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REVIEW

Recent progress in the development of Toll-like receptor (TLR) antagonists

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ABSTRACT

Introduction: Pattern recognition receptors (PRRs) of the innate immune system mediate and control the activation and progression of adaptive immunity. Toll-like receptors (TLRs) are the most notable of the PRRs: they play crucial roles in protecting the host body against invading pathogens or endogenously released hazardous molecules. Sustained TLR signaling even after the clearance of pathogens or failure to distinguish between 'self' and 'non-self' molecules can cause inflammatory disorders, autoimmune diseases, and cancer.

Areas covered: This review focuses on recently developed therapeutic agents with TLR-antagonistic activities.

Expert opinion: In recent years, several research institutes and pharmaceutical companies have achieved fundamental successes in inhibiting or reducing TLR signaling and associated effector mechanisms by using novel inhibitors. These inhibitory molecules include antibodies against TLRs, TLR-derived transmembrane (TM) peptides, bacterial-secreted proteins, and natural or synthetic small molecules, peptides, and proteins. Antagonist developers generally target the TLR ectodomain to block receptor activation. The TM and cytosolic Toll/IL-1 receptor domains also have regions that should be explored for the design of peptide-based and small molecule blocking agents. A number of preclinical and clinical breakthroughs may result in the availability of improved TLR immunomodulatory drugs to address important unmet medical needs.

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Toll-like receptor; innate immunity; antagonist; patent; clinical trial

1. Introduction

The vertebrate immune system is an ancient defense mechanism that protects the host from extracellular or endogenous pathogenic substances. It is sustained by complex crosstalk between the innate and adaptive immune systems, with innate constituents instructing the adaptive components.[1] The innate immune recognition mechanism is composed of several families of pattern recognition receptors (PRRs) as the primary defense against microbial infections.[2–4] These PRRs commonly recognize conserved pathogen-associated molecular patterns (PAMPs) from microbes or endogenous danger-associated molecular patterns (DAMPs) released from necrotic cells. Upon detecting a PAMP or DAMP, they trigger a signaling cascade that activates innate antimicrobial responses through the production of proinflammatory cytokines, type I interferons (IFNs), chemokines, and antimicrobial peptides.[5] These chemical signals activate B and T cells, thus linking the innate and adaptive immune responses.

Toll-like receptors (TLRs) were the first mammalian PRRs to be cloned.[6] They are the most well-characterized PRRs and subjects of considerable investigation.[7–10] TLRs include 10 functional subtypes in humans (TLR1–10), of which TLR1, 2, 4, 5, 6, and 10 are expressed on the cell membrane and TLR3, 7, 8, and 9 are located on endosomal membrane. The differential subcellular localizations of these homologous TLRs largely correlate with the type of molecular patterns they recognize (Table 1 of [11]). Several studies have indicated that UNC93B1, a 12-pass transmembrane (TM) chaperone protein, mediates trafficking of TLR3, 5, 7, and 9 from the endoplasmic reticulum to the endolysosome membrane through direct interaction with TLRs or by recruiting an adaptor protein complex 2 (AP-2), although the complete translocation mechanism is still not clearly understood.[12–14]

Each TLR recognizes a wide range of PAMPs derived from bacteria, viruses, fungi, and parasites,[9] including lipoprotein (TLR1, 2, and 6),[15,16] lipopolysaccharide (LPS) (TLR4),[17] flagellin (TLR5),[18] double-stranded (ds) RNA (TLR3),[19] single-stranded RNA (TLR7 and 8),[20] and CpG containing unmethylated DNA (TLR9).[21] The cognate ligand of TLR10 has not yet been defined; however, recently, researchers have noted its expression during certain bacterial and viral infections.[22–24]

TLRs are type I TM proteins with a tripartite domain architecture: (1) an extracellular recognition domain, also called an ectodomain, containing tandem copies of leucine-rich repeats (LRR), (2) a single-pass TM domain, and (3) a cytoplasmic Toll/IL-1 receptor (TIR) downstream signaling domain. Upon encountering their respective PAMPs/DAMPs, TLRs undergo either homodimerization or heterodimerization, recruit adaptor proteins, and initiate a complex process of downstream signal transduction events, leading to the expression of inflammatory cytokines and IFN (Figure 1). Additionally, TLR7, 8, and 9 exist as preformed inactive homodimers that undergo conformational reorganization after stimulation by an agonist, whereas antagonist binding does not affect their conformational status.[25,26] TLR signaling pathways have been reviewed in detail previously.[5,11]

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Article highlights

- Toll-like receptors (TLRs) are important regulators of innate and adaptive immune responses.
- Aberrant TLR signaling has been implicated in several autoimmune and inflammatory diseases, including cancer.
- Antagonists developed for downmodulating overactive TLR signaling include small molecules, aptamers, oligonucleotides, peptides, proteins, and antibodies.
- In addition to the extracellular domain, the transmembrane and Toll/IL-1 receptor domains have been explored to develop more effective TLR blockers.
- Some antagonists interfere with intracellular adaptors, alter the pH of endosomal compartments, or neutralize oligonucleotide agonists through weak interactions.
- Some antagonists have either completed or are in advanced phase III clinical trials and have demonstrated safety and efficacy.

This box summarizes key points contained in the article.

The innate immune system, governed by TLRs, performs a defensive role that contributes to the survival of the host by promptly neutralizing external or internal threats. However, overactivation or dysregulation of this system takes a heavy toll on the host, causing myriad diseases such as autoimmunity, inflammation, and even cancer.[27,28] Therefore, countering uncontrolled TLR-mediated signaling has been a major subject of research in the last decade or more.[29] Since the initial discovery of TLRs almost 18 years ago, our understanding of TLR structure and signaling pathways has improved greatly. Three-dimensional structural data showing the ligand recognition mechanisms of all human TLRs, except for TLR10, have been deposited in public domains.[30] This has led pharmaceutical industries and research institutions to develop TLR modulators (agonists and antagonists) to achieve various therapeutic goals. Some excellent reviews have been published on TLR agonists, and, to a lesser extent, on TLR antagonists.[31–33] However, no comprehensive reviews of antagonist patent records have been attempted, while reviews have been presented for agonists. In recent years, in response to detrimental inflammatory disorders, various patent-granting authorities have issued pharmaceutical patents for a number of anti-inflammatory compounds based on strong preclinical evidence. Currently, several TLR antagonists either are in or have completed clinical trials. Here, we present a review of recently developed TLR antagonists reported in the literature and patent publications (2011–2015) (Table 1).

