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Review

Immunomodulatory responses of toll like receptors against 2019nCoV

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Abstract: The present review discusses the immune signals via toll like receptors (TLRs) against 2019nCoV. We researched using different database, up to June 18th, 2020. All the included articles were published in English language. The outcome of this review, that some TLRs agonists or antagonists are progressed as drugs to combat and down regulating TLRs immune signals respectively. TLRs 3 and 4 recognized 2019nCoV spike protein through immune and molecular signals that leading to immune stimulation of pro-inflammatory cytokines and even the immune fever. While the TLRs7 and 8 recognized single-stranded ribonucleic acids (ssRNAs) leading to elevation of the tumour necrosis factor α (TNF-α), interleukin (IL)-6 and -12 levels. TLRs agonists or antagonists utilized as immunotherapeutic targets against 2019nCoV via TLRs signals. Chloroquine and hydroxychloroquine; the approval compounds for 2019nCoV therapy can be inhibiting the class II major histocompatibility complex molecules expression and antigen presentation and even immune suppressions of the pro-inflammatory cytokines profile.

Keywords: 2019nCOV, toll like receptors, immunotherapy, agonists, antagonists.

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Introduction

In December 2019, a novel severe acute respiratory syndrome (SARS)-like coronavirus (CoV) designated 2019nCoV broke up in Wuhan, China. It was causing approximately 60,000 cases and over 1,350 deaths [1]. In January 2020, World Health Organization (WHO) identified the new coronavirus as a novel coronavirus "2019 nCoV" and the disease is COVID-19 [2]. 2019nCoV are positive-stranded ribonucleic acid (RNA) viruses. According to sequence identity of the spike protein (S protien) or the non-structural proteins (ns protein), CoVs are categorized into *Alpha*-CoV, *Beta*-CoV, *Gamma*-CoV, and *Delta*-CoV. The CoVs infection transmitted from birds to mammals [3-6]. Approximately 10-20% of common cold cases annually are caused by CoVs [7-8]. Human CoV (h CoV) accompanying illness is frequently self-limited in immune competent patients and causing severe respiratory tract inflammation in the adolescent and elderlies [4, 9]; unfortunately, 2019nCov infections may be asymptomatic in the early stage until severe pneumonia, dyspnea, renal insufficiency, and even death [2]; However, Huang et al., [10] reported that the severity of 2019nCoV disease is associated with the clinical features and cytokine profile [10]. Chen et al., [11] verified the results of Huang et al., [10]; Nevertheless, enhancement of IL-2R and IL-6 levels in sera from 2019nCoV infected patients, estimated the extreme seriousness of the disease [11]; Additionally, a biopsy from dead 2019nCoV patient declared interstitial mononuclear inflammatory infiltrates in both lungs, dominated by lymphocytes and over activation of T cells accounted [12].

The innate immunity is self-possessed of several patterns of recognition receptors (PRRs) to recognize pathogen-associated molecular patterns (PAMPs); the PRRs involved toll like receptors (TLRs) were discovered 30 years ago interpreting the immune signals of innate immunity, inflammation and cytokines profile [13-14].

TLR1 to TLR10 were signified on primary bronchial epithelial cells, upon TLRs stimulation by specific ligands causing activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); furthermore, increased levels of IL-8, amplified the epithelial cell responsiveness, resulting in altered immune inflammatory responses [15]. On the human alveolar macrophages (MQ); the level of expression of TLRs 3, 5 and 9 was low while TLRs 1, 2, 4, 7 and 8 was high [16].

TLR4 is expressed on lung endothelial cells and is crucial for capillary concealment and neutrophil recruitment after systemic administration of lipopolysaccharide (LPS) [17].

TLR3-deficient mice showed reduction in the lung inflammatory responses [18]. Also, Le Goffic et al [19] demonstrated the immunological role of TLR3 deficiency in Influenza A virus infected mice. They perceived that the survival rates had increased in spite of elevated the viral burden in lungs, reduction in pro-inflammatory cytokines and decreasing in the number of CD8+ T lymphocytes in the bronchoalveolar airspace [19]. Respiratory syncytial virus modulates TLR3 up-regulation, leads to priming the lung epithelial cells for successive exposures to extracellular double-stranded RNA (dsRNA).

