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COMMENTARY

Epigallocatechin gallate: A useful therapy for cognitive disability in Down syndrome?

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ABSTRACT

Neurodevelopmental alterations and cognitive disability are constant features of Down syndrome (DS), a genetic condition due to triplication of chromosome 21. *DYRK1A* is one of the triplicated genes that is thought to be strongly involved in brain alterations. Treatment of Dyrk1A transgenic mice with epigallocatechin gallate (EGCG), an inhibitor of DYRK1A, improves cognitive performance, suggesting that EGCG may represent a suitable treatment of DS. Evidence in the Ts65Dn mouse model of DS shows that EGCG restores hippocampal development, although this effect is ephemeral. Other studies, however, show no effects of treatment on hippocampus-dependent memory. On the other hand, a pilot study in young adults with DS shows that EGCG transiently improves some aspects of memory. Interestingly, EGCG plus cognitive training engenders effects that are more prolonged. Studies in various rodent models show a positive impact of EGCG on brain and behavior, but other studies show no effect. In spite of these discrepancies, possibly due to heterogeneity of protocols/timing/species, EGCG seems to exert some beneficial effects on the brain. It is possible that protocols of periodic EGCG administration to individuals with DS (alone or in conjunction with other treatments) may prevent the disappearance of its effects.

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Cognitive disability: The hallmark of Down syndrome

Down syndrome is a genetic condition caused by triplication of chromosome 21. Excessive protein levels due to gene triplication cause a constellation of developmental abnormalities among which the most severe involve the heart, the intestinal system, and the nervous system.[1] Unlike the other developmental defects, the impaired development of the nervous system is a constant feature of DS that causes the cognitive disability characterizing this pathology. Cognitive impairment in individuals with DS represents the major concern for families and society. The IQ of individuals with DS ranges between 45 and 71 in children and worsens with age;[2] this, in most cases, is not compatible with an autonomous life. Brain abnormalities in DS were thought to be irreversible, but during the last decade several preclinical studies have shown that in mouse models of DS it is possible to improve or even rescue the major neurodevelopmental alterations of the trisomic brain.[3-5] These discoveries obviously give new hope for therapeutic interventions in individuals with DS.

The gene burden impairs brain development in Down syndrome

A patent feature of DS is a brain size reduction that is particularly prominent in some regions such as the cerebellum, the frontal cortex and the hippocampus. Regarding the causes of brain hypotrophy in DS, evidence in fetal material and mouse models of DS (the most widely-used of which is the Ts65Dn mouse) suggests that in the DS brain neurogenesis is impaired starting from the beginning of brain development (embryonic/fetal life stages). This defect translates into a diffused reduction in the number of neurons across the whole DS brain.[3,6] The increase in the repertoire of functions along the time course of mammalian evolution is due to an increase in the relative number of neurons populating the brain, culminating in the unique brain functions possessed by non-human and human primates. Therefore, the reduced neuron number in DS is very likely a key determinant underlying the poor performance of individuals with DS in many cognitive functions and behaviors.

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The second serious neurobiological issue affecting the brain of individuals with DS regards abnormalities in neuronal maturation.[3,6] Trisomic neurons have a dendritic tree with a reduced number of branches and, consequently, a reduced overall dendritic length. This defect is worsened by a reduction in the density of dendritic spines (the target of excitatory synapses). The outcome of both these abnormalities is impairment of normal connectivity among neurons. Therefore, dendritic hypotrophy is very likely a second key determinant underlying the poor performance of individuals with DS in many cognitive domains. In addition, the axons of trisomic neurons have a reduced myelination,[7] which implies slower conduction of the action potentials and further impairment in the cross talk among neurons.

The two patent developmental defects of the DS brain (neurogenesis and dendritogenesis alterations) are accompanied by more subtle changes that involve cell membranes, cell organelles, neurotransmitters and receptor systems, and numerous signaling pathways.[3,6] All these changes imply that the processing of signals in a brain that is already at a disadvantage, due to a poor neuroanatomical organization, is further compromised by impairment of the cellular machinery.