2. TLR antagonists

TLR antagonists are immune system regulators that inhibit or reduce activation of TLR-mediated cytokine cascades and check over-reactive, uncontrolled adaptive immune responses. They are being tested as novel therapies to treat or prevent autoimmune disorders, inflammatory diseases, and cancer. TLR antagonists, in general, are altered natural agonists or agonist imitators that competently bind TLRs, but that fail to induce the structural rearrangement required for signal transduction. The most well-known TLR antagonists are Eritoran (E5564) [34] and TAK-242 (Resatorvid),[35] which bind to the extracellular LPS binding pocket and intracellular TIR domain, respectively.

Table 1. Patent applications or patents published during the last 5 years (2011–2015*).

	Pub./Patent no.	Pub./Patent date	Title	Applicant/Assignee	Targeted TLR	Targeted diseases
1	US8734794	27 May 2014	Humanised antibodies to Toll-like receptor 2 and uses thereof	Opsona Therapeutics, Ltd.	TLR2	I/R injury, renal inflammation, diabetes, and uveitis
2	WO2013144345	14 November 2013	Toll-like receptor 2 binding epitope and binding members thereto	Opsona Therapeutics, Ltd.	TLR2	Chronic inflammatory conditions
3	WO2013034736	14 March 2013	Use of staphylococcal superantigen-like 3 (SSL3) as a TLR2 inhibitor	Umc Utrecht Holding B.V.	TLR2	TLR2-mediated inflammation
4	US8986712	24 March 2015	Peptides derived from HIV-1 gp41 transmembrane domain for T-immunomodulation	YRD Co., Ltd.	TLR2 and 4	Sepsis, Crohn's disease, atherosclerosis, neurodegenerative diseases, and RA
5	US20130225478	29 August 2013	Peptides based on the transmembrane domain of a Toll-like receptor (TLR) for treatment of TLR-mediated diseases	YRD Co., Ltd.	TLR1, 2, 4, or 6	TLR-mediated disease
6	WO2014022287	6 February 2014	Antagonists of the Toll-like receptor 1/2 complex	TRUC, A Body Corporate	TLR1/2	TLR1/2-mediated inflammation
7	US8153583	10 April 2012	Toll-like receptor 3 antagonists, methods and uses	Carton et al.	TLR3	Sepsis, inflammatory bowel disease, inflammatory pulmonary conditions, diabetes, and autoimmune diseases
8	US20140212426	31 July 2014	Toll-like receptor 3 antagonists	Janssen Biotech, Inc.	TLR3	Inflammation and metabolic diseases
9	WO2012099785	26 July 2012	Modulators of TLR3/dsRNA complex and uses thereof	TRUC, A Body Corporate	TLR3	Infectious and inflammatory diseases
10	WO2015016282	5 February 2015	Pharmaceutical composition for preventing or treating radiation-induced gastrointestinal syndrome	The University Of Tokyo	TLR3	Radiation-induced gastrointestinal syndrome

(Continued)

Table 1. (Continued).

	Pub./Patent no.	Pub./Patent date	Title	Applicant/Assignee	Targeted TLR	Targeted diseases
11	US8333978	18 December 2012	Poly-TLR antagonist	Cadila Pharmaceuticals	TLR3, 4, 5, 6, 7, 8, and 9	Sepsis, multiple sclerosis, optic neuritis, COPDs, and multiple myeloma
12	US9072760	7 July 2015	TLR4 inhibitors for the treatment of human infectious and inflammatory disorders	University of Pittsburgh – of the Commonwealth System of Higher Education	TLR4	Infectious, inflammatory and posttraumatic disorders
13	CN104546874	29 April 2015	Application of stibene glucoside in preparing toll like receptor 4 (TLR4) innate immune system inhibitor	Yunnan University of Traditional Chinese Medicine	TLR4	TLR4-mediated inflammation
14	CN103622982	12 March 2014	Application of ginsenoside Rg1 in preparation of medicine for preventing antiendotoxin from acting on Toll-like receptor 4	Zhejiang University	TLR4	Inflammation caused by LPS
15	US8642614	4 February 2014	Toll-like receptor modulators and uses thereof	TRUC, A Body Corporate	TLR4	Chronic pain and nociception
16	WO2014160077	2 October 2014	Compositions to reduce pain comprising an opioid/Toll-like receptor 4 antagonist, dextro enantiomers thereof, and methods of use therefor	Allodynic Therapeutics, LLC.	TLR4	Neuropathic pain
17	US8734790	27 May 2014	Anti-TLR4 antibodies and methods of use thereof	Novimmune SA	TLR4	Transplant rejection
18	US8153777	10 April 2012	Modulation of Toll-like receptor 5 expression by antisense oligonucleotides	Idera Pharmaceuticals, Inc.	TLR5	TLR5-mediated diseases
19	WO2011009015	20 January 2011	(+) Morphinans as antagonists of Toll-like receptor 9 and therapeutic uses thereof	Mallinckrodt, Inc.	TLR9	Neuropathic pain, inflammatory and autoimmune disorders, neurodegenerative disorders, and cancer
20	WO2014181131	13 November 2014	Treatment of cancer with naltrexone	Cancer Vaccine Institute	TLR9	Supportive care of subject having a tumor/cancer
21	US8940310	27 January 2015	Methods of treatment using TLR7 and/or TLR9 inhibitors	Dynavax Technologies Corporation	TLR7 and 9	Diseases caused by TLR7 and/or TLR9 activation
22	US9228184	5 January 2016	Human Toll-like receptor inhibitors and methods of use thereof	DT Corporation	TLR7, 8, and 9	Autoimmune disease or an inflammatory disorder
23	US20140193396	10 July 2014	Immune regulatory oligonucleotide (IRO) compounds to modulate Toll-like receptor-based immune response	Idera Pharmaceuticals, Inc.	TLR7, 8, and 9	Cancer, autoimmune and inflammatory disorder, airway inflammation, and allergy
24	US20120183564	19 July 2012	Inhibition of endosomal Toll-like receptor activation	Sullenger Bruce A.	TLR3, 7, 8, and 9	Autoimmune or inflammatory response
25	WO2012118911	7 September 2012	Oligonucleotide modulators of the Toll-like receptor pathway	Quark Pharmaceuticals, Inc.	TLR2, TLR4, MyD88, TICAM1, and TIRAP	*Inter alia*, acute and chronic inflammation, neuropathic pain, and primary graft dysfunction
26	CN102218058	6 March 2013	Application of sparstolenin B as TLR2 (Toll-like receptor 2)/TLR4 (Toll-like receptor 4) antagonist in pharmacy	Nanjing University of Chinese Medicine	TLR2 and 4	Atherosclerosis, I/R, cancer, inflammatory bowel disease, diabetes, Alzheimer's disease, chronic pulmonary disease, and sepsis

Data obtained from the European patent office database (URL: *http://worldwide.espacenet.com*).
*Only a single patent record (US9228184) in early 2016 has been included because of its importance.
IRO: Immune regulatory oligonucleotide; TLR: Toll-like receptor; LPS: lipopolysaccharide; SSL: staphylococcal superantigen-like 3; RA: rheumatoid arthritis; YRD: Yeda Research and Development; TRUC: The Regents of the University of Colorado; DT: Dynavax Technologies; COPD: chronic obstructive pulmonary disease; TIRAP: TIR domain containing adaptor protein; TICAM1: TIR domain containing adaptor molecule 1; I/R: ischemia/reperfusion.
The patents are listed according to their order of appearance in the article.