There are two dependent pathways for TLRs signals. For instance; TLR3 signals via TIR-domain-containing adaptor protein, inducing interferon beta (TRIF), while TLR7/8/9 rely on myeloid

differentiation factor-88 (MyD88) [15, 20-21]. In the TRIF-dependent pathway, the ubiquitin E3 ligase tumour necrosis factor receptor-associated factor 3 (TRAF3) is recruited and hence activates TANK-binding kinase-1 (TBK1) and Inhibitor-κB kinase ε (IKKε) [22]. Activated TBK1 and IKKε phosphorylate interferon (IFN) regulatory factor (IRF) transcription factors IRF3 and IRF7 to drive the expression of type I IFNs [23].

In the MyD88-dependent pathway, TRAF6 is attached to the MyD88 signal program, resulting in the stimulation of the kinase complex composed of IKKα and IKKβ. Activated IKK complex influences NF-κB activation, which induces the expression of pro inflammatory cytokines [6]. Furthermore, Myd88 also initiates IFN-α by immune promoting IKKα-dependent IRF7 phosphorylation, that is crucial for the antiviral survival in plasmacytoid dendritic cells (pDCs) [24-25]. TLR7 has been associated with stimulation of IFN type-1 which is essential for evacuation of viruses as the SARS CoV. TLR7-positive plamacytoid cells are copiously in lung tissue [26-28]. For example, upon activation of the TLR7 pathway by the influenza virus, pDC have the potential to assemble type I IFN [29]. TLR4 deficient mice exhibit immune suppression in IL-12 level and mononuclear cells infiltration leading to the immune reduction in viral clearance [30].

TLRs immune signals

The damage-associated molecular patterns (DAMPs) originated from the host damaged tissues acting as dangerous signals or ligands to release certain intracellular molecules, some of which specifically enhancing TLRs (as high mobility group box 1 (HMGB1), hyaluronan and heat shock proteins) [31-34]. TLRs signals are implicated in secretion of tumour necrosis factor α (TNF-α), interleukin (IL)-8, macrophage inflammatory proteins (MIP)-1α, -1β, RANTES (regulated upon activation, normal T cell expressed and presumably secreted), growth-regulated oncogene (GRO)-α, -β and -γ, IL-6, IL-5 and transforming growth factor (TGF)-β which are essential for promoting the immune influx of neutrophils, eosinophils, monocytes, natural killer (NK) cells, MQ and dendritic cells (DCs) to evade the pathogen [35-37]. In addition, TLRs immune signals are elaborated in of anti-microbial substances production (as defensins, lysozyme, nitric oxide and IL-37) in the lower respiratory tract [13]. TLRs involved proteins clan in recognition and initiating an appropriate response up against pathogenic attacks [38] as mucins which is the protein component of mucus and its expression is induced either directly or indirectly by TLRs signals [35, 39].

In the lower respiratory tract; TLR3 is expressed on the luminal and basal sides, whereas TLRs 2, 6 and 1 on basolateral side. TLR6 are plenteous. Whereas; TLRs 2, 4, 5 and 10 along with TLRs 3, 7 and 9 in the lower levels are on both cell surface as well as intracellular compartments [40]. TLR4 expressed in the lung endothelial cells is essential for neutrophil recruitment [41] and capillary sequestration [13, 17] while, TLRs 7 and 9 are highly expressed by lung pDCs that downregulate the allergic response. Alveolar MQ express TLRs 3, 5 and 9 and higher levels of TLRs 1, 2, 4, 7 and 8 [13, 16, 28, 42].

TLRs\ 2019 nCoV immune recognitions

2019nCoV are recognized by the three hereditary immune cytoplasmic receptors: toll-like receptors (TLRs), retinoic acid-inducible gene I-like (RIG-I) receptors (RLRs), and nucleotide-binding oligomerization domain-like receptors (NLRs) [43-46]. Immune responses to viral infection are negotiated through TLRs 3, 7, 8, 9, 4 and 2 signals. Each TLR is contributing differently depending on the virus, cell type and infection model examined [47-48]. TLRs7 and 8 detect GU-rich and AU-rich single-stranded ribonucleic acid (ssRNA) sequences of RNA viruses [47-48]. TLRs7 and 8 used as endosomal PRRs for a number of ssRNA viruses as coronaviruses [48-52].