DYRK1A: A candidate gene participating in brain alterations in Down syndrome

Intense efforts are being made to identify the genes whose triplication determines the developmental alterations that characterize the DS brain. This knowledge will make it possible to specifically target pathways downstream of these genes, thereby counteracting their negative effects. Yet, the large number of triplicated genes in DS makes the search for the "culprits" very challenging. It is likely that many triplicated genes concur to impair neurogenesis, dendritic development and synaptic organization. The problem is further complicated by the fact that gene triplication may have a generalized impact on the whole genome. Nevertheless, some triplicated genes are emerging as key (although not unique) actors that may play a particularly prominent role in altering proper brain development. One of these genes is DYRK1A [Dual specificity Tyrosine(Y) Regulated Kinase 1A], a mammalian ortholog of minibrain in drosophila. This gene is fundamental for normal neurogenesis and is overexpressed in fetuses and adults with DS.[8] The idea that the triplicated gene DYRK1A is involved in brain developmental alterations in DS derives from evidence that Dyrk1A transgenic mice exhibit brain and behavioral abnormalities that are similar to those of DS.[8] In addition, Dyrk1A transgenic mice exhibit neurogenesis alterations, confirming the role of DYRK1A in this process.[9] This notion has prompted studies in Dyrk1A transgenic mice aimed at establishing whether it is possible to correct their phenotype through modulation of DYRK1A activity.

Inhibition of DYRK1A activity has a positive impact in Dyrk1A transgenic models

Among the several inhibitors of DYRK1A, epigallocatechin gallate (EGCG) is one of the most specific.[10] EGCG, which is the major catechin in the leaves of green tea, can cross the blood- and placental barrier.[11] Treatment of Dyrk1A transgenic mice with a polyphenol-based diet from gestation to adulthood has been shown to correct brain morphogenesis alterations.[12] In addition, Dyrk1A transgenic mice treated with EGCG for one month starting from 21 d of age undergo restoration of hippocampal neurogenesis.[9] In adult Dyrk1A transgenic mice, a 4–6 week administration of green tea extracts rescues defective long-term potentiation in the prefrontal cortex.[13] Finally, treatment with extracts containing EGCG in adult mBACtgDyrk1A mice restores components of GABAergic and glutamatergic pathways in the cortex and hippocampus, and improves behavioral deficits.[14] All this evidence obtained in Dyrk1A transgenic mice suggests that treatment with EGCG may be exploited in DS to improve brain functional alterations due to triplication of DYRK1A. Details regarding components of treatment, dosage, treatment timing, route of administration, and age of mice at treatment are reported in Table 1.

Effects of EGCG in the Ts65Dn model of Down syndrome

A pioneering study suggesting a possible benefit of EGCG in DS examined the effect of EGCG in hippocampal slices from Ts65Dn mice.[15] This study showed that it was possible to rescue levels of long-term potentiation (LTP) at the Schaffer collateral-CA1 synapse in hippocampal slices from Ts65Dn mice incubated with EGCG (10 μM). Since LTP is a form of synaptic plasticity that is thought to be a cellular correlate of memory, this effect suggests a potential benefit of EGCG in the rescue of memory in DS. A recent study[16] analyzed

Table 1. Studies that have examined the effect of EGCG on neurogenesis and/or behavior in rodent models.

