number, intended to reflect a significant, but incomplete cognitive gain toward the IQ range of the general population). First, let us assume a normally distributed population of 300,000 people with DS (which is a conservative estimate of the number of persons with DS in the US), with an  $IQ = 44 \pm 15$  (mean  $\pm$  standard deviation), which are numbers based on educational records in the US and the UK [66, 67]. Mathematically, this results in approximately: 82,000 individuals with  $IQ < 35$  (severe and profound intellectual disability); 148,000 individuals with  $35 < IQ < 55$  (moderate intellectual disability); 57,000 with  $55 < IQ < 70$  (mild intellectual disability); 12,000 individuals with  $70 < IQ < 85$  (learning disability), and 1,000 individuals with  $IQ > 85$  (typically developing). This situation is depicted in figure 1a.

In clinical and educational settings, the IQ intervals chosen above are commonly associated with practical consequences in terms of amount of one-to-one attention and supervision required for raising, educating, and caring for individuals with intellectual disabilities. As a general rule, children and adults functioning in the severe and profound intellectual disability range have very limited spoken language skills. Children and adults functioning in the moderate range of intellectual disability have very limited written language skills. Whereas children and adults functioning in the mild range of intellectual disability may have somewhat limited written and spoken language skills, with significant intervention, these skills can become quite functional and a reasonable level of independence and work productivity can be achieved in adult life. Lastly, individuals with learning disabilities (again, with significant therapeutic and educational intervention) often go on to live lives indistinguishable from that of many of their typical peers.

If we now envision that a hypothetical therapy were to be developed in the field of DS, with the significant, but not completely unimaginable effect of increasing the av-



Color version available online

**Fig. 1.** Normal probability density functions of IQ in the population of typically developing individuals (TD) (mean IQ = 100; standard deviation = 15) and theoretical normal distributions calculated for persons with DS before and after the implementation of a hypothetical pharmacotherapy. **a** Side-by-side comparison of IQ distributions before the implementation of a hypo-

thetical pharmacotherapy (the assumptions for the population of persons with DS are mean IQ = 44; standard deviation = 15). **b** Side-by-side comparison of IQ distributions after the implementation of a hypothetical pharmacotherapy (the assumptions for this population of persons with DS are mean IQ = 64; standard deviation = 15).

erage IQ by 20 points, the resulting numbers (assuming a simple, across-the-board, shift in IQ) would be approximately: 6,000 individuals with  $IQ < 35$ ; 64,000 with  $35 < IQ < 55$ ; 112,000 with  $55 < IQ < 70$ ; 88,000 with  $70 < IQ < 85$ , and 31,000 individuals with  $IQ > 85$ . This hypothetical scenario is depicted in figure 1b. It is easy to see, even from the pure perspective of savings in educational resources and personnel costs, how those results would be very significant. For example, as a consequence of such hypothetical therapy, we would only rarely (<5%) see persons with DS functioning in the severe and profound intellectual disability range. Also, DS would be seen as a genetic disorder associated with a mild (as opposed to moderate) degree of intellectual disability. Finally, because a rather large number of individuals with DS would start to function in the typical range, the expectation of parents and professionals would certainly be enhanced, which, by itself, tends to correlate with better outcomes in educational and daily living skills.

