Small-Molecule Modulators of Toll-Like Receptor Signaling and Their Clinical Applications

Naoko Matsunaga

Independent Researcher and Consultant, San Diego, California 92111, USA

Correspondence to: Naoko Matsunaga, yff33165@gmail.com

Additional material is published online only. To view please visit the journal online.

Cite this as: Matsunaga N. Small-Molecule Modulators of Toll-Like Receptor Signaling and Their Clinical Applications. Premier Journal of Science 2025;4:100031

DOI: https://doi.org/10.70389/PJS.100031

Received: 16 September 2024

Revised: 6 November 2024

Accepted: 6 November 2024

Published: 14 November 2024

Ethical approval: N/a

Consent: N/a

Funding: No industry funding

Conflicts of interest: N/a

Author contribution: Naoko Matsunaga – Conceptualization, Writing – original draft, review and editing

Guarantor: Naoko Matsunaga

Provenance and peer-review: Commissioned and externally peer-reviewed

Data availability statement: N/a

ABSTRACT

Toll-like receptors (TLRs) are pattern recognition receptors expressed in various immune cells on the cell surface or within endosomes. TLRs play a key role in the innate immune response by detecting pathogen-associated molecular patterns. TLRs also recognize damage-associated molecular patterns that are endogenous molecules produced from damaged or stressed cells. TLRs are involved in the pathogenesis of a variety of inflammatory, infectious, and autoimmune diseases. Moreover, TLRs regulate immune response in the tumor microenvironment. The pivotal role of TLRs in the pathophysiology of these diseases makes them attractive targets for the development of innovative therapy for these immune disorders and cancer as well as preventive measures, such as vaccination. Manipulating the TLR signaling pathway by a small-molecule drug is one of the promising approaches for the development of effective treatments that can be translated into a clinical setting. Small-molecule drugs are chemically synthesized and are more economically sustainable than biologics. This affordability makes the drugs more accessible to patients including those in developing countries. Besides, small-molecule drugs can be more stable and easier to administer. Their oral bioavailability and accessibility to cytosol are the distinct advantages over biologics. Nevertheless, only a limited number of small-molecule modulators have been developed due to the difficulty in finding appropriate drug candidates with acceptable selectivity, toxicity, and physicochemical profile. Here, we overview the pathophysiological roles of human TLRs and structural features to discuss the efforts, current promising opportunities, and challenges in developing small-molecule modulators of TLRs.

Keywords: Toll-like receptors, Autoimmune disease, Cancer, Inflammation, Innate immunity, Small molecule

Abbreviations

TLR, Toll-like receptor; TIR, Toll/IL-1 receptor; PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; MAMPs, microbe-associated molecular patterns; MD-2, myeloid differentiation factor 2; TRIF, TIR domain-containing adaptor inducing IFN-β; MyD88, differentiation primary response 88; Pam3CSK4, Pam3-Cys-Ser-Lys4; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis

Highlights

- Toll-like receptors (TLRs) are pattern recognition receptors that connect innate immunity to adaptive immunity in response to pathogens and the damaged self.
- TLRs are involved in a variety of diseases, including infections, inflammations, autoimmune diseases, cardiovascular diseases, and cancers. Manipulating the TLR signaling pathway remains a hot topic in preclinical and clinical research.
- Among the 10 human TLRs, the small-molecule modulators of TLR4, TLR7, and TLR9 have been relatively well studied.
- Most of the small-molecule modulators are ligand analogs or molecules that bind to the ectodomain of TLRs.
- Structural analyses of the intracellular domain of TLRs lag behind compared to those of the extracellular domain.

Introduction

Toll, the initial member of the Toll-like receptor (TLR) family, was identified as a developmental gene in *Drosophila melanogaster* in 1996. The Toll pathway was shown to play a critical role in the antifungal response of fruit flies.[1] This finding aroused a huge interest in similar systems in mammals and accelerated the epoch-making discovery of the roles of various TLRs in innate immunity. Currently, a total of 10 TLRs (TLR1–TLR10) have been found in humans, while 12 members (TLR1–TLR9 and TLR11–TLR13) have been found in mice. TLRs are type I transmembrane receptor proteins. They have a large extracellular horseshoe-shaped ligand-binding domain consisting of leucine-rich repeat (LRR) motifs and a globular intracellular Toll-interleukin-1 receptor (TIR) homology domain (Figure 1). TLRs are activated upon binding of ligands to their ectodomain, i.e., the extracellular domain of the receptor. The initial step of the activation is the dimerization of the receptor. Dimerization means to form a functional protein complex composed of two TLRs.