Moreover; TLR7 signaling is well characterized in human and mice for type I IFN production by pDCs in response to ssRNA viral stimuli [48]. TLR-3 may recognize the virus intermediates during intracellular replication dsRNA. The realization of pathogen associated molecular patterns (PAMPs) as viral RNA, particles, or danger signals via endosomal RNA receptors, TLRs 3 and 7 and the cytosolic RNA sensor, RIG-I/MDA5 (melanoma differentiation-associated gene 5). This recognition leads to stimulation of the downstream signaling cascade NF-κB and IRF3 that influence the expression of type I IFN and IL-1β, -6, -18, and TNF-α that activates the janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway that initiate the transcription of IFN type 1 [53-55].

Interestingly; sometimes, accessory proteins of CoV during replication can interfere with TLR3 immune signal and prevent TLR3 stimulation; therefore, CoV evade the immune house. Only TLRs7 and -8 recognize ssRNA viral genomic. Nonetheless, 2019nCoV infects lung alveolar epithelial cells through S-protein containing a ligand binding domain with the angiotensin-converting enzyme II (ACE2) [56]. The TLRs 3 and 4 recognize S-protein and leads to activation of pro-inflammatory cytokines [2, 57-58]. The TLRs7 and 8 recognize SARS-CoV-ssRNAs leading to elevation of the TNF-α, IL-6 and IL-12 levels [58-59].

2019nCoV immune fever is the most common symptom of 2019-nCoV infection. It is resulted from the binding of 2019nCoV with the TLRs causing the release of pro-IL-1b that is cleaved by caspase-1, followed by inflammasome activation and release of active mature IL-1b that is a mediator of lung inflammation, fever and fibrosis [60]. The TLR4 expression is resulted due to the immune fever [61] and identifies S-protein via MyD88, this introduction will provoke the stimulation of NF-κB transcription factors and the pathogen-activated protein kinases (MAPKs) pathway to induce proinflammatory proteins [2, 62-63].

TLRs\2019 nCoV immunotherapy

However, various antiviral drugs have no productive role compared with standard guideline care [64]. TLRs agonists or antagonists utilized as immunotherapeutic targets to up and down regulate TLRs immune responses respectively; Therefore, TLRs are being directed in a dual way by small molecule ligands (agonists) development which have the ability to bind with TLRs and augment host immune capabilities; On the other hand, TLRs act as adjuvants in vaccine therapies and by inhibiting TLRs through specified antibodies, small molecule inhibitors, oligopeptides and by improving endogenous anti-TLR molecules. Also, negative regulation of signal pathways involving TLRs are crucial as a novel therapeutic strategy [65].

2019nCoV Immunotheraby by TLRs agonists

Our review summarizes some essential TLRs agonist: *Mycobacterium bovis* BCG Tokyo 172 (SMP-105). Which is a TLR2 agonist from *Mycobacterium bovis* containing mycolic acids, and peptidoglycans. SMP-105 can be served as an immune adjuvant as

anti-inflammatory agent. The mode of action of SMP-105 starts by upregulating NF-κβ in a TLR2 dependent and TLR4 independent manner resulting in the deficiency of TNF-α and IL-6 levels [66].

Lipoprotein 1 and Lipopeptide-CGP40774 are TLR2 agonists capable of managing asthma and airway inflammation by inhibiting immunoglobulin E (IgE), IL-4 and IL-13 levels [67-68].

Rintatolimod is a TLR3 agonist being investigated for treatment of acute respiratory syndrome, influenza, hepatitis infections and cancer. It regulates the cellular RNase L enzyme levels in patients [69].

Another one is LPS that is a potent functional TLR4 ligand [70]. The LPS stimulating action is initiated by binding the disulfide high-mobility group protein B1 (HMGB1) at low nanomolar avidity with the TLR4 co-receptor myeloid differentiation factor 2 (MD-2), in an analogous way to LPS but at different position [71].

Poly-γ-glutamic acid (γ-PGA) is a TLR4 agonist seems to cause downregulation of Th2 cytokines, airway inflammation and eosinophilia while elevation the manifestation of co-stimulatory molecules, CD80, CD86 and CD40 on dendritic cells [72].