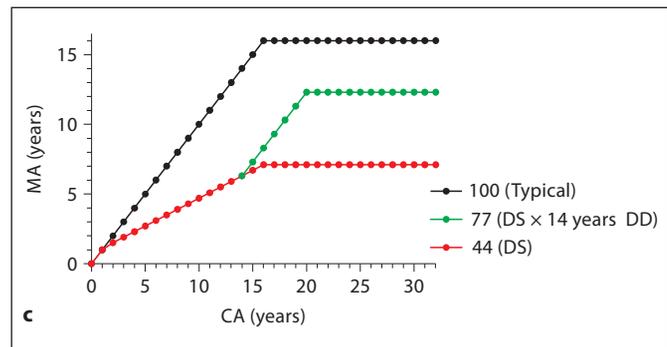
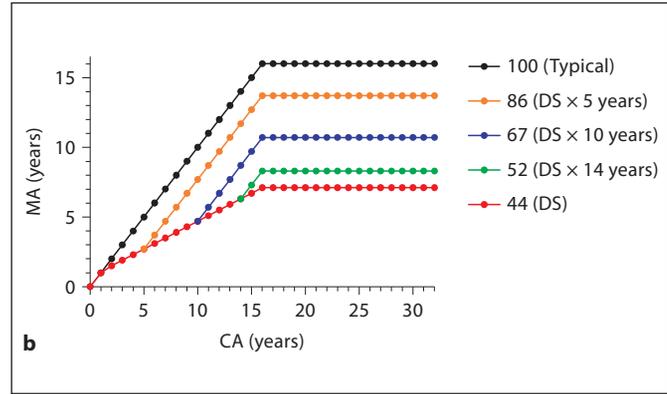
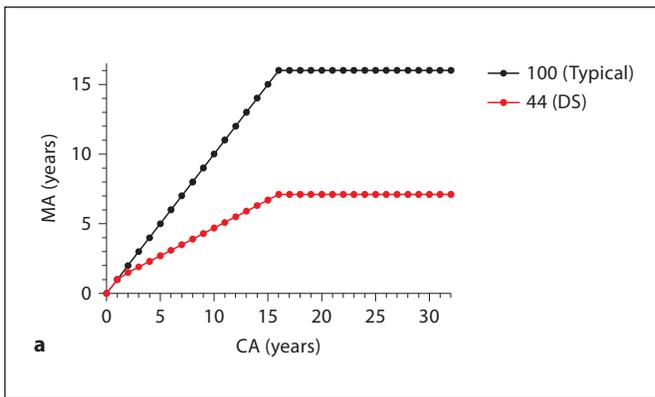
The simpliminded model described above helps to illustrate the idea that the development of pharmacotherapies designed to counteract the cognitive component of DS could have a significant impact not only on the quality of life of individuals with this disorder, but also on their families and communities. For example, multivariate analyses of cost variations carried out for 930 adults with intellectual disabilities [88] found strong, nonlinear, interdependent links between degree of intellectual disability, behavior, service use and costs. In this study, the

authors have found that higher costs were associated with more severe intellectual disabilities and more challenging behavior. Hence, a hypothetical therapy (please notice that, in this entire discussion, I am not talking about any specific therapy) that were capable of decreasing the number of individuals with severe and profound degrees of intellectual disability would clearly provide significant cost savings to society.

#### *The Neurodevelopmental Perspective*

The very simplistic hypothetical scenario described above certainly has its merits as a heuristic tool. However, by ignoring the neurodevelopmental dimension, this hypothetical scenario may not only be simpliminded, but completely unrealistic in the context of therapy design for older children, adolescents, and adults with DS.

The neurodevelopmental perspective has been extremely useful in the clinical setting, such as in developmental pediatrics and developmental neuropsychology. Physicians and therapists involved in the care of individuals with intellectual disabilities use various forms of developmental charts to interpret the timing, rate, and pattern of achievement of sets of specific milestones as diagnostic and prognostic tools. True cognitive capability, which mirrors more closely actual biological development, however, can only be inferred from measures of individual performance during the execution of specific tests, which in turn are heavily dependent on experience and training. For example, some of the most