Since they are transmembrane proteins, there are technical challenges to purify full-length receptors. As a result, the structure of the only extracellular domains of TLRs was well determined by X-ray crystallography.[2–10] To date, a limited number of crystal structures of the intracellular domain of TLRs have been determined.[11, 12]

TLR1, 2, 4, 5, and 6 are primarily expressed on the plasma membrane, also called cell membrane, and recognize a variety of microbial components. On the other hand, TLR3, 7, 8, and 9 are expressed on endosome, intracellular sorting organelles in cells, and are involved in the recognition of nucleic acids (Figure 2). The natural and synthetic ligands of TLRs are summarized in Table 1.

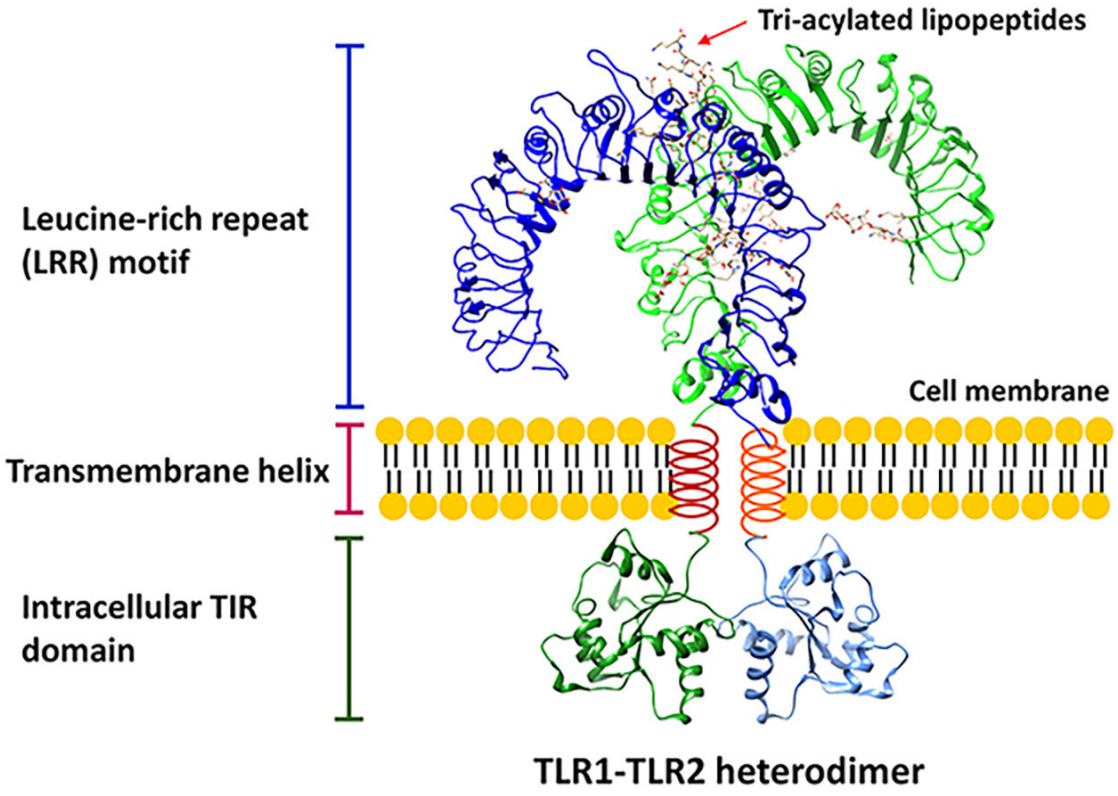

Fig 1 | A representative structure of TLR. The conserved structural features of all TLRs consist of three critical components: (1) leucine-rich repeat (LRR) motif; (2) transmembrane helix; (3) intracellular TIR domain. The LRR structure is based on the model of TLR1-TLR2 heterodimer (Protein Data Bank, PDB, ID: 2z7x) interacting with 6 tri-acylated lipopeptides, Pam3CSK4, whereas the TIR domain homology model is based on TLR2 TIR structure (PDB ID: 1fyw). © 2017 Gao W et al. Front Physiol 2017;8:508