ER803022 is a synthetic TLR4 agonist, it has immune potential against asthma in murine models in a TLR-dependent MyD88 upregulation and IL-12/IFN-γ profile [73].

Diebold et al. [48] have stated that the ssRNA of influenza virus is a TLR7 agonist, and TLR7 has a preference for a particular RNA motif that is uridine. In addition, adenosine- and uridine-rich oligoribonucleotides (ORNs) are capable of stimulating TLR8, without affecting TLR7, while guanosine-rich ORNs activate TLRs7 and 8-dependent signaling [48]. In quest of TLRs 7 and 8 agonists, a range of chemical modifications was done to natural ORN ligands to augment their selectivity for TLR7 and/or TLR8 and stability against nucleases [74-77]. In 2015, Tanji et al. [78] stated for the first time, that TLR8 recognizes the ssRNA degradation products at two different sites are uridine and short ORNs such as UG and UUG. Uridine alone is not capable of stimulating TLR8 in the absence of the ORNs at the second site. The ORN binding with the second site proliferates the affinity of uridine at the first site; Therefore, it leads to stimulation of TLR8 [78]. TLR7 also recognizes the degraded form of ssRNA guanosine binds with the first site while the oligonucleotide (polyU) binds with the second site, and the conjunction of these two products activates the dimerization of TLR7 in a synergistic way [79]. Up regulating TLR7 not only suppresses Th2-mediated airway inflammation but also suppresses the manifestation of IgE titre; Additionally, it seriously inhibits IL-17 and IL-13 production through IL-10 mediated pathway [80]; Therefore, TLR7 is designed to cure airway diseases such as asthma and allergic rhinitis. Heil et al. [47] investigated the effect of ssRNAs on the immune stimulation the APCs via TLRs7 and 8. They proved that guanosine (G) and uridine (U)-rich oligonucleotides enhance the production of TNFα by human PBMCs and that ssRNAs are the natural ligands of TLRs 7 and 8 [47].

TLRs7 and 8 respond to imidazoquinoline compounds. These synthetic compounds have been stated to mediate the TLRs7 and 8 inflammatory responses [53].

Imiquimod is a clinically approved TLR7 agonist against asthma and viral induced lung and airway infection. Imiquimod augments the IFN-α level via TLR7–MYD88 dependent pathway.

Resiquimod (R-848) is a TLR7 and TLR8 agonist enhance IFN-α, IL-12 and TNF-α production while reducing lung eosinophilia and airway inflammation models [81-82]. A synthetic TLR7 agonist SA-2 regulates T cell production through IFN-α, IL-27 and IL-10 [83]. ANA773 is a tiny molecule TLR7 agonist has anti-viral immune potential [84].

Asthma exacerbation is treated by CpG-ODN, which is a TLR9 agonist [85-86]. CpG-ODN interacts with TLR9 present on leukocytes, B cells and T cells and stimulates the secretion of IL-6, IFN-γ, IL-12 and CD4 T lymphocytes, successively stimulating Th1 type of inflammatory response along with enhanced IL-12 and IL-10 production [87]. CpG-ODN also suppresses Th2 cytokine, airway eosinophilia, IgE levels, and bronchial hyperreactivity *in vitro* and *in vivo* models [88-90]. Indoleamine 2, 3-dioxygenase, IMO-2055, MO-2125 and IMO-2134 TLR9 agonists [91-92] are revealed to have anti-inflammatory and anti-allergic responses (*Table* 1).

2019nCoV Immunotherapy by TLR antagonists

This review gives an outline of some natural or synthetic TLRs antagonists (*Table* 2). A small-molecule TLR4-specific antagonist (P5779) prevents HMGB1-MD-2 interaction, protects mice from influenza virus-induced lethality and downregulate pro inflammatory cytokine gene expression in the lungs [93]. Targeting TLR4 with small-molecule antagonists, like TAK-242, or with anti-TLR4-specific antibodies is considered as protection against influenza virus infections [94-95].