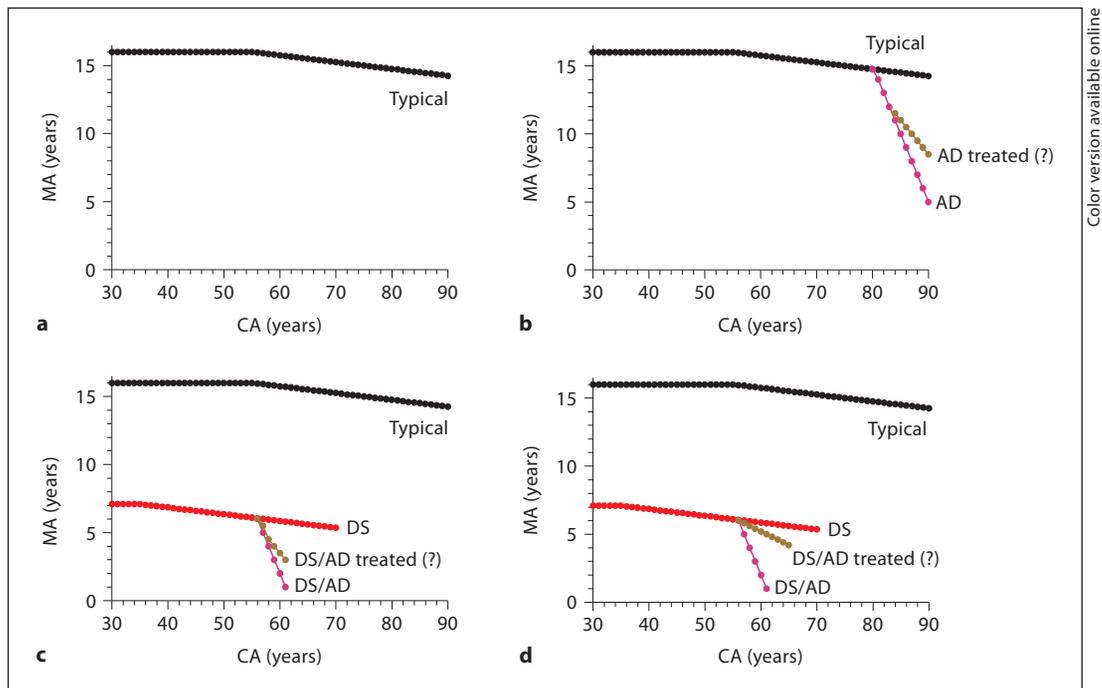
**Fig. 2.** Developmental pattern graphs for a hypothetical ‘average typically developing individual’ versus the developmental pattern for a hypothetical ‘average person with DS’. **a** Developmental patterns for individuals who were not subjected to any form of specific pharmacotherapy. **b** The theoretical outcomes of a hypothetical therapy that completely normalizes the rate and pattern of measurable neurodevelopment, which was commenced at different CAs (5, 10, and 14 years of age). **c** A significant change in the theoretical outcome of the same pharmacotherapeutic intervention started at a CA of 14 years, if we allow a change in the constraints of the developmental model in which MA development was allowed to continue increasing up to 20 years of CA.

useful measures of the cognitive capabilities of individuals with intellectual disabilities are arguably those involving daily living skills and other practical everyday skills affecting independence and social competence. In the course of either normal or abnormal neurodevelopment, however, the quantitative assessment of these very domains may serve only as a lagging indicator of actual cognitive capability to develop such skills. A similar scenario should be expected in the embryonic field of pharmacotherapeutics of neurodevelopmental disorders. In other words, although a certain pharmacological agent might normalize the actual cognitive capability of a given population of individuals with intellectual disability, the desired positive changes in some of the most useful neurodevelopmental measures may lag several years from the beginning of the pharmacotherapeutic intervention.

To illustrate this perspective, let us examine another greatly simplified hypothetical model of pharmacotherapeutic intervention. Figure 2a depicts the average composite MA (from developmental quotient and IQ assessments) as a function of CA for typically developing individuals and for persons with DS. This hypothetical ‘average typically developing person’ of course follows

the typical MA developmental pathway, which is generally accepted to be a linear growth at a 1:1 MA/CA ratio until age 16, at which time, development (as assessed by standardized IQ tests) is supposed to flatten or plateau. For our hypothetical ‘average person with DS’, we have started with a close-to-typical rate of neurodevelopment in the first 2 years of life, which is progressively replaced with a slower rate of development that is close to 1:2 MA/CA ratio (based on data from Nadel [60], Wishart [61], Turner and Alborz [69], Pueschel and Hopmann [70], and Carr [71]). Again, as for the typically developing individual, the assumption is that our hypothetical average person with DS is supposed to plateau his/her MA development at CA 16. The result of this combination of delay and dissociation in the developmental curve is an IQ of 44 during most school years and young adult life, which is in agreement with the general literature in the field.