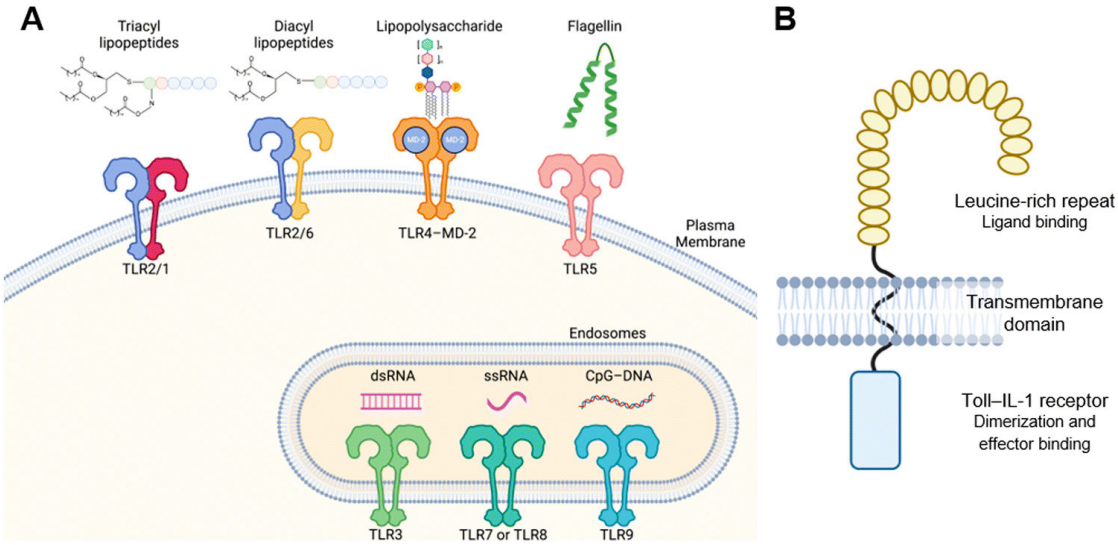

Fig 2 | Toll-like receptors. (A) Sub-cellular localization and MAMPs, PAMPs and/or DMAPs of TLRs. (B) Domain structure of TLRs. Tsukidate T et al. RSC Chem Biol. 2023;4(12):1014-36 © The Royal Society of Chemistry 2023

The immunological roles of TLRs are mainly sensors of pathogens as pattern recognition receptors and activators of the innate immune signaling pathway in response to the danger signal by pathogens[13] that are called pathogen-associated molecular patterns or microbe-associated molecular patterns. Moreover, TLRs can recognize a variety of other molecules released from dying cells or damaged tissues that are called damage-associated molecular patterns (DAMPs). Activation of TLR signaling has a critical role in connecting

DOI: https://doi.org/10.70389/PJS.100031 | Premier Journal of Science 2025;4:100031

Table 1 | Natural and Synthetic Ligands for TLRs

TLR	Expression	PAMPs	DAMPs	Nature of Molecules Investigated in Clinical Trials
TLR2 (1/6)	B, Mo, Mac, DCs, Plt N, MyDCs, Mc	Lipoproteins, zymosan, peptidoglycan	HSPs, HMGB1, hyaluronan, HDL (modified)	Lipopeptide, recombinant protein, antibody
TLR3	DC, B, Plt	Viral dsRNA	Self dsRNA	Polyinosinic-polycytidylic acid (polyIC, poly-ICLC, polyIC12U), anti-TLR3 antibody
TLR4	Mo, Mac, N, MyDCs, Mc, B, IE, Plt	LPS	HSPs, fibrinogen, heparin sulfate, fibronectin, HA, HMGB1, hyaluronan, oxidized LDL, ANG II	Lipid A derivates (glycolipids), anti-TLR4 antibody, polysaccharide
TLR5	Mo, Mac, DC, IE	Flagellin	HMGB1	Flagella and flagella-based molecules
TLR7	Mo, Mac, pDC, B, Plt	Viral ssRNA	Self ssRNA	SM
TLR8	Mo, Mac, DC, Mc	Viral ssRNA	Self ssRNA	SM
TLR9	Mo, Mac, pDC, B, Plt	Bacterial and viral CpG DNA	Self DNA	DNA-based, synthetic ssDNA molecules
TLR10	LN, Mo, S, B, L	NA	NA	NA