Species/Strain		Treatment	Timing of treatment	Effect on neuroanatomy	Effect on behavior or LTP	Reference
Transgenic Dyrk1a mice	YACtg152F7 mice	Daily green tea infusion (equivalent to administering 0.6–1 mg/day pure EGCG) or daily administration of polyphenon 60 solution (0.8 g/l, Sigma) containing a mixture of polyphenolic compounds. The dose is equivalent to 1.2 mg EGCG per day	From gestation to adulthood	Rescue of brain weight, brain volume and hypothalamus-thalamus volume	Rescue in the NOR test	12
	TgDyrk1A mice	EGCG in drinking water (EGCG concentration: 90 mg/ml for a dose of 2–3 mg per day). EGCG solution was prepared from a green tea leaf extract (Mega Green Tea Extract, Decaffeinated, Life Extension®, USA) standardized to 98% polyphenols with 45% EGCG per capsule	1 month in 21 day-old mice	Normalization of the density of Ki-67 positive cells and the proportion of cells exiting the cell cycle	N.D.	9
	mBACtgDyrk1a mice	Green tea extract solution in drinking water containing 0.25% green tea decaffeinated extract (0.8 mg/ml EGCG) and 0.25% glucose. Green tea extract contained 45% EGCG. Daily EGCG assumption ranged between 120 and 200 mg/kg	4–6 weeks in 3–4 month-old mice	Rescue of spine density in prefrontal cortex pyramidal cells	Rescue of LTP in the prefrontal cortex	13
	TgDyrk1A mice	EGCG in drinking water (EGCG concentration: 90 mg/ml for a dose of 2–3 mg per day). EGCG solution was prepared from a green tea leaf extract [Mega Green Tea Extract, Lightly Caffeinated (0.8% caffeine), Life Extension®, USA] standardized to 98% polyphenols with 45% EGCG per capsule	1 month in 3 month-old mice	N.D.	Rescue in the MWM and NOR tests	17
	mBACtgDyrk1a	Polyphenon 60 (POL60, Sigma) solution in drinking water (final concentration of POL60: 225 mg/kg/day) containing green tea polyphenols with 27% EGCG, 42% other catechins (EC, ECG, EGC, and GC) with no effect on DYRK1A activity, and 8% caffeine. 1% sucrose was added	4 weeks in 3–4 month-old mice	Rescue of components in GABAergic and glutamatergic pathways in cortex and hippocampus	N.D.	14
	mBACtgDyrk1a	Administration of solid food pellets containing Decaffeinated Mega Green Tea Extract, Life Extension® (contains 45% EGCG and 53% other catechins). Mega Green Tea Extract was produced at a dose corresponding to 60 mg/kg/day EGCG	4 weeks in 3–4 month-old mice	N.D.	Improvement in the rate of spontaneous alternation in the Y-maze test	14
Ts65Dn mice	Hippocampal slices from Ts65Dn mice	10 μM pure EGCG (purity $\geq$95%)	1h prior to the experiment	N.D.	Rescue of LTP in CA1	15
	NPCs from the dentate gyrus of 6–8 week-old Ts65Dn mice	20 μM pure EGCG (purity $\geq$95%)	24h	Proliferation enhancement and rescue of mitochondrial biogenesis	N.D.	16
	Ts65Dn mice	EGCG in drinking water (EGCG concentration: 90 mg/ml for a dose of 2–3 mg per day). EGCG solution was prepared from a green tea leaf extract [Mega Green Tea Extract, Lightly Caffeinated (0.8% caffeine), Life Extension®, USA] standardized to 98% polyphenols with 45% EGCG per capsule	1 month in 3 month-old mice	N.D.	Rescue in the MWM and NOR tests	17
	Ts65Dn mice	Pure EGCG in drinking water (EGCG concentration: ~100 mg/kg/day; purity $\geq$95%)	From postnatal day 24 to postnatal day 75	N.D.	No improvement in the radial-arm maze, delayed non-matching-to-place pattern separation tests	19
	Ts65Dn mice	Pure EGCG in drinking water in a concentration of 0.124 mg/ml (~20 mg/kg/day; purity $\geq$95%)	3 or 7 weeks starting from 24 days of age	N.D.	No improvement in the OF, NOR , BB, DMNP or MWM tests	18
	Ts65Dn mice	Polyphenon 60 (POL60, Sigma) solution in drinking water (final concentration of POL60: 225 mg/kg/day) containing green tea polyphenols with 27% EGCG, 42% other catechins (EC, ECG,	6 weeks in 3–4 month-old mice	Rescue of levels in GABAergic and glutaminergic markers in cortex and hippocampus	Rescue in the Y-maze test	14

(*Continued on next page*)

Table 1. (Continued)

