Patent Highlights June–July 2015

A snapshot of noteworthy recent developments in the patent literature of relevance to pharmaceutical and medical research and development.

WO/2015/078477

Fatty acid derivatives of dimeric inhibitors of PSD-95

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Keywords: chronic pain • DLG4 protein, human • nitric oxide synthase type I • PDZ domains • pharmacokinetics

The postsynaptic density protein-95 (PSD-95) is a scaffold anchor protein, and a member of the membrane-associated guanylate kinase family. Its three PDZ domains interact with several proteins [1] including NMDA receptors and also neuronal nitric oxide synthase, which itself is activated by the influx of calcium ions through opened NMDA receptor channels. Therefore, PSD-95 is a key facilitator of glutamate-mediated neurotoxicity. Specific inhibition of excitotoxicity can be achieved by perturbing the

intracellular neuronal nitric oxide synthase/PSD-95/NMDA receptor complex using PSD-95 inhibitors. Dimeric ligands that consist of peptides linked by a polyethylene glycol linker and simultaneously bind to the PDZ1 and PDZ2 domains of PSD-95, such as UCCB01-125 (claimed in WO 2010/004003), have shown some effect in animal models of chronic pain [2], but the plasma half-life of these constructs is very short. The present invention uses modified linkers where at least one of the polyethylene glycol backbone oxygen atoms is replaced by nitrogen, to which a fatty acid is attached. This greatly enhances the affinity of the construct to human serum albumin, and thereby increases its plasma residence time without interfering substantially with binding to the PDZs of PSD-95. For small molecule inhibitors see [3].

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- Lee WH, Xu Z, Ashpole NM *et al.* Small molecule inhibitors of PSD95-nNOS protein-protein interactions as novel analgesics. *Neuropharmacology* pii: S0028-3908(15)00228-2 (2015) [Epub ahead of print] [PMID: 26071110].

WO/2015/078935

TAL effector means useful for partial or full deletion of DNA tandem repeat

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Keywords: DNA repeat expansion • endonucleases • genetic diseases, inborn • myotonic dystrophys

Short tandem repeats with a length of a few nucleotides are quite frequent in mammalian DNA. They are prone to recombination and/or random integration events, and are considered to be at the center of species evolution [4]. Only if they greatly expand in length (which can happen through basically any repair mechanism involving *de novo* DNA synthesis) a situation is created that underlies about 20 severe neurological or musculoskeletal genetic diseases. Removing these massive tandem repeat expansions would offer a causative treatment, but the tools that are available so far, such as zinc finger endonucleases, are not sufficiently specific and cause frequent off-target mutations. Customized transcription activator-like effector endonucleases (TALENs), which can be engineered to bind practically any desired DNA sequence, are promising tools for genome editing [5] and should be much better suited for partial or complete elimination of excessively multiplexed tandem nucleotide sequences; apparently this had not been reported before. A TALEN designed to

recognize and cut a CAG/CTG trinucleotide repeat was assayed in a dedicated yeast experimental system. The assay revealed a 100% efficacy in trinucleotide repeat contractions, no effect on unrelated triplet repeats and no effect on the overall mutation rate of the yeast genome. All karyotypes were normal, showing no evidence for aneuploidies, large segmental duplications or translocations. This could ultimately open possibilities for germline gene therapy of myotonic dystrophy type 1, where an expansion of the CTG triplet repeat occurs in the 3'-untranslated region of the *DMPK* gene [6]. Even somatic treatments might provide some relief to affected individuals if the TALENs could be administered together with a proper nuclear targeting signal.

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- 6 Meola G, Cardani R. Myotonic dystrophies: An update on clinical aspects, genetic, pathology, and molecular pathomechanisms. *Biochim. Biophys. Acta* 1852(4), 594-606 (2015) [PMID: 24882752].

WO/2015/081407

Use of chemical compounds that can inhibit the toxic activity of sphingomyelinase D from venoms of *Loxosceles* spiders and pharmaceutical composition comprising said compounds

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Keywords: brown recluse spider • phospholipase D • sphingomyelin phosphodiesterase