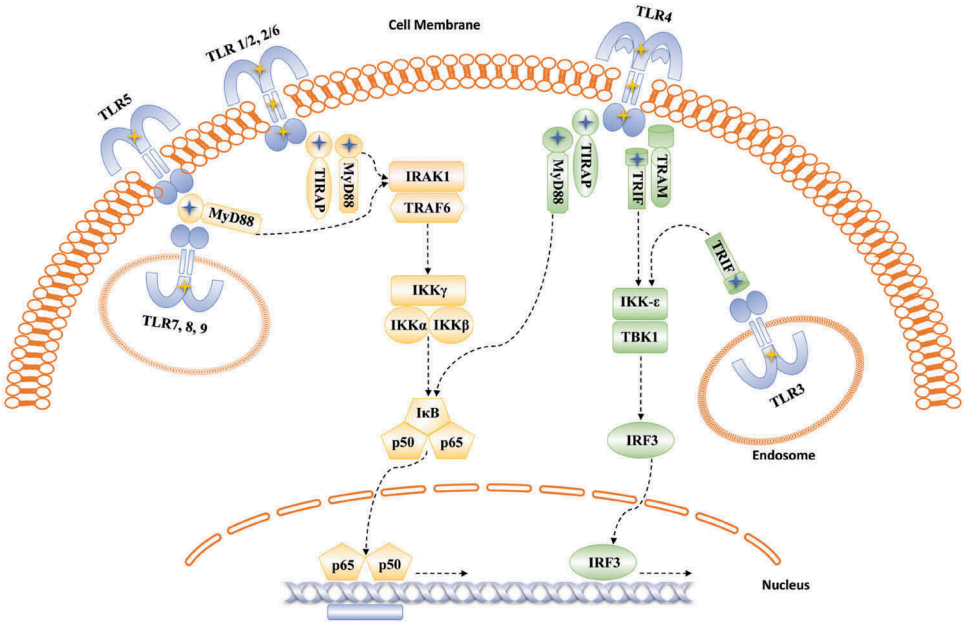

Figure 1. A general overview of TLR signaling pathways. The full names of proteins shown in the figure are as follows. MyD88: Myeloid differentiation primary response protein 88; TIRAP: TIR-domain-containing adaptor protein; IRAK1: IL-1R-associated kinase 1; TRAF6: Tumor-necrosis-factor receptor-associated factor 6; IKK: Inhibitor of nuclear factor-κB (IκB)-kinase; IRF3: Interferon regulatory factor 3; TBK1: TRAF family member-associated NF-kappa-B activator (TANK) binding kinase; TRIF: TIR domain-containing adaptor–inducing interferon-β; TRAM: TRIF-related adaptor molecule. Targets of agents patented within last five years are indicated by a "star" symbol. Multiple star symbols on certain TLRs indicate the respective domains have been targeted by the agents. See text for description.

Two different but simultaneous pathways of signal transduction are known by which TLRs activate an adaptive immune response. One is through the adaptor MyD88, which is used by all TLRs except for TLR3. TLR3 operates through the TRIF pathway. TLR4 signals through both TRIF and MyD88 pathways. In the MyD88 pathway (also called the canonical pathway), conformational alteration of TLR homo/heterodimers allows binding of the TLR-TIR domain to the MyD88-TIR domains through homotypic TIR-TIR interaction. Subsequently, MyD88 recruits the kinase IRAK4 by death domain (DD)-DD interactions. IRAK4 binds and phosphorylates IRAK1, a natural substrate of IRAK4. Phosphorylated IRAK1 brings TRAF6 to the receptor complex. TRAF6 and IRAK1 dissociate from the receptor complex and form another complex with transforming growth factor β activated kinase 1 (TAK1), which coexists with the adaptor TAK1-binding protein 2 (TAB2) and activator TAB1 at the plasma membrane. IRAK1 phosphorylates both TAB1 and TAK1, and is degraded afterward, facilitating the translocation of TRAF6, TAK1, TAB1, and TAB2 to the cytoplasm where ubiquitin-conjugating enzyme 13 (UBC13) and ubiquitin-conjugating enzyme E2 variant 1 (UEV1A) ubiquitinate TRAF6, inducing activation of TAK1. Next, TAK1 phosphorylates mitogen-activated protein kinase (MAPK) and IKK complexes. The IKK complex, in turn, phosphorylates the IκB subunit of NF-κB, leading to its ubiquitination and subsequent degradation. This allows nuclear translocation of NF-κB to induce transcription of target genes. MyD88 works in concert with an upstream DD-lacking adaptor TIRAP, also called MyD88 adaptor-like (MAL), particularly during TLR1/2, 2/6, and TLR4 signaling. The TRIF pathway, also called the MyD88-independent or alternate signaling pathway, is specifically activated by TLR3 and TLR4. TRIF, along with a second, upstream adaptor, TRAM, stimulates IKK-ε and TBK1. IKK-ε, the counterpart of IKK in the canonical pathway, and TBK1 mediate activation and nuclear translocation of IRF3 to induce transcription of antiviral IFNs.

Oligonucleotide ligands similar to nucleic acid agonists have been successfully tested as downregulatory inflammatory mediators. In addition, several small natural or synthetic molecules that are quite distinct from natural TLR agonists have proven to be efficient TLR signaling blockers. In addition, peptide- and protein-based inhibitors, including antagonistic antibodies, have shown promise in reducing TLR effector functions. Some notable TLR antagonists recently reported in the literature are summarized in Table 2.