Figure 2b illustrates the theoretical outcomes of a pharmacotherapy commenced at different CAs (5, 10, and 14 years). For simplicity, it was assumed that this hypothetical pharmacotherapy completely normalizes the rate and pattern of measurable neurodevelopment for the person with DS from the time it is administered. The only other assumption here is that MA development would



**Fig. 3.** Graphs representing the patterns of decline in mental status for hypothetical ‘representative typically developing individual’ compared to a hypothetical ‘representative individual with DS’. **a** The patterns of decline for an uncomplicated age-related mild cognitive decline in a ‘representative typically developing individual’. **b** The typical accelerating effect of AD in the rate of decline in mental status and the hypothetical result of the implementation of AD pharmacotherapy [AD treated (?)]. **c** The same pattern of decline shown in **a** compared to rates of

decline for a person with DS with uncomplicated age-related mild cognitive decline (DS), a person with DS with AD (DS/AD), and the hypothetical result of the implementation of AD pharmacotherapy with memantine [DS/AD treated (?)]. **d** A more optimistic perspective on the hypothetical result of the implementation of AD pharmacotherapy with memantine [DS/AD treated (?)], based on the premise that this therapy might be more directly targeted to the specific pathogenesis of AD in persons with DS.

still plateau at CA 16. The theoretical outcomes of such a simple model of pharmacotherapeutic intervention would be adult IQs of 52, 67, or 86, for interventions starting at ages of 14, 10, or 5 years of CA, respectively. Obviously, small changes in the constraints of this model could result in significantly different outcomes. For example, in figure 2c, MA development was allowed to continue increasing up to 20 years of CA. This simple change would result in an adult IQ of 77, instead of 52, for a pharmacotherapeutic intervention starting at a CA of 14 years.

Once again, the model just described is a very simplistic heuristic tool meant to illustrate factors typically not brought up in the discussion of potential pharmacotherapies in the field of intellectual disabilities. Development obviously is not a linear process, and the existence of sensitive periods cannot be ignored. For example, in more mature areas of neuropsychopharmacology, such as the fields of schizophrenia, depression, and attention deficit hyperactivity disorder in which investigators have had

decades of experience, studying the long-lasting implications for brain structure and function of exposure to drugs early in life are just starting to emerge (see Stanwood and Levitt [89] for an excellent review on the topic). Because these long-lasting effects can be either positive or negative, the old adage ‘start low, go slow’ should apply to reduce possible harm. Hence, early clinical trials in these vulnerable populations should involve only the minimal numbers of subjects necessary to demonstrate potential efficacy, and continued preclinical investigations on animal models is a must.

#### *Adding Neurodegeneration to the Equation*

Figure 3 illustrates the effect of neurodegeneration, in the form of mild cognitive decline and clinical AD, as examined using a simplistic model similar to the one discussed in conjunction with figure 2. Figure 3a represents the hypothetical and not-so-uncommon case of a typically developing adult who maintained a stable level of

cognitive function up to age 55 (as assessed by some general and blunt diagnostic instrument, such as the Mini-Mental State Examination). At age 56, this hypothetical person had started to display a slow rate of decline in cognitive functioning, which resulted in a clinically detectable mild cognitive decline in his/her 70th and 80th decades of life. Such a pattern is easily distinguishable from a person who starts displaying clinical AD, which is illustrated in figure 3b. Typically, the cognitive decline in persons with untreated AD is quite precipitous, and leads to death in an average of 10 years from the onset of this fast declining mental status [90]. The two classes of approved pharmacotherapies for AD, i.e. anticholinesterase agents (donepezil, rivastigmine, and galantamine) and the NMDA receptor antagonist memantine both produce measurable but small positive changes in cognition, and a slowing down in the rate of cognitive decline (as assessed by several instruments, such as the Mini-Mental State Examination and/or the Alzheimer's Disease Assessment Scale). Accordingly, figure 3b includes an alternative (and perhaps somewhat optimistic) pattern of decline for patients with AD being treated with one or a combination of these agents.