Abbreviations: ANG II, angiotensin II; B, B cell; DAPM, danger-associated molecular pattern; DC, dendritic cell; dsRNA, double-stranded RNA; HA, hyaluronic acid; HDL, high-density lipoprotein; HMGB1, high mobility group box 1; HSPs, heat shock proteins; IE, intestinal epithelium; L, lung; LC, liver cell; LDL, low-density lipoprotein; LN, lymph node; Mac, macrophage; Mc, mast cell; Mo, monocyte; MyDC, myeloid dendritic cell; N, neutrophil; NA, not available; PAMP, pathogen-associated molecular pattern; pDC, plasmacytoid DC; Plt, platelet; S, spleen; SM, small molecule; ssRNA, single-stranded RNA; TLR, Toll-like receptor; LPS, lipopolysaccharide; CpG DNA, cytidine-phosphate-guanosine deoxynucleotide.

Source: Anwar MA et al. Med Res Rev. 2019;39(3):1053-90. © 2018 The Authors. Medicinal Research Reviews Published by Wiley Periodicals, Inc.

innate and adaptive immunity. However, prolonged and excessive activation of TLRs could disrupt host immune homeostasis by enhancing pro-inflammatory mediators, which results in inflammatory and autoimmune diseases such as sepsis,[14] atherosclerosis,[15] systemic lupus erythematosus (SLE),[16] osteoarthritis,[17] and central nervous system disorders including Alzheimer's disease.[18] TLRs are also associated with cancer by promoting mutagenesis, angiogenesis, and metastasis.[19] Therefore, inhibitors of the TLR signaling pathway have the therapeutic potential for the treatment of infections, inflammations, autoimmune diseases, and cancers. On the other hand, TLR agonists have been applied to boost vaccine effectiveness. They are also applied to promote a T helper (Th)1-predominant immune response for the treatment of Th2-associated disorders such as asthma[20] and to induce a preferable anti-tumor effect in the tumor microenvironment. Considering the pathological role of TLRs in a diverse of disease states, manipulating TLR signaling remains a hot topic in preclinical and clinical research.

Currently, a limited number of compounds are available for clinical uses despite considerable efforts to discover drug candidates targeting TLR signaling pathways. Among the 10 human TLRs, TLR4, TLR7, and TLR9 have been studied well in terms of drug discovery and development. Here, we focus on the disease etiology of TLRs, novel small-molecule specific modulators of TLRs, and the prospect for their clinical application. Although we compare the small molecules with some biologics, the biologics targeting TLRs have been reviewed elsewhere.[21–23] Several approved molecules with different mechanisms of action have been reported to have activity against multiple TLRs, such as melatonin (TLR3/4 inhibitor), fluvastatin (TLR2/3/4/8 antagonist), and chloroquine (TLR7/8/9 inhibitor). These compounds are beyond the scope of this review and are not discussed here. In this review, we discuss the recent progress, limitations, and trends in the research activities of specific small-molecule modulators of TLR signaling.

TLR1/2, TLR2/6

Biological Function

TLR2 acts by forming heterodimers with either TLR1 or TLR6, thereby recognizing lipoproteins derived from pathogens such as bacteria, viruses, fungi, and parasites. The microbial and endogenous ligands of TLRs are shown in Table 1. TLR2 is expressed on the surface of a wide range of immune cells and epithelial cells. TLR1/2 mainly recognizes tri-acylated lipopeptides, while TLR2/6 recognizes di-acylated lipopeptides.[24, 25] Co-crystal structures of Pam3-Cys-Ser-Lys4 (Pam_3CSK_4) or Pam_2CSK_4 bound to the TLR1/2 or TLR2/6 heterodimers revealed the mechanism of dimerization. Multiple studies have demonstrated that TLR2 is associated with the pathogenesis of sepsis, atherosclerosis, hypertension, chronic obstructive pulmonary disease, and SLE.[26] In addition, TLR1 and TLR2 are associated with epithelial homeostasis in the intestine.[27]

Structural Features

The structural analysis demonstrated that TLR2 in the TLR2/1 or TLR2/6 complex exhibits a pocket that allows a structure-based drug design. All the TLR2 modulators discovered up to date are analogs of the ligands that interact with the ectodomain of TLRs. For the intracellular domain, crystal structures of the TIR domain of TLR1 and TLR2 were resolved in 2000.[12] However, small molecules targeting the TIR domain of TLR2 are not available for clinical trials until now. TIR domains are important to transduce the activation signal into cytosol. Adaptor molecules adjacent to TLRs such as myeloid differentiation primary response 88 (MyD88) and MyD88 adapter-like (Mal, also termed TIRAP) also have a TIR domain. These adaptor

molecules assemble via the TIR domain, form TIR-domain signalosomes, and play a key role in signal transduction.[32]