A TLR4 is the receptor for advanced glycation end products (RAGE) antagonists, further supports that the HMGB1/RAGE/TLR4-axis is central in the pathogenesis of influenza infections [96]. Disulfide HMGB1-TLR4 stimulation induces a substantial production of proinflammatory cytokines both *in vivo* and *in vitro* [97]. The clinical end results of murine influenza infection indicated that it can be improved by TLR4-specific antagonists.

Table 1. 2019nCoV immunotherapy by TLRs agonists

Agonist	*TLR*	*Reference*
SNIP-105	TLR2	68
CGP40774	TLR2	6, 82
Rintatolimod	TLR3	72
lipopolysaccharide	TLR4	115
γ-PGA	TLR4	56
ER803022	TLR4	11
ssRNA	TLRs7 &8	39, 24
Imiquimod	TLR 7	35, 75
Resiquimod	TLRs7&8	35, 75
SA-2	TLR7	47
ANA773	TLR7	10
CpG-ODN	TLR9	105,91
IMO-205 5, MO-2125 and IMO-2134	TLR9	1, 73

TLR, toll like receptor; γ-PGA, poly-γ-glutamic acid; LPS, lipopolysaccharide.

Table 2. 2019nCoV immunotherapy by TLRS antagonists

Antagonists	*TLR*	*References*
P5779	TLR4	97
TAK-242	TLR4	77, 78
RAGE	TLR4	3
RS-LPS	TLR4	38
N1-0101	TLR4	67
1A6	TLR4	120
Resveratrol	TLRs 3&4	121, 122
Eritoran TM (E5564)	TLR4	97, 98. 88.
OPN-305	TLR2	41
Capsazepine	TLR3	62
R837	TLR7	40

TLR, toll like receptor.

A TLR4 antagonist, under acylated form of rhodobacter sphaeroides LPS (RS-LPS) when inhaled, reduce the symptoms of asthma by inhibiting eosinophilia and lymphocytosis, also by limiting the levels of Th2 cytokines and lower airway hyper responsiveness [98].

RS-LPS acts as a TLR4 antagonist by suppressing the induction of IkBz (gene NFKBIZ, a member of the NF-kB family, play a consequential role in functioning of epithelial cells) in bronchial epithelial cells, consequently leading to inhibition of contribution of pro-inflammatory cytokines in a coculture based study of bronchial epithelial cells and monocytes stimulated with house dust mite mix (HDM) [99].

One of the rare synthesized antibodies that target TLRs is NI-0101, which is specific to TLR4 and blocks TLR4 dimerization. It has the potency to minimize cytokine secretion and averts flu and its symptoms in *ex vivo* and *in vivo* models. This drug has accomplished phase I clinical trial successfully and was proved to be a safe, low-risk and non-toxic drug at various concentrations [100].

1A6 is anti-TLR4 monoclonal antibody effectively in minimizing inflammatory disorders and has real role in lung injury models [101]. Lipid A analogues specifically targets TLR4 signals and has become potential therapeutic tool against airway diseases.

Additionally, eritoran is the TLR4-blocking compound ameliorated murine influenza-induced lung injury by inhibiting the cytokine storm. It has been proved to prevent high mobility group box 1 (HMGB1)-mediated TLR4-dependent signaling *in vitro*, and to suppress extracellular HMGB1 release *in vivo* by preventing necroptotic cell death in respiratory epithelial cells [93, 102]. Eritoran TM (E5564) is a TLR4 antagonist that affects TLR4/MD-2/LPS complex formation which is the most advanced TLR agonist [103]. A fully humanized IgG4 monoclonal TLR2 specific antibody, OPN-305 blocks TLR2-mediated pro-inflammatory cytokine production [104]. R837 is TLR7 antagonist is that plays a critical role in relaxing the airway passage through release of nitric oxide in a TLR7 dependent manner *in vitro* as well as *in vivo* models [105].

Capsazepine and its analogues have been considered as TLR3 inhibitors and they have shown to repress the production of pro-inflammatory cytokines such as IL-8 and TNF-α in asthmatic patients [106].

Resveratrol suppresses TLR4 expression [107] and activated SIRT1 to inhibit HMGB1/TLR4/ MyD88/NF-κB signaling [108]. Apart from synthesized compounds, a natural product known as Resveratrol that is found in grapes and peanuts down regulates TLR3 and one of its adapter molecule (TRIF) and assigns a defensive mechanism against asthma development [109].