2.1. TLR1, 2, and 6

Based on molecular phylogenetic analysis, TLR1, 2, 6, and 10 have been classified into one family, designated the TLR1 family.[59] They have highly similar primary sequences and cooperate with one other for PAMP recognition.[60] TLR2 can heterodimerize with either TLR1 to detect triacyl lipopeptides of Gram-negative bacteria [16] or TLR6 to recognize diacyl lipopeptides of Gram-positive bacteria.[15] In addition, TLR2 recognizes endogenous molecules released during stress, cell death, and various diseases. Dysregulated PAMP/DAMP recognition by TLR2 causes the continuous production of inflammatory cytokines, leading to the onset of autoimmune and inflammatory diseases, such as rheumatoid arthritis (RA), atherosclerosis, systemic lupus erythematosus (SLE), sepsis, ischemia/reperfusion (I/R) injury, and pulmonary tumor metastasis (PTM).[61] Therefore, there is a pressing need to develop TLR2 antagonists to modulate TLR2 signaling and inhibit cytokine production during inflammatory and autoimmune diseases.

Several efforts are underway to develop antibodies that react with TLR2. For example, Opsona Therapeutics has developed a humanized TLR2 antagonistic antibody, named OPN-305,[62] which prevents I/R injury associated with organ transplantation. It is currently undergoing a phase II clinical trial to evaluate its safety, efficacy, and tolerability in delayed graft function (NCT01794663).[39,63] The three-dimensional structure of the antibody has revealed that the antibody binds to an epitope consisting of residues from LRR11 to 14 of TLR2 and interferes with agonist-induced dimerization.[64] This indicates that further attempts to target this epitope may provide novel TLR2-specific antagonistic binding members homologous to OPN-305.

Immune modulation of TLR2 has also been attempted using a unique inhibitor of bacterial origin called staphylococcal superantigen-like 3 (SSL3) protein.[38] SSL3 surrounds the lipopeptide binding pocket on the TLR2 ectodomain (LRR11–13), preventing agonist access to the receptor cavity. Further,

Table 2. List of selected TLR antagonists recently reported in the literature (2011–2015). The listed antagonists are small molecules unless otherwise indicated.

No.	Antagonist name	Target receptor	Binding region	Disease models	Reference
1	CU-CPT22	TLR2	Pam3CSK4 binding site	TLR2-mediated immune responses	[36]
2	C29 and Ortho-vanillin	TLR2	TIR domain (BB loop)	TLR2-mediated immune responses	[37]
3	SSL3 protein	TLR2	Lipopeptide binding pocket (LRR11–LRR13)	TLR2-mediated immune responses	[38]
4	OPN-305 (antibody)	TLR2	Ectodomain	I/R injury; acute myocardial infarction	[39]
5	SsnB	TLR2, 4	TLR2 and TLR4 TIR domains or TIRAP/MAL	TLR2- or TLR4-mediated inflammatory diseases	[40]
6	Vizantin	TLR4	TLR4/MD-2 complex	Endotoxin-mediated immune responses	[41]
7	(+)-*N*-phenethylnoroxymorphone	TLR4	LPS binding pocket	Potentiate morphine analgesia	[42]
8	VB3323 (peptide)	TLR4		Neuroinflammation and neurodegenerative diseases	[43]
9	Monosaccharide 3	TLR4	LPS binding pocket	Sepsis	[44]
10	(+)-Naltrexone and (+)-naloxone	TLR4	LPS binding pocket	Neuropathic pain; opioid reward and reinstatement	[45]
11	HT52, HTB2 (antibody)	TLR4	LRR2 (Phe^{75}, Ser^{76}, Pro^{79})	LPS-induced septic shock	[46]
12	Compound 4a	TLR3	TLR3/dsRNA binding interface	Viral infection and radiation-induced gastrointestinal syndrome	[47,48]
13	CNTO2424 (antibody)	TLR3	TLR3 ectodomain	Downregulates poly(I:C)-induced production of IL-6, IL-8, MCP-1, RANTES, and IP-10	[49]
14	TH1020	TLR5	TLR5/flagellin interface	TLR5-mediated immune disorders	[50]
15	INH-ODN (nucleotide)	TLR9	Undetermined	Autoimmune diseases, such as SLE	[51]
16	E6446	TLR8, 9	DNA or RNA and accumulates in acidic compartments	Malaria	[52,53]
17	AT791	TLR7, 9	DNA or RNA and accumulates in acidic compartments	Autoimmune diseases, such as SLE	[52]
18	CpG ODN 2088 (nucleotide)	TLR9	TLR9-DNA interface	Traumatic spinal cord injury	[54]
19	COV08-0064 (nucleotide)	TLR9	TLR9	Acute liver injury and acute pancreatitis	[55]
20	Oligonucleotides	TLR7, 8, 9	TLR7, 8, 9	Autoimmune and inflammatory diseases	[56]
21	Anti-microRNA oligonucleotides	TLR7, 8	Off target effect	Viral and bacterial infections	[57]
22	2R9 (peptide)	TLR2, 4, 7, 9	TIRAP	Influenza	[58]

TLR: Toll-like receptor; SSL: staphylococcal superantigen-like 3; MAL: MyD88 adaptor-like; LPS: lipopolysaccharide; SsnB: sparstolonin B; SLE: systemic lupus erythematosus; I/R: ischemia/reperfusion.

SSL3 was shown to obstruct activation of the already lipopeptide-bound TLR2, limiting the conformational change required for downstream adaptor recruitment. In addition, an SSL3-mimicking antibody or peptidomimetic has been identified that is effective in alleviating TLR2-mediated inflammatory conditions.[65]

In a novel approach, Yeda Research and Development (YRD) has used 10–30 residue long TM peptides derived from HIV gp41 protein to treat pathologies associated with T cell and/or monocyte activation. The peptides were shown to inhibit TNF-α secretion from macrophages by interfering with the activation of TLR2 and 4 in response to lipoteichoic acid and LPS.[66] Interestingly, YRD also claims that TM peptides selected from TLR1, 2, 4, and 6 with or without cytoplasmic and extracellular juxtamembrane residues prevent cell activation by these TLRs.[67] The 4–30 amino acid long synthetic peptides can be used to treat TLR1, 2, 4, and 6-mediated inflammatory diseases, including septic shock, Crohn's disease, atherosclerosis, neurodegenerative diseases, and RA. These antagonists are in preclinical stages, and clinical trials are required to prove their efficacy for human use. Some of the most common issues with these antagonists could be their accurate delivery to TM targets in sufficient quantities to get the desired effect. The TM peptides have a tendency to self-aggregate forming dimers and trimers, they can cause immunogenicity, and due to short half-life and fast elimination profile their actions can be short-lived.