Current Modulators

Synthetic phospholipid Compound 1 and its derivatives have been discovered as the antagonists of TLR2 signaling.[28] On the other hand, its agonist Pam_2CS derivatives have been reported with a structure-activity relationship study.[29] Synthetic lipopeptide TLR2 agonist CBLB612 was developed for the treatment of cancer and chemotherapy-induced myelosuppression.[30]

They are lipopeptide analogs of the TLR2 ligand, which interact with the ectodomain of TLRs. For non-lipopeptide analogs of TLR2 modulators, SMU-Y6 (antagonist), SMU-C80 (agonist), and several other chemotypes have been discovered.[31] They are in the discovery and pre-clinical research phase. To find an innovative modulator, novel approaches such as virtual screening of chemical library and computational chemistry have the potential to discover active compounds with a drug-like profile that interact with the TIR domain as well as the ectodomain of TLRs.

TLR3

Biological Function

TLR3 recognizes double-stranded RNAs (dsRNAs) from pathogens in early endosomes in immune cells and initiates an antiviral response (Figure 2). It also recognizes the RNA released from necrotic synovial fluid cells and is involved in the pathology of rheumatoid arthritis (RA).[33]

Recently, TLR3 has been reported to be expressed in some types of cancer cells related to viral infections.

Structural Features

From the crystal structure study, it was shown that each TLR3 ectodomain binds dsRNA at two sites located at the opposite ends of the TLR3 horseshoe, and an intermolecular contact between the two TLR3 ectodomain C-terminal domains coordinates and stabilizes the dimer.[5] TLR3 interacts with the backbone of RNAs rather than bases. After forming the TLR3 homodimer, it signals in an exclusively TIR domain-containing adaptor inducing interferon (IFN)-β (TRIF)-dependent manner.

Current Modulators

Polyinosinic-polycytidylic acid (poly(I:C)) and polyadenylic-polyuridylic acid (poly(A:U)) are synthetic analogs of dsRNA. A ligand analog IPH-3102 was identified as a TLR3 agonist which is a synthetic dsRNA.[34] Since TLR3 is expressed by a large subset of patients in multiple cancers, it has been suggested as an immune-stimulating agent for potential cancer treatment. For a TLR3 antagonist, a high-throughput cell-based screening identified a novel ellipticine derivative SMU-CX24. It showed anti-inflammatory effects and protected mice against atherosclerosis.[35] Several small-molecule modulators of TLR3 with different chemotypes have been reported; however, only few of them show sufficiently promising pharmacological, physicochemical, and toxicological characteristics to become a marketed drug.

TLR4

Biological Function

TLR4 was originally identified as the signaling receptor for lipopolysaccharides (LPS) or endotoxins from the outer membrane of Gram-negative bacteria.[36] Binding of lipid A, the hydrophobic anchor of LPS, to the signaling cofactor myeloid differentiation factor 2 (MD-2) initiates TLR4 signaling. TLR4 is expressed in several immune cells such as dendritic cells (DCs) and macrophages on the plasma membrane (Figure 2). It recognizes multiple DAMPs as shown in Table 1. TLR4 and TLR2 are involved in inflammatory diseases, such as atherosclerosis, hypertension, and colitis-associated colorectal tumors.[26] They are also contributors to the pathogenesis of sepsis and septic shock, a serious condition with an abnormal response to an infection. Activation of TLR4 signaling plays a pivotal role in a wide range of pathologies. For this reason, TLR4 has attracted great attention as a therapeutic target.

Structural Features

TLR4 is the only TLR among the 10 human TLRs that require a co-receptor MD-2 to recognize its ligand. The complex of TLR4 and MD-2 recognizes LPS. The co-crystal structure shows that the TLR4/MD-2/LPS complex is an m-shaped dimer bridged by two molecules of LPS.[6] A lipid A analog eritoran (E5564) binds to the hydrophobic pocket of MD-2 which is associated with the ectodomain of TLR4. Structural analysis showed that there is a suitable ligand-binding pocket in the TLR4/MD-2 complex rather than in the ectodomain of TLR4. Thus, TLR4/MD-2 antagonists with non-LPS-like structures were reported by computational virtual screening[37] and high-throughput cell-based screening using a reporter assay[38] although they are in the discovery and preclinical stage.