Recluse spiders (*Loxosceles* spp.), which occur in the southern and central USA, South America and the Mediterranean countries, produce a venom that can cause necrotic skin lesions with or without visceral and vascular symptoms, known as loxoscelism [7,8]. Sphingomyelinase D is the primary factor of the venom [9]. While earlier approaches to loxoscelism treatment were directed to neutralizing antibodies and therapeutic vaccines [10], proteases (WO/2007/149343) and immune response modifiers (WO/2001/74343), benzenesulfonamide and benzenesulfonate inhibitors of sphingomyelinase D were never claimed for util-

ity in loxoscelism. 4-Bromo-N-[(E)-(2-methyl-1H-indol-3-yl) methylene amino]benzenesulfonamide and 4-methyl-3-oxo-2-(3-pyridylmethylene)benzo[3,4-b]furan-6-yl-4-chlorobenzenesulfonate are the preferred compounds. When human keratinocytes were co-incubated with *Loxosceles laeta* venom and these compounds (10 μ M), cell viability increased by 30% and by over 100%, respectively, compared with toxin exposure alone. Importantly, these compounds are also inhibitors of phospholipase D (which also degrades sphingomyelin) from *Corynebacterium pseudotuberculosis*, *Corynebacterium ulcerans* and *Arcanobacterium haemolyticum*, and of sphingomyelinases from *Staphylococcus aureus* and *Bacillus cereus*; they could therefore have much more widespread applications than loxoscelism.

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- 9 Gremski LH, Trevisan-Silva D, Ferrer VP *et al.* Recent advances in the understanding of brown spider venoms: From the biology of spiders to the molecular mechanisms of toxins. *Toxicon* 83, 91-120 (2014) [PMID: 24631373].

WO/2015/083871

Composition containing osmotin as active ingredient for improving cognitive function Kim MO

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Keywords: adiponectin • Alzheimer disease • amyloid beta-peptides • *Nicotiana tabacum* • osmotin protein

Osmotin, a plant defense protein widely distributed in fruits and vegetables, protects the cells from various environmental stresses and invading fungal pathogens [11]. Amazingly, osmotin shares structural and functional properties with the mammalian hormone, adiponectin, although no sequence similarity is present at the amino acid level. For example, the effects of both proteins on human fibroblasts are similar, confirming the *in silico* predictions [12]. Adiponectin has antidiabetic, antiproliferative and cardioprotective effects, and therefore related actions might be expected from osmotin. This invention goes considerably beyond that (and also beyond WO/2015/108372 from the same applicant, claiming osmotin to treat synaptic dysfunction caused by excitotoxic stress) by showing that the plant protein reverses A β ₁₋₄₂-induced synaptic deficits, A β accumulation and BACE-1 expression; hyperphosphorylation of the tau protein is ameliorated. In rats, spontaneous alternation behavior in the Y-maze showed improvement after intracerebral administration of osmotin. Such findings have not yet been reported for adiponectin, although it is known that this hormone stimulates hippocampal neurogenesis [13]. For the peer review companion paper see [14].

Published 11 June 2015

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- 12 Miele M, Costantini S, Colonna G. Structural and functional similarities between osmotin from *Nicotiana tabacum* seeds and human adiponectin. *PLoS One* 6(2), e16690 (2011) [PMID: 21311758].
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- 14 Ali T, Yoon GH, Shah SA *et al.* Osmotin attenuates amyloid beta-induced memory impairment, tau phosphorylation and neurodegeneration in the mouse hippocampus. *Sci. Rep.* 5, 11708 (2015) [PMID: 26118757].

WO/2015/088162

Composition for promoting differentiation from stem cells to chondrocyte Lee JW, Lee SG, Yoon DS

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Keywords: cartilage • chondrocytes • mesenchymal stromal cells • microRNA-495, human

In the emerging field of microRNA-related therapy concepts, miR-495 takes a prominent place; much of the research has focused on cancer where its role is ambiguous [15,16]. In keeping with evidence published by a different group that miR-495 suppresses differentiation of mouse embryonic stem cells [17], the present invention identifies miR-495 as an inhibitor of differentiation from mesenchymal stem cells to chondrocytes and demonstrates that its inhibition allows chondrocytes to be produced from pluripotent cells. miR-495 directly binds

to the 3'-untranslated region of Sox9, a transcription factor that regulates not only male sexual development but also chondrocyte differentiation. Inhibition of this specific binding might offer perspectives for osteoarthritis, and for the reconstruction of nonskeletal cartilage tissue. The companion peer review paper is [18]. Note that Sox9 is also a target for miRNA-101 [19].

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- 16 Jiang X, Huang H, Li Z *et al.* MiR-495 is a tumor-suppressor microRNA down-regulated in MLL-rearranged leukemia. *Proc. Natl Acad. Sci. USA* 109(47), 19397-19402 (2012) [PMID: 23132946].
- 17 Yang D, Wang G, Zhu S *et al.* MiR-495 suppresses mesendoderm differentiation of mouse embryonic stem cells

via the direct targeting of Dnmt3a. *Stem Cell Res.* 12(2), 550-561 (2014) [PMID: 24530979].