Species/Strain		Treatment	Timing of treatment	Effect on neuroanatomy	Effect on behavior or LTP	Reference
		EGC, and GC) with no effect on DYRK1A activity, and 8% caffeine. 1% sucrose was added				
	Ts65Dn mice	Daily injection of pure EGCG (25 mg/kg; purity: ≥95%)	From postnatal day 3 to postnatal day 15	Rescue of proliferation, and cellularity in the dentate gyrus and rescue of connectivity in hippocampus and neocortex at P15. Disappearance of these effects at P45	No improvement in the Y-maze and MWM tests at P45	20
Transgenic APP mice	APP/PS1 transgenic mice	Daily intragastric administration (pure EGCG concentration: 2 mg/kg; purity ≥95%)	1 month in 9 month-old mice	Attenuation of neuronal apoptosis in the hippocampus; deceleration of Aβ(1–40) plaque formation	Improvement in the PA and MWM tests	29
Normosomic mice	C57BL/6J mice	Green tea catechins (purity: 93%; EGCG: 71%; caffeine: 0.3%; Orient Tea Development Co., Ltd) in drinking water at different concentrations: 0.25, 0.5 and 1 g/l	6 months in 14 month-old mice	Prevention of age-related reductions of PSD-95 in the hippocampus. No effect on granule cell density	Improvement in the MWM test at the concentration of green tea catechins of 0.5 and 1g/l; no effect on OF	27
	C57BL/6J mice	Daily administration of pure EGCG (25 mg/kg; purity ≥95%) through oral gavage	1 month in 8 week-old mice	Proliferation enhancement in the dentate gyrus	N.D.	24
	C57BL/6J mice	Daily intraperitoneal injection of pure EGCG (20 mg/kg; purity ≥95%)	2 months in 2 month-old mice	Proliferation enhancement in the dentate gyrus	Improvement in the MWM test	26
	NPCs from the hippocampus of 8–10 week-old C57BL/6J mice	5, 10, 20, 40 μM of pure EGCG (purity ≥95%)	24h	Proliferation enhancement of adult NPCs (10, 20, 40 μM)	N.D.	26
	BALB/cJ mice	Food pellets made with Teavigo® (>90% EGCG, DSM Nutritional Products, Basel, Switzerland). Estimated EGCG dose ~250 mg/kg/day	39 days in 10 week-old mice	No effect on proliferation in the dentate gyrus	No improvement in the CFC test	31
Rats	Wistar rats	Green tea in drinking water (EGCG concentration: 299.56 μg/ml) and exercise	3 months and 7 days in 9 month-old rats	N.D.	No improvement in the OF, NOR, IA tests in rats treated with EGCG; improvement in these tests in rats treated with EGCG and exercise	32
	Sprague–Dawley rats	Daily injection of pure EGCG (100 mg/kg; purity ≥95%) in stressed rats	21 days in 8 week-old rats	N.D.	Rescue in the OF and MWM tests	28
	Wistar rats	Green tea catechins, Polyphenon E (63% (-)-epigallocat-echin gallate, 11% (-)- epicatechins, 6% (-)-epigallocatechin and 6% (-)-epicatechin gallate) in drinking water	26 weeks in adult rats	Proliferation enhancement in the dentate gyrus	N.D.	25
	SH rats	Daily intraperitoneal injection of pure EGCG (100 mg/kg; purity ≥95%)	28 days in 8 week-old rats	N.D.	Rescue in the OF and MWM tests	30

Abbreviations: BB: Balance Beam; CFC: Contextual Fear Conditioning; DMNP: T-maze delayed non-matching-to-place; EC: epicatechin; ECG: epicatechin gallate; EGC: epigallocatechin; EGCG: epigallocatechin gallate; GC: gallocatechin; GTC: green tea catechins; IA: inhibitory avoidance/aversive memory; MWM: Morris Water Maze; OF: Open Field; N.D.: not done; NOR: Novel Object Recognition; NPCs: neural progenitor cells; PA: Passive Avoidance; SH: spontaneously hypertensive.

mitochondrial functions in neural progenitor cells (NPCs) isolated from the hippocampus of Ts65Dn mice. Results show that, during NPC proliferation, mitochondrial bioenergetics was compromised and that EGCG (20 μM) restored oxidative phosphorylation efficiency and mitochondrial biogenesis, and improved proliferation (Table 1). This suggests that EGCG has the potential to improve neurogenesis alterations in DS.