Current Modulators

Eritoran, a lipid A analog, was developed as a TLR4 antagonist for the treatment of sepsis. It showed promising results in phase I and II clinical trials; however, it failed to show benefits than the existing treatment regimens in phase III clinical trials.[39] Several reasons for the failure have been considered. There might have been oversights in the study protocol design and patient stratification. The inadequate understanding of the biology of TLRs also might have contributed to the failure.[55] Similarly, a better understanding and an improved study protocol might have been required for the clinical study of anti-TLR4 monoclonal antibody NI-0101 which did not improve the disease parameters of patients with RA.[23] Other lipid A analogs, monophosphoryl lipid A derivatives, are clinically applied as

DOI: https://doi.org/10.70389/PJS.100031 | Premier Journal of Science 2025;4:100031

TLR4 agonists. They are used as adjuvants of vaccines for hepatitis B and cervical cancers.

The derivatives of pyrimido[5,4-b]indoles also showed TLR4 agonist activity. The interaction of the compound with MD-2 is suggested based on a docking study.[40] They are in the early stage of drug discovery and development.

There are some other non-LPS-like TLR4 signaling modulators. One of them is TAK-242 (also called resatorvid or CLI-095) which was identified in 1998 from a small-molecule library by phenotypic screening to inhibit LPS stimulation of macrophages. Coincidentally, TLR4 was identified as the LPS receptor in the same year.[36] Later, TLR4 was identified as the target molecule of TAK-242 using a radiolabeled compound. Notably, it binds irreversibly to the Cys747 in the TIR domain of TLR4.[41,42] TAK-242 is a very potent and the most studied selective small-molecule TLR4 inhibitor to date. TAK-242 was evaluated in a clinical trial to treat patients with sepsis and was found to be generally well tolerated.[43] Although the clinical trial for sepsis was discontinued by their sponsor for strategic reasons, numerous reports showed the efficacy of TAK-242 in a variety of animal models, such as intracerebral hemorrhage,[44] LPS-induced lung inflammation,[45] osteoarthritis,[17] and RA.[46]

TLR5

Biological Function

TLR5 recognizes flagellin, a bacterial protein that is a key component of bacterial flagella. It is expressed on the surface of monocytes, immature DCs, and epithelial cells (Figure 2). Among human TLRs, TLR5 is the only TLR that recognizes a protein ligand (Table 1). Increasing evidence suggests that TLR5 plays a crucial role in the control of intestinal homeostasis[47] since it is one of the most significant extracellular receptors interacting with the human gastrointestinal microbiome. Flagellin and TLR5 have reported to be involved in the pathogenesis of inflammatory bowel disease (IBD), especially Crohn's disease.[48]

However, some reports indicate that the TLR5 expression level is not positively correlated with the incidence of IBD. Moreover, it has been reported that TLR5 expression on monocytes from patients with RA correlates with RA disease activity and tumor necrosis factor (TNF)-α levels .[49] Further studies are needed to clarify the multiple roles of TLR5 in the etiology of autoimmune diseases.

Structural Features

The ligand flagellin binds directly to the lateral surfaces of TLR5 in a symmetrical arrangement, resulting in the formation of a 2/2 complex. After a conformational change in the TIR domain, a signal is transmitted into the cells through the interaction between the TIR domain of TLR5 and cytosolic adaptor molecules.

Current Modulators

There are very few small-molecule modulators of TLR5 reported although some biologics including entolimod (CBLB502),[50] a recombinant flagellin protein fragment targeting TLR5 activation, are being developed for the treatment of cancer. Flagellin-based vaccines are studied extensively, which is discussed elsewhere.[51]