- 18 Lee S, Yoon DS, Paik S *et al.* microRNA-495 inhibits chondrogenic differentiation in human mesenchymal stem cells by targeting Sox9. *Stem Cells Dev.* 23(15), 1798-1808

WO/2015/091647

3-(5-Chloro-2-oxobenzo[d]oxazol-3(2H)-yl)propanoic acid derivatives as KMO inhibitors Bouillot AMJ, Mirguet O, Liddle J, Walker AL

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Keywords: central nervous system diseases • inflammation • kynurenine 3-monooxygenase

In mammals, tryptophan is mainly metabolized through the kynurenine pathway, ultimately leading to the production of nicotinamide adenosine dinucleotide. Several enzymes in this pathway are under tight control of inflammatory mediators, allowing for their involvement in a broad range of pathologies [20]. The recent resolution of the crystal structure of kynurenine 3-monooxygenase (KMO; a flavin adenine dinucleotide-dependent mitochondrial hydroxylase) [21] has greatly assisted the search for inhibitors. A structurally novel class of such inhibitors has now been identified, and may be useful in the treatment of acute pancreatitis and systemic inflammatory response syndrome,

(2014) [PMID: 24654627].

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but especially in a wide range of neurodegenerative and psychiatric disorders: the primary product of KMO, 3-hydroxykynurenine, and its downstream metabolites quinolinic acid and kynurenic acid, are neuroactive [22]. The preferred compound of the invention is 3-(5-chloro-6-methyl-2-oxobenzo[d]oxazol-3(2H)-yl)propanoic acid, a commercially available compound which exhibited a pIC₅₀ value of 7.9 for KMO in a RapidFire mass spectrometry assay.

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WO/2015/092035

CD300a receptors as virus entry cofactors

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Keywords: bacteriocins • CD300a molecule, human • flavivirus • receptor, dengue virus

Dengue virions make initial contact with the host cell by binding to heparan-sulfate proteoglycans on the cell membrane, which are thought to concentrate the virus at the target cell surface before its interactions with entry factors and by clathrin-mediated endocytosis. Some C-type lectin receptors (DC-SIGN, L-SIGN [23], and the macrophage mannose receptor) and TIM and TAM (proteins of two receptor families that mediate the phosphatidylserine-dependent phagocytic removal of apoptotic cells [24]) are characterized as dengue virus entry factors; however, the virus also infects cell types that do not express any of these proteins. The inven-

tors have found that CD300a [25] expressed at the surface of the host cell can mediate interaction between phosphatidylserine and/or phosphatidylethanolamine at the surface of the viral envelope and the receptor, and that CD300a blockade inhibits dengue virus entry into host cells. In addition they have found that this interaction is also used by other aminophospholipid-harboring viruses, in particular other flaviviruses such as West Nile virus and Yellow Fever virus. The interaction can be blocked by antibodies directed at CD300a, or by duramycins, lantibiotics which are phosphatidylethanolamine-binding proteins [26]. Other members of the CD300 antigen family do not have viral entry-mediating effects.

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virus entry. *Cell Host Microbe* 12(4), 544-557 (2012) [PMID: 23084921].

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for Phosphatidylserine and Phosphatidylethanolamine. *J. Immunol.* 194(11), 5053-5060 (2015) [PMID: 25980030].

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WO/2015/093933

Use of kisspeptin and agonists to regulate serotonin

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Keywords: depressive disorder • KISS1 receptor, human • kisspeptins • serotonin

Kisspeptins, encoded by the metastasis suppressor gene *KISS1* and with a core sequence that is highly conserved across many animal species, are the endogenous agonists at what used to be called the orphan receptor GPR54 [27]. They play a critical role in reproduction and sexual maturation [28], have limited anticancer effects, bind beta-amyloid and might be useful in neuropsychiatric disorders that involve impairment of sensory gating function, including some forms of schizophrenia that are linked to congenital immune deficiencies [29]. The present invention claims another utility, one that attempts modulating serotonin in the brain to treat depression, anxiety and panic disorder. When synthetic zebrafish Kiss-1 core peptide (pyroglut-NVAYYNLNSFGLRY-NH₂) was administered to the brains of anesthetized zebrafish, increased c-fos mRNA levels were detected in the raphe nuclei. The inventors argue that, since c-fos is a reliable marker for neuronal activity, this implies increased activity of the raphe nuclei, which in turn has the established function of releasing serotonin to the rest of the

brain. Levels of mRNA transcribed from serotonin-related genes such as *Pet1* and *slc6a4a* were significantly increased (3.3-fold, $p < 0.01$ and 2.2-fold, $p < 0.01$, respectively) relative to levels in the control at 8 h after injection. Intracerebral administration would not be required in man because kisspeptins and their synthetic analogs such as TAK-683 [30] readily penetrate the blood–brain barrier. However, the profound hormonal suppression would preclude extended administration.