In recent years, several efforts have been made to develop small molecule or antibody antagonists for TLR2.[36,39,68] However, none of these molecules have been found suitable for human use. Antagonist developers target different domains on TLRs; for instance, the extracellular, TM or TIR domains. The BB loop on the surface of the TIR domain is a crucial structural scaffold for MyD88 recruitment. A computer-aided screening study targeting the BB loop pocket identified a small molecule antagonist C29 and its derivative *ortho*-vanillin that potently blocked interactions between TLR and MyD88. This suggests that additional efforts to target the BB loop pocket within the TLR2 TIR domain may yield novel small-molecule therapeutics with improved antagonistic properties.[37] The TLR1/2 complex has been shown to induce cytokine production in response to cytomegalovirus (CMV), lymphocytic choriomeningitis virus (LCMV), and herpes simplex virus-1 (HSV-1) infection, causing inflammatory conditions such as acne, sepsis, and PTM. In a filing from The Regents of the University of Colorado (TRUC), small molecules based on a benzotropolone scaffold that shows TLR1/2 complex inhibitory properties were claimed to attenuate the effects of both acute and chronic inflammatory conditions.[69] The molecules are suitable for oral administration and can be used to treat PTM and inflammation caused by CMV, LCMV, and HSV-1 infection. The benzotropolone antagonist (CU-CPT22) competes with synthetic triacylated lipoprotein (Pam3CSK4) and binds at the interface between TLR1 and TLR2 dimers, similar to the agonist.[36]

2.2. TLR3

TLR3 recognizes viral dsRNA released following the death of infected cells. Activation of TLR3 signaling culminates in the production of type 1 IFN and proinflammatory cytokines TNFα, IL1, and IL6. These immune signaling molecules further activate innate and adaptive immune cells, allowing the host to mount a protective response during viral infections. TLR3 deficiency is associated with severe outcomes, such as

susceptibility to herpes simplex encephalitis and coxsackievirus infection.[70,71] On the other hand, a dysregulated and sustained TLR3-mediated immune response can contribute to severe pathogenesis and death during certain viral infections. Therefore, controlling dysregulated TLR3 signaling in several diseases might improve the lives of suffering patients.

Carton et al. have disclosed that a TLR3-reactive monoclonal antibody (mAb) inhibits production of RANTES and/or IL6, IL8, and MIP1α, which are secreted as a result of TLR3 activation.[72] This therapy can be used in cases of sepsis, inflammatory bowel disease, inflammatory pulmonary conditions, diabetes, metabolic syndrome, and autoimmune diseases. In addition, Janssen Biotech claims that a number of TLR3-specific mAb antagonists reduce NFκB activation and inhibit IL6 and IFNγ production by >50%.[73] Binding assays, crystallography, and protein–protein docking on selected variants have revealed critical binding epitopes on the surface of TLR3. Further, mutagenesis studies have shown that two N-terminal residues, D116 and K145, and three C-terminal residues, K467, R488, and R489, which are proximal to the dsRNA recognition sites, are essential for the inhibitory effect of these mAbs.[49]

TRUC has developed a small-molecule inhibitor (compound 4a) that competitively binds to the TLR3–dsRNA binding groove with high affinity and specificity, thus blocking the accessibility of dsRNA to TLR3.[47,74] TLR3 has been implicated in radiation-induced gastrointestinal syndrome, a lethal illness where radiation-sensitive hematopoietic stem cells are severely affected upon exposure to ionizing radiation. This exposure leads to cell death and immune deterioration because of an insufficient supply of platelets and leukocytes.[48] The University of Tokyo claims that a small-molecule inhibitor of the TLR3/dsRNA complex, (R)-2-(3-chloro-6-fluorobenzo [b]thiophene-2-carboxamido)-3-phenylpropanoic acid ameliorates radiation-induced gastrointestinal syndrome.[75] However, all of these antagonists are in preclinical developments. Clinical trials could evaluate the key issues concerning TLR3 antagonists, such as their accurate delivery to the endosomal compartment through appropriate carriers/conjugates showing safety and efficacy.

2.3. TLR4

TLR4 has therapeutic importance because of its role in severe sepsis associated with Gram-negative bacterial infection, autoimmune diseases, inflammation, and posttraumatic disorders. It recognizes the bacterial endotoxin LPS, which is released from bacteria because of cell wall repair, biosynthesis, or death. LPS binding protein carries and delivers LPS to a coreceptor, cluster of differentiation 14, which loads it on myeloid differentiation protein-2 (MD-2) attached to TLR4. TLR4 signaling that follows LPS recognition induces a strong immune reaction. The signaling cascade downstream of TLR4 involves recruitment and activation of two adaptor proteins, MyD88 and TRIF, which enable nuclear translocation of NFκB and IRF3, respectively, leading to the production of large amounts of inflammatory cytokines and IFNs, a response termed as 'cytokine storm.' LPS is such a potent stimulator of TLR4 that even a tiny amount can cause septic shock in patients. Cadila Pharmaceuticals has disclosed Mycobacterium w (Mw) or its constituents having antagonistic effects on several TLRs, including TLR3, 4, 5, 6, 7, 8, and 9 activated by agonists such as virus particles, LPS, CpG DNA, and oligodeoxynucleotides.[76] Administration of Mw to patients suffering from sepsis for 28 days significantly reduced mortality, days on a ventilator, days in an intensive care unit, length of hospital stay, and sequential organ failure.[77] A phase III clinical trial to evaluate its efficacy in patients with severe sepsis is ongoing (NCT02330432).

University of Pittsburgh has developed a number of TLR4 antagonists (referred to as T4ICs) based on the compounds isopropyl 3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxyhexopyranoside, 2-(acetylamino)-2-deoxy-D-galactopyranose hydrate, and 2-(acetylamino)-2-deoxy-4-*O*-hexopyranosylhexopyranose. These molecules were found to reduce NFκB activation and cytokine production during infections and inflammatory disorders, including sepsis, RA, ulcerative colitis, Crohn's disease, and posttraumatic disorders.[78] Stilbene glycoside is a plant-derived natural compound that has shown antioxidant,[79] antiangiogenic,[80] and antimalarial [81] properties in experimental settings. Recently, a novel stilbene glycoside was found to effectively reduce TLR4-mediated NFκB activation to a greater extent and to inhibit inflammatory cytokine release.[82] In addition, a natural plant (genus *Panax*; ginseng) product, Ginsenoside Rg1, prevents endotoxins from activating TLR4.[83]

Accumulating evidence suggests that TLR4 can directly recognize opioid ligands, such as morphine, and trigger neuroinflammatory response.[84] Therefore, blocking the TLR4-MD2 complex using inhibitor/antagonists would be beneficial in treating morphine-induced proinflammation. A series of small molecule inhibitors of the TLR4/MD-2 complex (compound 1 and its analog) have been designed through structure–activity relationship (SAR) studies that potentiate morphine analgesia by blocking opiate-induced TLR4 activation.[85,86] Moreover, the recognition that opioid antagonists function as TLR4 antagonists suggests that targeting TLR4 to treat neuropathic pain may be effective. The dextroenantiomers of opioid/TLR4 antagonists, naltrexone, naloxone, and nalmefene, have been used for the treatment, prevention, and reversal of neuropathic and nociceptive pain.[87] They specifically antagonize TLR4 without affecting the opioid receptors, thus evading the side effects caused by opioid receptor antagonism. Furthermore, the use of at least one α2 adrenergic receptor agonist, acetyl para aminophenol, a cyclooxygenase (COX) inhibitor, and an α2δ ligand boosts the antagonists' effect. Toledano (Allodynic Therapeutics, LLC) is developing a synergistic composition of these compounds, designated ATNC05, as an investigational drug. It has completed a phase II trial showing good safety and efficacy profiles in patients with chronic, neuropathic back and cervical pain (NCT01415895).