TLR7, TLR8

Biological Function

TLR7 and TLR8 are phylogenetically similar receptors in the endosomal membrane and recognize single-stranded RNAs (Table 1). TLR7 is expressed primarily in the endosome of plasmacytoid dendritic cells (pDCs), B cells, and monocytes/macrophages (Figure 2). Low levels of the expression of TLR7 have been observed in non-immune cells, such as hepatocytes, epithelial cells, and keratinocytes. TLR8 is known to be primarily expressed in monocytes/macrophages and myeloid dendritic cells.[52] TLR7 and TLR8 recognize bacterial RNAs and human RNAs as well as viral RNAs that are endocytosed. Because TLR7 and TLR8 recognize RNAs from damaged cells, their signaling pathways have been implicated in the development of several autoimmune diseases and tumor immune surveillance. Importantly, the activation of TLR7 and TLR8 signaling pathways results in the induction of type I and type III IFN. Overproduction of type I IFN is implicated in the pathogenesis of SLE, a chronic autoimmune disease characterized by the production of autoantibodies. Accumulating evidence suggests that TLR7 and TLR8 are associated with inflammatory responses in SLE.

Structural Features

There are two distinct ligand-binding sites in both TLR7 and TLR8. TLR7 exists as a monomer and forms a dimer upon binding guanosine, its derivatives, or synthetic ligands. On the other hand, TLR8 exists as a preformed dimer and binds uridine, short oligonucleotides, or synthetic ligands at a dimerization interface. TLR7, 8, and 9 have an insertion loop called a Z-loop between LRR14 and LRR15 in the ectodomain of the receptors. A proteolytic cleavage of the Z-loop is required for the activation of the receptors.[53]

Current Modulators

The imidazoquinoline amine analog TLR7 agonist imiquimod (R837) has been approved by the U.S. Food and Drug Administration for treating malignant skin cancer. It is used for the topical treatment of anogenital warts, superficial basal cell carcinomas, and actinic keratoses. The TLR7/8 agonist resiquimod (R848) was developed for potential use as an antiviral treatment, including topical treatment of herpes simplex virus 2[54] and oral treatment of chronic hepatitis C virus infection.[55] The reduction of virus level was observed; however, adverse effects similar to IFN-α were the issue. Similarly, resiquimod, which showed potent adjuvant activity in animal models, suppressed the virus level only at concentrations that induced systemic cytokines, which led to systemic side effects.[56] Other agonists with non-imidazoquinoline derivatives such as motolimod (VTX-2337) and vesatolimod (GS-9620)

have been studied in clinical trials.[57] Since toxicity remains a challenge, the research focus is advancing toward formulations to enhance safety and efficacy by changing biodistribution, pharmacokinetics, and cellular targeting.

For TLR7/8 antagonists, the discovery, research, and development fall behind compared to agonists, probably because antagonist binding does not induce remarkable structural changes of the ectodomain of the TLRs except for the local loop regions. In such a situation, phenotypic cell-based screening and structure-based drug design identified the oral TLR7/8 antagonist MHV370.[58] Its clinical trials in patients with Sjögren's syndrome and mixed connective tissue disease are ongoing. Also, the anti-TLR7 antagonistic monoclonal antibody DS-7011a is in the clinical stage for the treatment of SLE though it is administered subcutaneously. A functional TLR8 antagonist CU-CPT8m was identified through a reporter assay.[59] The antagonist binds to a unique binding site on the protein-protein interface of the TLR8 homodimer, thereby stabilizing the preformed TLR8 dimer in its resting state. Further studies on the structural mechanisms of activation and inhibition may offer new approaches for the design of TLR7/8 inhibitors.

TLR9

Biological Function

TLR9 is primarily expressed in the endosome of pDCs, B cells, and eosinophils (Figure 2). Recent studies have revealed that TLR9 can also be expressed on the cell surface of neutrophils, B cells, and erythrocytes.[60] TLR9 recognizes the pathogen-derived non-methylated cytidine-phosphate-guanosine deoxynucleotide (CpG DNA) motifs from bacteria or artificial synthetic non-methylated CpG-containing oligodeoxynucleotides.

The activation of TLR9 is associated with a variety of diseases, including cardiometabolic disorders,[61] SLE, and Sjögren's syndrome. TLR9 is suggested to have a protective role in SLE because TLR9-/- murine models displayed higher TLR7-mediated inflammation.[62] Diverse therapeutic approaches currently target TLR9.

Structural Features

Crystal structure analysis revealed that the agonistic CpG DNA-bound TLR9 formed a symmetric complex with a 2:2 stoichiometry, whereas the inhibitory DNA-bound TLR9 was a monomer,[10] suggesting that the regulation of dimerization is the mechanism of action. TLR9 contains a Z-loop, similar to TLR7/8. The cleavage of the Z-loop plays an important role in the activation of TLR9.