Via TLRs signals the immunomodulatory effects of chloroquine (CQ) and hydroxychloroquine (HCQ) synergize their antiviral effects in the treatment of 2019nCoV [110]. The bioimmune action of CQ and HCQ depends on changing the intracellular pH, TLR 7, TLR 9 and cGAS response of interferon genes. Whereas; CQ/HCQ causes irregular obligation between TLRs 7 and 9 and their RNA/DNA ligands, due to changing of pH towards the basic, in the cellular endosomal environment, which suppressed TLR signaling [111-113]. The approaching of STING pathway (TLR and cGAS response of interferon genes) results in the attenuation of pro-inflammatory cytokines [114]. Noteworthy; the activation of TLRs 7 and 9 by their ligands requires the low pH of endosomes and lysosomes and an active autophagic pathway, the CQ/HCQ, through vesicle alkalinization, may inhibit this interaction and TLR signaling, in turn inhibiting the inflammatory response [115]. Matrix metalloproteinases (MMPs) are a family of zinc endopeptidases that regulate inflammation and tissue repair at several levels. On the contrary; CQ has been shown to downregulate MMP-9 expression through the inhibition of TLR9 signaling [116]. In particular, HCQ can enhance the intracellular pH and inhibit lysosomal activity in APCs. Meanwhile, due to the altered pH of endosomes and interrupted binding between TLR7 and TLR9 and their RNA/DNA ligands, TLRs signal are suppressed by administration of HCQ [111-112].

Remdesivir is a broad spectrum anti-viral drug that has shown to inhibit SARS-CoV-2, *in vitro* and *in vivo*. In absence of any effective treatment for SARS-CoV-2 infection (COVID-19), remdesivir has been tried for a compassionate use in severe COVID-19. Remdesivir hold promises as treatment for 2019 nCoV supported by a recent study on 2019nCoV [117] and multiple research studies on SARS and MERS-CoVs [118-120] and a single clinical report [121]. The Chinese government has started a clinical trial using remdesivir for treating 2019nCoV patients with mild to severe symptoms (NCT04252664, NCT04257656). Finally, the current situation also provides an opportunity to evolve innovative treatments to target infectious diseases [1].

2019nCoV Immunotherapy by TLRs-vaccine

Enayatkhania et al, [122] have testified and prognosticated NOM recombinant protein against HLA-A11:01 and TLR4 receptors. The vaccine could be considered as a possible vaccine candidate against 2019nCoV by stimulating cellular and humoral immune responses.

Conclusion

In this paper, we researched a literature (over 300 ones) using different database as the PubMed, up to June 18th, 2020. We used a specific key word as: COVID19, toll like receptors and COVID19, TLRs immunotherapy, antiviral compounds etc. All the included articles were published in English language. Our studies on the immune signals via the toll like receptors (TLRs) against 2019nCoV. The outcome of this review, that some TLRs agonists or antagonists are utilized as immunotherapeutic targets against 2019nCoV. The TLRs 3 and 4 recognize S-protein and leads to activation of pro-inflammatory cytokines [2, 57-58]. 2019nCoV-immune fever is the most common symptom of 2019nCoV infection resulted from the binding of 2019nCoV with the TLRs causing the release of pro-IL-1b. It is cleaved by caspase-1, followed by inflammasome activation and release of active mature IL-1b that is a mediator of lung inflammation, fever and fibrosis [60]. Via TLRs signals, the CQ and HCQ were inhibiting the immune responses. The bioimmune action of CQ and HCQ is causing irregular obligation between TLRs 7 and 9 and their RNA/DNA ligands, depends on changing the intracellular pH modifying the intracellular pH towards the basic, in the cellular endosomal environment. Recently in 2020, Enayatkhania, et al, [122] have testified NOM recombinant protein against HLA-A11:01 and TLR4 receptors and prognosticated that it could be considered as a possible vaccine candidate against 2019nCoV. Substantially; in 2019nCoV- immune fever; the high light must be focused on TLRs immunotherapy as an innate immune innovative alternative to some antivirals resist drugs by utilizing TLRs agonists or

antagonists to up and down regulate TLRs immune responses respectively

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Conflict of interest

No competing financial interests exist

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