The major risk factor after organ or tissue transplantation is rejection of the transplanted biological material by the host immune system because of the adaptive immune response of T and B lymphocytes, resulting in organ failure, further morbidity, and mortality. TLRs expressed on nonimmune cells (such as kidney cells) can recognize endogenous molecules, such as HMGB1, which are released into the intercellular milieu

after normal apoptotic events, and can initiate inflammation through B and T cells.[88] Novimmune SA has generated an anti-TLR4 antibody that successfully inhibits endogenous DAMP detection by the TLR4/MD2 complex, thereby preventing inflammatory transplant rejection and/or transplanted tissue/organ damage. The therapeutic antibody can be administered during and/or after the transplantation.[89] Another therapeutic antibody by Novimmune, NI-0101, designed to suppress inflammation in the presence of systemic LPS challenge, has completed a phase I clinical trial with good safety, tolerability, distribution, and elimination profiles (NCT01808469).

2.4. TLR5

TLR5, expressed on antigen presenting cells (especially dendritic cells), plays a vital role in host protection against various bacterial infections.[90] Lack of TLR5 signaling is associated with accelerated malignant growth in tumor-bearing hosts [91] and altered gut microbiota-related metabolic syndrome. [92] In addition, polymorphisms in the TLR5 gene have been linked to human colorectal cancer.[93] Flagellin protein, the main component of bacterial flagella, is the natural agonist of TLR5.[18] The complex formed between TLR5 and flagellin induces an innate immune response mediated by proinflammatory gene expression that can lead to inflammation, autoimmunity, and malignancy.[94] In addition to natural flagellin, a modified *Salmonella* flagellin, CBLB502, stimulates TLR5 and activates NFκB signaling in mouse and monkey models, protecting them against acute radiation syndrome.[95]

Idera Pharmaceuticals has disclosed the development of synthetic antisense oligonucleotides that downregulate human TLR5 gene expression by complementary base pairing.[96] Nevertheless, use of immunomodulatory compounds to target the TLR5-flagellin binding interface remains largely unexplored because of the inherent difficulty of disrupting protein–protein interactions.[97] Recently, Yan et al. identified by a high-throughput screen the first small-molecule inhibitor, TH1020 (4-((4-benzyl-5-(pyridin4yl)-4*H*-1,2,4-triazol-3-yl)thio) pyrido[3′,2′:4,5]-thieno[3,2-d]pyrimidine), that competitively binds TLR5 with high affinity (IC_{50} = 0.85 μm ± 0.12 μm) and disrupt its association with flagellin.[50]

2.5. TLR7, 8, and 9

TLRs expressed on glial cells of the central nervous system (CNS) can be activated by several microbe-associated molecular patterns, such as LPS of Gram-negative bacteria, unmethylated CpG DNA of viruses, and many more. Prolonged use of morphine, an opioid receptor agonist, is also known to activate TLRs on neuroglia, causing the release of proinflammatory cytokines and initiating chronic pain states called neuropathic pain. Consequently, there is a need to develop clinically effective agents that target TLRs on glial cells to control inflammation in the CNS. Mallinckrodt has found that (+)-Morphinan compounds antagonize TLR9 and block glial cell activation.[98] In one study, an enantiomeric analog of (+)-morphinan, named COV08-0064, showed broad-range bioavailability and greater specificity for TLR9 than other oligonucleotide-based inhibitors.[55] These compounds are envisioned to be used in the treatment of posttraumatic and neuropathic pain, inflammation, neurodegenerative and autoimmune disorders, acetaminophen toxicity, cancer, and sterile inflammatory injury. Naltrexone is an opioid receptor antagonist prescribed for use in the treatment of opioid and alcohol addiction. However, low-dose naltrexone (LDN) can be used to treat diseases such as multiple sclerosis and Crohn's disease. [99,100] In addition, clinical trials indicate, though not strongly, that LDN may be used to regulate the chronic pain associated with glial cells.[101] TLR9 overexpression or overactivation has been associated with a number of disorders, including some cancers. Cancer Vaccine Institute has disclosed LDN as a novel therapeutic that antagonizes TLR9.[102] The disclosure claims that LDN can be used in the supportive care of a cancer patient who was administered a chemotherapeutic agent such as Revlimid® (Celgene Corporation).

Dynavax Technologies (DT) has designed immunoregulatory oligonucleotides (IROs) having 2′-*O*-methyl or methoxyethyl sugar modifications as inhibitors of TLR7 and/or TLR9.[103] Further, DT has developed IROs with unique inhibitory sequences for TLR7, 8, and 9 that suppress autoimmune and inflammatory diseases.[104] The novel approach can be applied to treat a variety of autoimmune diseases, such as SLE or cutaneous lupus erythematosus (CLE), systemic sclerosis, polymyositis, dermatomyositis, RA, and Sjorgren's syndrome. Idera Pharmaceuticals has designed unique CpG oligonucleotide sequences containing an immune stimulatory motif with a 7-deaza-dG or arabino-G modification and an immune regulatory motif with 2′-*O*-methylribonucleotides that act as antagonists of TLR7, 8, and 9.[105] IROs have shown promising results in preclinical studies and effectively prevented hyperactivation of these TLRs in response to agonists. They are foreseen as future therapies for TLR 7, 8, and 9-mediated inflammatory disorders, including various autoimmune and inflammatory diseases and cancers initiated by pathogens. Chemical modifications and/or internucleotide linkages introduced into the IROs were found to increase inhibition or suppression of TLR7, 8, and 9.[56] Idera Pharmaceuticals is conducting clinical trials for its lead candidate, IMO-8400, to determine its safety and efficacy in patients with dermatomyositis (NCT02612857), diffuse large B-cell lymphoma (NCT02252146), Waldenstrom's macroglobulinemia (NCT02092909), and plaque psoriasis (NCT01899729).