The first study describing the effects of EGCG in the Ts65Dn model of DS was performed in adult mice.[17] This study examined the effects of green tea extracts containing EGCG on learning and memory in Ts65Dn and in TgDyrk1A mice. Adult TgDyrk1A and Ts65Dn mice (3 months of age) that were treated for one month with green tea extracts containing EGCG exhibited a memory improvement, assessed using the Morris Water Maze and Novel Object Recognition tests[17] A subsequent study by Stringer et al.[18] tested the effects of pure EGCG (20 mg/kg/day) in Ts65Dn mice starting from weaning, for either three weeks (i.e. until adolescence) or seven weeks (i.e., until adulthood). Since EGCG undergoes rapid degradation at ambient temperature, which may be a confounding effect in preclinical studies with EGCG, EGCG consumption was adjusted for loss due to degradation. This study shows that neither the short- nor long-term treatment with EGCG improved performance in a battery of behavioral tasks. In an attempt to establish whether a higher dose was effective, the same group[19] tested the effects of ~100 mg/kg/day of EGCG from postnatal day 24 to adulthood (75 days). Ts65Dn mice, examined with a radial-arm maze delayed non-matching-to-place pattern separation task did not show any improvement in their deficits.

Since the DS brain starts with a disadvantage, attempts to rescue neurogenesis, neuron maturation and connectivity should be prompt. For this reason, our group decided to treat Ts65Dn mice with EGCG in the neonatal period with the premise that if brain defects are restored from the initial phases of brain development the brain may remain in its restored state after treatment cessation. Mice received a daily injection of pure EGCG (25 mg/kg) in the postnatal period P3-P15 and the effects were examined both at treatment cessation (at P15) and after one month (at P45).[20] We found positive effects of the acute treatment, with restoration of neurogenesis, cellularity and connectivity in the hippocampus and neocortex. Treatment, however, had no long-term effects, and hippocampal neurogenesis and connectivity were once more impaired when mice reached 45 d of age. Moreover, the Y-maze and Morris Water Maze tests in P45 mice showed no beneficial effects of neonatal treatment on behavior. Details regarding components of treatment, dosage, treatment timing, route of administration, and age of mice at treatment are reported in Table 1.

Effects of EGCG in individuals with Down syndrome

Mitochondrial function is notably impaired in DS,[21] which may contribute to several DS-linked developmental abnormalities. In lymphoblast and fibroblast cultures from DS subjects treatment with EGCG (20 μM) has been shown to rescue mitochondrial function and promote mitochondrial biogenesis.[21] This study prompted research aimed at establishing whether a similar effect was replicated in vivo.[22] A child (10-year and 3-monh-old) with DS was treated with ECCG (10 mg/kg/day) plus fish oil daily for six months. This treatment was safe and improved mitochondrial function. However, since EGCG was co-administered with fish oil, the extent to which fish oil contributes to this effect remains to be established.

In a pilot study, young adults with DS (29 subjects) aged 14–29 years were treated with either green tea extracts in capsule form (Mega Green Tea Extract, Lightly Caffeinated, Life Extension®, USA) containing EGCG (mean EGCG oral dose of 9 mg/kg/day) (6 females, 7 males) or a placebo (8 females, 8 males) for three months.[17] The effects of treatment on indices of neuropsychological performance were examined after 3 months of treatment and 3 months after treatment discontinuation. After 3 months of treatment, EGCG-treated individuals showed a significantly higher percentage of correct answers in visual memory recognition compared with those who had been given the placebo. Three months after treatment discontinuation this effect declined, and treated subjects had a performance that returned to baseline measures. This evidence indicates that treatment with EGCG has a positive effect on cognition in DS, albeit relatively moderate and transitory. In a subsequent study,[23] the same group examined the effect of cognitive training alone or cognitive training plus green tea extract supplement in capsule form containing 45% EGCG (Life Extension Decaffeinated Mega Green Tea Extract; Life Extension ®, USA). The mean EGCG oral dose was 9 mg/kg/day. In this study a larger group (84 subjects) of adults (age: 16–34 years) with DS was enrolled and