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WO/2015/100331

Phenazine derivatives as antimicrobial agents

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Keywords: antibacterial agents • phenazines • reactive oxygen species

The bromophenazines of the invention exhibit antibacterial activity but are not antibiotics in the classical sense. Rather, they are believed to generate reactive oxygen species in, or in the immediate vicinity of, any pathogen including protists, fungi and parasites. The inventors were inspired by the observation that many young cystic fibrosis patients first develop *Staphylococ-*

cus aureus lung infections; in later years *Pseudomonas aeruginosa* successfully displaces *S. aureus* using natural redox-active phenazines (such as pyocyanin and phenazine-1-carboxamide) as ‘chemical warfare’ agents [31,32]. In culture tests with *S. aureus* and *Acinetobacter baumannii*, most bromophenazines that are presented here inhibit both suspension (‘planktonic’) growth and biofilm formation; some however exclusively inhibit biofilms. The four compounds possessing the most promising biofilm inhibition activity all have 4-substituted phenyl ester groups on the bromophenazine scaffold. Interestingly, several of the more potent *A. baumannii* ‘biofilm formation inhibitors’ gave slight

(~15%) increases of planktonic growth when tested at 100 μ M. Several compounds also had good dispersal activity against established biofilms at micromolar concentrations. For the peer review companion paper see [33]. Potential deleterious effects on host tissue would have to be considered if such compounds were developed as antibiotics. Also, they might promote opportunistic growth of *P. aeruginosa* in semi-anaerobic environments; inhibitors of *P. aeruginosa* pyocyanin production are being considered for exactly this reason [34].

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- 31 Jayaseelan S, Ramaswamy D, Dharmaraj S. Pyocyanin: production, applications, challenges and new insights. *World*

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Antileishmanial compositions containing fullerol and use thereof

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Keywords: fullerenes • glucantime • Leishmania • liposomes

Spherical carbon nanostructures can enclose and transport drugs, and the fact that they are known to cross cell membranes makes them even more interesting in this respect [35]. They can also have several direct effects; for example, fullerenes are among the most effective radical scavengers while fullerols (caged C60 fullerene hydroxides) are glutamate receptor antagonists [36] and bind well to a broad range of proteins. The present invention adds antileishmanial activity of fullerol. In the standard animal model of visceral leishmaniasis (BALB/c mice infected with *Leishmania infantum chagasi*), fullerol (five doses of 0.05 mg/kg every 4 days) reduced liver parasite burden in a manner equivalent to that observed with the standard therapy, meglumine antimoniate which required much longer

J. Microbiol. Biotechnol. 30(4), 1159-1168 (2014) [PMID: 24214679].

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- 33 Borrero NV, Bai F, Perez C *et al.* Phenazine antibiotic inspired discovery of potent bromophenazine antibacterial agents against *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Org. Biomol. Chem.* 12(6), 881-886 (2014) [PMID: 24389824].
- 34 Miller LC, O'Loughlin CT, Zhang Z *et al.* Development of potent inhibitors of pyocyanin production in *Pseudomonas aeruginosa*. *J. Med. Chem.* 58(3), 1298-1306 (2015) [PMID: 25597392].

administration. Encapsulation of fullerol in liposomes further improved its activity. In addition, hepatotoxic side effects of meglumine antimoniate were reduced when it was given together with liposomal fullerol, without interference with effectiveness of the pentavalent antimonate. The mechanism of the antileishmanial effect is not discussed; it might be related to specific properties of fullereneol interaction with surface proteins and ubiquitin [37], or it might be more general in nature [38].

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- 38 Maleki Dizaj S, Mennati A, Jafari S *et al.* Antimicrobial activity of carbon-based nanoparticles. *Adv. Pharm. Bull.* 5(1), 19-23 (2015) [PMID: 25789215].

For many years much ingenuity has been invested in the development of biocompatible synthetic or semi-synthetic oxygen carriers, with the aim of reducing reliance on donated blood. Besides achieving a high oxygen carrying capacity, proper allosteric responses to O₂ partial pressure are at the center of these efforts: unless the carrier's affinity for O₂ does not diminish in an environment of low oxygenation, it would not release enough of its payload to achieve the purpose.