More recently, a 2-aminoimidazole derivative was found to have pan-TLR antagonistic activity. In an attempt to design a TLR8 agonist containing benzimidazole core as vaccine adjuvant, Beesu et al. (2016) have discovered that an inactive agonist (4-(2-(benzyloxy)phenyl)-1-pentyl-1*H*-imidazol-2-amine) competitively inhibited TLR8, while it inhibited TLR2, 3, 4, 5, 7, and 9 by an unknown mechanism.[106] The micromolar affinity of this molecule toward multiple TLRs in various cell lines and whole blood systems suggests that it may evolve as a promising anti-inflammatory agent if subjected to further pharmacological development.

2.6. Indirect TLR antagonists

In addition to directly blocking TLR activation by targeting different domains of a TLR, the activation of several TLRs has

been indirectly inhibited. Lamphier et al. showed the inhibition of TLR7 and 9 by two small molecules (AT791 {3-[4-(6-(3-(dimethylamino)propoxy)benzo[*d*]oxazol-2-yl)phenoxy]-*N*,*N*-dimethylpropan-1-amine} and E6446 {6-[3-(pyrrolidin-1-yl)propoxy)-2-(4-(3-(pyrrolidin-1-yl)propoxy)phenyl]benzo[*d*]oxazole}), which weakly interact with nucleic acid agonists (DNA or RNA) in acidic intracellular compartments such as endolysosomes.[52] E6446 has previously been reported to act as antimalarial compound, preventing hyperinflammation and lethality caused by the parasite *Plasmodium berghei* in a mouse model of cerebral malaria.[53]

Chloroquine, hydroxychloroquine, and quinacrine can block nucleic acid access to endosomal TLRs.[52,107] Hydroxychloroquine is an established antimalarial drug sold in the market and used in the treatment of SLE, RA, and Sjogren's syndrome.[108] Sjogren's syndrome is a systemic autoimmune disease that seriously affects salivary and lachrymal glands, causing xerostomia (dry mouth), keratoconjuctivitis sicca (dry eye), pain, and fatigue.[109] Although the effectiveness of hydroxychloroquine in Sjogren's syndrome has been reported, a randomized controlled trial involving 120 patients for 48 weeks duration raised critical questions about its efficacy. Further studies are therefore recommended to clarify the clinical outcomes (NCT01601028).[110]

Neutralization of endosomal TLRs (3, 7, 8, and 9) has been achieved by nucleic acid binding agents. These novel molecules bind to exogenous or endogenous nucleic acids responsible for the induction of TLR activation and signaling irrespective of sequence, structure, or chemistry of the agonists.[111] The neutralizing antagonists are proteins, polypeptides, peptides containing mostly basic amino acids or cationic lipids, or positively charged natural or synthetic polymers. These agents can be used in the treatment of inflammatory and autoimmune diseases, such as cardiovascular disease, cancer, bacterial sepsis, multiple sclerosis, SLE, RA, chronic obstructive pulmonary disease, obesity, and psoriasis, resulting from endosomal TLR activation. These agents can be thought as unconventional TLR antagonists that do not make physical contact with the receptor. Furthermore, these agents can antagonize the activation of cytoplasmic nucleic acid recognizing PRRs as well. Quark Pharmaceuticals has developed dsRNA molecules that hybridize to TLR2, TLR4, MyD88, TRIF, and TIRAP mRNA through imperfect complementary base pairing and downregulate their expression in response to agonists. These novel inhibitors can be used to treat injuries and diseases associated with lung transplantation.[112]

Transmission of an immune signal by TLRs is solely dependent on agonist-induced receptor dimerization. All of the antagonists developed to date prevent TLR dimerization or activation of inactive dimers by blocking the dimerization interfaces located on the ectodomain, TM domain, or TIR domain. The BB loop on the TLR TIR domain is an important structural scaffold that assists in downstream adaptor recruitment by means of homotypic TIR–TIR interaction. This loop has been exploited by antagonist developers to design novel blocking agents. D-helix peptides from the TLR2/TLR4 TIR domains were found to inhibit TLR2/TLR4 activation without directly binding to them.[113] Piao et al. reported a cell permeable peptide, designated 2R9, derived from helix D of the TIR domain of TLR2 that specifically targets TIRAP and blocks its association with TLR2, 4, 7, and 9.[58] This indicates that the BB loop peptide sequences from the TLR2/TLR4 TIR domains fused to cell penetrating peptides are potent inhibitors of TLR signaling mediated through MyD88 and NFκB.

Sparstolonin B (SsnB), a natural compound derived from the Chinese herb *Spaganium stoloniferum* has shown anti-inflammatory properties.[114] The antagonist effectively blocks TLR2- and TLR4-mediated NFκB activation in mouse macrophages induced by LPS, Pam3CSK4, and Fsl-1 (TLR4, TLR2, and TLR2/6 ligands, respectively); however, the exact binding mechanism is undetermined. The observation that SsnB selectively inhibits recruitment of MyD88 to TLR2 and TLR4 indicates that it may directly target TLR2 and TLR4 TIR domains or the helper adaptor TIRAP, which bridges MyD88 and TIR interactions.[40] Further efforts to develop TLR2/4 antagonistic molecules may lead to therapies for sepsis, atherosclerosis, IR, SLE, diabetes, and Alzheimer's disease.

3. Conclusion

Over the past half-decade, we have witnessed a number of preclinical breakthroughs in the field of TLR immunomodulation. Although clinical success is still limited, some antagonistic ligands have performed exceptionally well in advanced clinical trials. As the detailed mechanism of TLR signaling continues to be unveiled, unique strategies have been implemented to neutralize aberrant innate and adaptive immune responses. Occasionally, direct targeting of TLRs has been bypassed and intracellular downstream adaptors or nucleic acid agonists themselves have been blocked to reach the desired therapeutic goals. Continuing pharmaceutical research in the area of TLR biology will lead to important discoveries and might yield novel therapies to address urgent and unmet medical needs.