treatment lasted 12 months. The percentage of males and females was similar in each treatment group (placebo plus cognitive training group: males = 48%, females = 52%; EGCG group: males = 56%, females = 44%). Subjects were tested with a battery of neuropsychological tests periodically during treatment, at 12 months (i.e., immediately following treatment cessation) and at 6 months after treatment discontinuation. At 12 months, there were significant differences between the two groups in two of the 15 tests developed for testing cognitive performance and in one of the nine adaptive skills. Subjects that received cognitive training plus EGCG had a better performance in these three tests in comparison with the group that received cognitive training only. Some of these effects persisted in the cognitive training plus EGCG group. Since in this and in the previous study[17] cumulative results for all enrolled subjects are reported, it is not possible to establish whether treatment has gender- and/or age-dependent effects. It should be noted that in the 2016 study by De la Torre et al., treatment with EGCG was combined with cognitive training and no group received EGCG only. Thus, it remains to be established whether EGCG worked by itself in terms of improving scores in cognitive measures in individuals with DS or whether it had to be combined with cognitive training. In addition, since the green tea extracts contain various polyphenols in addition to EGCG, the contribution of these polyphenols to cognitive improvement remains to be clarified. For instance, in the earlier study by De la Torre et al.,[17] lightly-caffeinated extracts were used; it has been shown that caffeine by itself is able to normalize the excitatory-inhibitory imbalance in mBACtgDyrk1A mice.[14] Consequently, the effects of green tea extracts may depend on individual components present in the extracts and/or their combination. Thus, although the groups receiving green tea extract were defined as "EGCG" groups, it cannot be ruled out that the effects ascribed to EGCG were due to interactions with other constituents in the extracts.

Potential usefulness of EGCG treatment of Down syndrome

The available data in Ts65Dn mice show that treatment with EGCG may[17] or may not[18,19] have a positive effect on behavior, and that it restores hippocampal development, but has no long-term impact on hippocampal neuroanatomy and behavior.[20] It is important to note that the only study that examined DYRK1A activity in the Ts65Dn mouse showed marginal differences in comparison with euploid mice and marginal effects of EGCG on its activity.[18] However, due to the relatively small sample size in conjunction with the intrinsic phenotypic variability of Ts65Dn mice, further observations are required to confirm this finding. In addition, protein expression, including that of DYRK1A, may change across brain regions and ages. Therefore, systematic studies are needed to draw more definitive conclusions regarding the role of DYRK1A in DS. If in Ts65Dn mice, unlike in TgDyrk1A mice, DYRK1A activity is less severely affected, this may imply that DYRK1A is not a major cause of brain and behavior impairment and may thus explain the inconsistent effects on behavior found in Ts65Dn mice treated with EGCG. Finally, it must be observed that in wild-type mice EGCG may have an adverse effect on behavior[17] and synaptic connections,[20] suggesting some caveats in treatment with EGCG as supplementation to enhance brain function. Conflicting results have also been reported for other rodent models. Some studies show beneficial effects of EGCG (alone or with other catechins) on neurogenesis and memory.[24-30] Other studies, however, show no effects of treatment on neurogenesis and memory (Table 1).[31,32] It must be observed that all these studies differ from each other concerning several aspects that may influence the effects of EGCG supplementation: i) administration of other green tea extracts in combination with EGCG; ii) dosage; iii) means of administration; iv) duration of treatment; v) age of animals; vi) species/strain (Table 1). Although some animal studies seem to suggest that EGCG may be a good candidate drug for DS, in view of the heterogeneity of the experimental evidence available, definitive conclusions cannot yet be drawn.

In conclusion, the available data in mouse models of DS show that EGCG may partially improve trisomy-due brain and behavioral defects, but does not rescue them. Data in individuals with DS show that three months of treatment with green tea extracts containing EGCG exert some benefits on cognitive performance but that these effects disappear with time.[17] This fits in with the disappearance of the effects of EGCG in the Ts65Dn mouse model.[20] However, it is important to note that in individuals with DS treatment with EGCG plus cognitive training for 12 months leads to effects that are partially retained after

treatment discontinuation.[23] Although the effects of treatment are of small magnitude and involve a limited number of behaviors, this study suggests that EGCG may be used to ameliorate some aspects of cognitive performance in individuals with DS. None of the therapies tested so far in clinical trials in individuals with DS have significantly improved behavior. It is possible, however, that treatments that are ineffective or scarcely effective if administered alone elicit some benefits when they are given in combination. If so, EGCG may represent a useful treatment to be used alone or in combination, to improve cognitive performance in individuals with DS. We believe it is now important to perform systematic preclinical studies to elucidate the therapeutic potential of EGCG alone and of EGCG in combination with other treatments. This will allow for an identification of the optimum therapy for individuals with DS.

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No potential conflicts of interest were disclosed.

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