WO/2015/106242

Bisamide compounds as allosteric effectors for reducing the oxygen-binding affinity of hemoglobin

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Keywords: • allosteric regulation • hemoglobin • propionates

In devising a way to improve local tissue oxygenation by reducing O₂ affinity of normal hemoglobin in a patient's own blood, to treat or prevent organ ischemia and to enhance the efficacy of radiation therapy for cancer, the inventors follow a creative approach that has already been tried with organic phosphates, aromatic propionates such as efaproxiral [39,40], and oligopeptides of tryptophan and phenylalanine derivatives. At 200 μ M, (S)-2-((R)-3-([1,1'-biphenyl]-4-yl)-2-(N,3,5-trimethylbenzamido)propanamido)-3-(1H-indol-3-yl)propanoic acid increased the P₅₀ (the O₂ partial pressure at which the oxyhemoglobin level is 50%) from 17 $\pm$ 0.3 to 24 $\pm$ 0.4 Torr, with an allosteric effect that was greater than that of 2,3-diphosphoglycerate (200 μ M, P₅₀: 19 $\pm$ 0.1 Torr, $p < 0.001$),

with which it acted in synergy. x-ray crystallography of deoxyhemoglobin complexed with this compound (which is related to efaproxiral) revealed its noncovalent binding at the β -cleft on the dyad axis, within the 2,3-diphosphoglycerate binding site.

Published 16 July 2015

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WO/2015/107340

Materials and methods for modulation of tendon healing

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Keywords: alternative splicing • collagen type I • microRNA-29, human • tendons

Tendons consist mostly of viscoelastic collagen fiber bundles; they have evolved for tensile strength, but their healing properties are vastly inferior to those of muscle or bone, although the tenocytes (spindle-shaped fibroblast-type cells that are responsible for synthesis and turnover of the fibers and the extracellular matrix) initiate a process that follows the usual sequentially overlapping pattern of inflammation, proliferation and remodeling. In part, the slow pace of healing can be attributed to a shift in collagen synthesis from type 1 collagen to type 3 during early tendinopathy under the influence of elevated IL-33 expression. The invention rests on the inventors' discovery that tenocytes contain alternatively spliced forms of type 1 collagen which do not contain binding sites for miR-29, a microRNA that is involved in liver fibrosis [41] and systemic sclerosis [42].

As a result, miR-29 affects synthesis of type 1 collagen in tenocytes to a much lesser degree than synthesis of type 3 collagen, and upregulation of miR-29 activity shifts the balance of *de novo* synthesis to 'healthy' type 1 collagen. Extensive immunocytochemical data were obtained using tendon samples from patients undergoing shoulder surgery for tendon cuff tears, and with tendon-derived cells from hamstring tissue of patients undergoing tendon reconstruction. A murine patellar tendon injury model was employed for biomechanical analysis following injection of a transfection complex containing 7.5 ng miR-29a mimic. For the peer review companion paper see [43].

Published 23 July 2015

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Goblet cell replacement therapy

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Keywords: chemokine CXCL10 • CXCR3 receptors • dry eye syndrome • goblet cells

Keratoconjunctivitis sicca (dry eye syndrome) not only develops if the lacrimal glands fail to secrete sufficient

fluid, but also if the lipid composition of the tear film is disturbed. Another important cause for dry eye is dysfunction of the goblet cells in the conjunctiva, which are responsible for secreting the mucins that constitute the inner mucous layer of the tear film. The C-X-C chemokine receptor CXCR3, which is expressed on Th1 effector T cells and epithelial cells (such as goblet cells), is required for the pathogenesis of dry eye disease [44]. Chronic graft-versus-host disease of the conjunctiva is characterized by the expression of Th1-

associated chemokines that bind to CXCR3 [45]. It is therefore of special interest to see an invention claiming activating ligands of CXCR3, such as peptide fragments of the proteins IP-10 (also known as CXCL10), IP-9 (CXCL11) and PF-4 (CXCL4) to increase the number of goblet cells. Modified trabeculectomy was performed in New Zealand white rabbits; bleb tissue treated with two different fragments of IP-10 by intraoperative topical application exhibited an increase in the number and density of goblet cells. This was also observed when the peptides were administered to injured blebs together with mitomycin C, which is used in glaucoma surgery to prevent scarring [46] and increases the risk of developing dry eye syndrome.

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The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies,

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