4. Expert opinion

TLRs, the most important external or internal danger sensors, provide the first line of defense against external or internal threats. However, beyond innate host protection, TLRs have been implicated in several autoimmune and inflammatory diseases, including cancer, diabetes, cardiovascular disease, atherosclerosis, RA, SLE, Alzheimer's disease, and chronic neuropathic pain. Currently, most of these life-threatening diseases do not have any treatments available in the market, representing unmet medical needs. As our understanding of TLR biology has increased, scientists around the globe have made considerable progress in developing novel methods to downmodulate TLR signaling in several disease conditions. A number of preclinical breakthroughs have been registered in patent offices around the world, although clinical success is still limited. Preclinical trials are based on animal models, which are usually inbred strains with less genetic variability. Therefore, a difference in expression and activity of TLRs can significantly alter the induction or suppression of proinflammatory cytokines and IFNs. Furthermore, the differential expression profile of TLRs between mouse and human may

not reflect the true function in response to a drug in human. This compels to consider a large margin of error while predicting safety doses for human trials. For example, in spite of their exceptional TLR4 inhibitory properties *in vitro* and *in vivo*, two potent antagonists, Eritoran and TAK-242, did not improve signs of sepsis after 28 days of administration in phase III clinical studies.[115,116] These failed clinical trials have made the investigators rethink of the clinical trial strategies, such as inclusion criteria, dosage regimen, severity of infection and inflammation, immune status, and genetic history of the participants.[117] Preventing LPS recognition by TLRs or inhibiting post-recognition downstream signaling is considered a promising approach to treat severe sepsis or sepsis syndrome. Therefore, the failed clinical studies of Eritoran and TAK-242 should not pose a setback for other molecules under investigation. Rather, second and third generation derivatives may be designed for better clinical outcome. Moreover, alternate antagonists with increased potencies have improved clinical symptoms. Antibodies and peptide-based antagonists require further development efforts to obtain less toxic and more efficient drugs for human use. Meanwhile, some of the newly developed antagonists that show safety, efficacy, and tolerability are now in phase III testing, suggesting hope for the coming years.

X-ray crystallography has made a profound contribution to our understanding of TLR three-dimensional structure, ligand recognition, and downstream signaling mechanisms. This understanding has led to computer-aided drug design methods, such as virtual library screening, to find novel candidates that possess inhibitory pharmacophores for TLRs and to expedite drug discovery process. Although computational screening methods reduce the number of molecules to be tested in the laboratory, they are quite error prone and require highly skilled personnel. Improved algorithms and force-field developments are required for more accurate predictions. Furthermore, routine chemical library screening and SAR studies have provided novel leads with increased potency to disrupt TLR signaling.

This review has presented recent preclinical and clinical achievements in the safety and effectiveness of TLR antagonists for the treatment of autoimmune and inflammatory diseases caused by microbes, self-metabolites, and radiation. Among the few TLR antagonists that have entered clinical trials, hydroxychloroquine, a TLR9 antagonist intended for the treatment of Sjogren's syndrome, has completed phase III study, and Mw, a TLR4 antagonist, is in phase III testing to evaluate its efficacy in treating severe sepsis. Other inhibitors are still in phase I or II testing; for example, Idera Pharmaceuticals is examining its investigational drug, IMO-8400 (TLR7, 8, and 9 antagonist), separately for the treatment of dermatomyositis, diffuse large B-cell lymphoma, Waldenstrom's macroglobulinemia, and plaque psoriasis. Allodynic Therapeutics has successfully completed a phase II clinical trial of its investigational drug, ATNC05, which is a synergistic mixture of two generic drugs containing an opioid/TLR4 antagonist, for the treatment of chronic neuropathic back pain in the cervical, thoracic, and lumbar regions. Furthermore, mAb antagonists have entered clinical trials. Notably, a TLR2 antagonist, OPN-305, for the treatment of delayed graft function is in a phase II trial, and a TLR4 antagonist, NI-0101, for the treatment of Gram-negative sepsis has completed a phase II study, demonstrating safety, efficacy, and tolerability (Table 3).

One of the major concerns about the TLR family members is their redundancy, that is TLRs exist in multiple isoforms in different species with different cellular expression patterns and downstream signal transduction effects. Human TLRs can elicit overlapping yet distinct immune responses because of their ability to recognize an overlapping set of PAMPs/DAMPs. Therefore, potent poly TLR antagonists need to be developed to block multiple TLRs. Oligonucleotide inhibitors of endosomal TLRs have shown preclinical successes in controlling a range of immune disorders. However, such pharmaceutical agents show low bioavailability and have limited specificity for their receptors, potentiating broad innate and adaptive immunologic reactions. This is evident by the fact that TLR9-specific oligonucleotide antagonists can also be detected by the cytosolic DNA sensors AIM2,[118] STING,[119] and IFI16.[120] Further, the nucleic acid binding specificities are different among endosomal TLRs; for instance, RNA and synthetic molecules recognized by TLR8 are slightly different from those detected by TLR7. In addition, the type of

Table 3. Clinical trial status of some investigational new TLR antagonists.

Antagonist	Target TLR	Investigator	Condition	Phase	ClinicalTrials.gov Identifier	Status
OPN-305	TLR2	Opsona Therapeutics Ltd.	Delayed graft function	II	NCT01794663	Ongoing
ATNC05	TLR4	Annette C. Toledano MD, Allodynic Therapeutics, LLC	Back pain Lower back pain Cervical pain	II	NCT01415895	Completed
NI-0101	TLR4	NovImmune SA	Healthy volunteers	I	NCT01808469	Completed
IMO-8400	TLR 7, 8, 9	Idera Pharmaceuticals, Inc.	Dermatomyositis	II	NCT02612857	Ongoing
IMO-8400	TLR 7, 8, 9	Idera Pharmaceuticals, Inc.	Diffuse large B-cell lymphoma	I/II	NCT02252146	Ongoing
IMO-8400	TLR 7, 8, 9	Idera Pharmaceuticals, Inc.	Waldenstrom's macroglobulinemia	I/II	NCT02092909	Ongoing
IMO-8400	TLR 7, 8, 9	Idera Pharmaceuticals, Inc.	Plaque psoriasis	II	NCT01899729	Completed
Hydroxychloroquine	TLR9	Seoul National University Hospital	Autoimmune diseases Sjogren's syndrome Dry eye	III	NCT01601028	Completed
Mycobacterium w	TLR4	Postgraduate Institute of Medical Education and Research	Sepsis	II/III	NCT02330432	Ongoing

The information was obtained from *https://clinicaltrials.gov/*.

cytokines produced differs depending on the activated TLR. [121] This suggests that a single-specific ligand for each TLR may be more effective than a poly-TLR antagonist. Moreover, nucleic acid binding agents that directly bind to the nucleic acid agonists through weak interactions and block their access to endosomal TLRs are innovative and may be more effective in dampening cytokine gene expression. Use of mAb antagonists to block the overexpression or overactivation of TLRs is a preferred method of treatment, because antibodies are highly specific to their target, causing a little or no side effects. In the 2015 list of FDA-approved drugs, one-third are biologics, including peptides, enzymes, antibody fragments, and antibodies.[122] Based on the current pace of antagonist development, we expect that more antagonists will proceed through clinical trials and that newly potent TLR immunomodulators will soon enter the pharmaceutical market.

Declaration of interests

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