In figure 3c, we can appreciate the equivalent scenarios for persons with DS compared with the curve for the hypothetical 'representative typically developing individual' with uncomplicated age-related mild cognitive decline. First, let us examine the hypothetical and also not-so-uncommon case of an adult with DS who maintains a stable level of cognitive function up to age 35, which is followed by a mild and progressive cognitive decline. This pattern is then compared to that of a person with DS who starts displaying clinical AD, which again involves a precipitous and inexorable decline in function and cognitive abilities. This shift to the left by 20 years in the age of onset of cognitive decline reflects the observations reported by Chicoine et al. [91]. In addition, Margallo-Lana et al. [92], in London (UK), have reported that people with DS over the age of 40, without clinically detectable dementia, experienced an average decline of 11% on neuropsychological measures of attention, executive function, and memory over 1 year. Actually, these observations have led these investigators to start an ambitious, randomized, placebo-controlled clinical trial, involving 180 persons with DS, on the efficacy and tolerability of memantine in preventing age-related cognitive deterioration and dementia in people with DS aged 40 and over (NCT00240760 at <http://www.clinicaltrials.gov>). It should be noted that, because these authors are investigating memantine actions on preventing age-related cog-

nitive deterioration (as opposed to cognitive enhancement in hippocampus-dependent measures) in middle-aged adults with DS (as opposed to adolescents and young adults), this study is a nonoverlapping and complementary clinical trial to the one we are conducting presently.

One of the possible results of the London study is illustrated in the pattern in figure 3c labeled as 'DS/AD treated (?)', which somewhat mirrors the current findings on the effect of memantine in slowing the rate of cognitive and functional deterioration in persons without DS. However, one has to remember that one of the crucial limitations associated with clinical research in AD is lack of reliable biomarkers that would identify subpopulations of affected individuals by specific etiologies. Therefore, clinical trials on AD almost certainly involve persons who acquired AD through different mechanisms. Consequently, it is unlikely that any single pharmacological agent will be similarly efficacious across a randomly chosen cohort of clinical trial participants. In contrast, because all persons with DS share the same underlying chromosomal disorder, it is much more likely that, in any given clinical trial, most, if not all individuals will have acquired AD through the same mechanism. Therefore, it is possible that an NMDA antagonist such as memantine might indeed prove superior in the treatment of neurodegenerative dementia in persons with DS than it has been in the treatment of AD in the general population. This more hopeful vision is shown by the line in figure 3d also labeled 'DS/AD treated (?)'.

## Summary and Conclusions

The present report briefly reviewed the prominent role of the Ts65Dn mouse model in basic DS research and, more recently, in translational DS research. Particular emphasis was placed on some of our recent results showing that the FDA-approved AD drug memantine can reverse learning and memory deficits in Ts65Dn mice. Then, a short discussion on the neurodevelopmental and neurodegenerative components of DS, with emphasis on the pivotal work by Pennington et al. [22], was presented. This was followed by a brief description of our clinical research effort at The Children's Hospital in Denver. This report was then concluded with a speculative analysis on the potential impact of this and future translational work in DS.

Three main ideas emerged from the analysis. First, even modest gains in cognitive function in individuals with DS through pharmacotherapeutic intervention could potentially lead to vast collective gains to the popu-

lation of persons with DS, their families, and their communities. Second, even simple-minded models for the potential pharmacotherapeutics for the cognitive deficits associated with DS should take into account the effect of neurodevelopment. A practical consequence of this idea has to do with the effective design of clinical trials. For instance, for short clinical trials (i.e. trials in which the study medication is administered for weeks or months, and not years), one should not choose efficacy end points that are only expected to be significantly affected in a period of years, such as the acquisition of certain daily living skills. For example, generally it takes several months or even years before typically developing children learn how to brush their teeth or tie their shoelaces like an adult. Therefore, it would be unreasonable to expect that these and other skills would simply emerge out of nowhere in a period of a couple of months from someone who has never mastered them; even if the necessary brain subsystems supporting the learning of such skills suddenly became completely normalized by the use of some form of pharmacointervention. Instead, one should aim for end points that more directly assess the functioning of target brain subsystems, such as the hippocampal formation or the prefrontal cortex. The third, and final con-

clusion, is that, to appreciate the full benefit of the potential pharmacotherapeutics for the cognitive deficits associated with DS, one also has to take into account the added benefit of counteracting the neurodegenerative aspects of this chromosomal disorder.

Finally, one should emphasize what should be obvious, but unfortunately sometimes gets lost or distorted in certain discussions: the development of pharmacotherapies for the cognitive disabilities associated with DS (or any other neurodevelopmental disorders resulting in intellectual disability) should be considered an adjuvant therapy, and never as a replacement, to more traditional and proven forms of interventions such as speech, physical, and occupational therapies and innovative special education strategies.

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