Current Modulators

All of the small-molecule TLR9 modulators studied in clinical trials are ligand analogs, i.e., either nucleotides or nucleotide derivatives, and several agonists have been evaluated in clinical trials. An inhaled TLR9 agonist AZD1419 was developed for the treatment of asthma. It was safe and well tolerated, but there were no statistically significant differences between AZD1419 and placebo for time to loss of asthma control, a primary endpoint, despite reducing markers of type 2 inflammation.[63] EMD 1201081 was evaluated in phase 2 trials in patients with head-and-neck cancer. It was found to be ineffective in the tested dose regimen.[64] CYT003 showed no additional benefit in patients with insufficiently controlled moderate-to-severe allergic asthma receiving standard inhaled glucocorticosteroid therapy.[65] Poor efficacy, unfitting hypothesis, or improper study design for TLR9 might have prevented their success in clinical trials.

TLR 7/8/9

TLR7, TLR8, and TLR9 are expressed intracellularly within endosomes (Figure 2). Since the ligands for these TLRs are nucleic acids and structurally similar, there are some small-molecule modulators that interact with multiple TLRs. CpG-52364, a quinazoline derivative, is a TLR7/8/9 antagonist studied in clinical trials for the treatment of SLE.[66] Another antagonist IMO-8503 attenuated the cachectic phenomenon in tumor-bearing mice.[67] Another antagonist IMO-8400 inhibited disease development in mouse models of psoriasis; however, efficacy was not observed in clinical trials for the treatment of dermatomyositis.[68] Further biological studies are needed to understand the complex crosstalk of the TLR signaling pathway and other signaling pathways involved in the innate immune response and inflammation.

Concluding Remarks

In this review, we summarize the pathophysiological roles of TLRs in related diseases and highlight the potential and limitations of small-molecule drug candidates that regulate TLR signaling. Our understanding of the structure and function of TLRs has increased dramatically in the past two decades. Identification of small-molecule compounds with a drug-like property and potential therapeutic applications for the treatment of TLR-related diseases is imperative to improve public health and the quality of life of patients.

Small molecules have a shorter half-life span in the body in general. They need to be taken more often to keep an effective concentration in the plasma. At the same time, it is relatively easier to control the plasma concentration by getting patients to comply with treatment since they are stable and orally bioavailable. Small molecules can target the intracellular part of the TLRs as well as the ectodomain of the receptor. In comparison, biologics such as antibodies are heat-sensitive and membrane-impermeable, which means they cannot target intracellular molecules. Biologics typically exhibit longer half-lives due to slower absorption and clearance mechanisms. They must be injected, which creates an obstacle for patients. However, biologics provide more targeted treatment options. Novel small-molecule modulators of TLR signaling can offer a variety of clinical treatment choices in combination with biologics.

DOI: https://doi.org/10.70389/PJS.100031 | Premier Journal of Science 2025;4:100031

In order to understand the disease etiology of TLRs and explore novel biomarkers, reliable preclinical animal models are essential. To establish superior disease models and evaluate drug candidates in an appropriate protocol is the key to a successful transition of the candidates from preclinical to clinical trials. Small-molecule drugs can have potential off-target effects due to selectivity issues that can be predicted in the early stage using animal models. Biologics have twice the final success rate in clinical trials compared with that of small molecules,[30] which underlines the need for further research in appropriate animal models to address the issues at an earlier drug discovery stage.

Recent advances in crystallography and in silico techniques give us promising opportunities for a structure-based drug design. Nevertheless, structures of the intracellular domain of most of the TLRs are still unknown. TIR domain, an intracellular domain of TLRs, plays a key role by interacting with the adaptor molecules that initiate cytoplasmic signaling pathways. The TIR domain exhibits a weak self-association and association with other TIR domains, which leads to the formation of a signalosome complex such as Myddosome.[69] Shifting the research focus from ligand-receptor interaction to protein-protein interaction will offer a solution to identify innovative drugs distinct from conventional TLR ligand analogs. Further research on the biology, pathophysiology, and protein structure of TLRs including the TIR domain may contribute to breakthrough findings to achieve successful drug discovery and development.

Acknowledgment

The author would like to thank the support from Mr. Claude A Duncan for providing a great work environment and infrastructure.

References

1 Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell. 1996;86(6):973–83.
2 Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, et al. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell. 2007;130(6):1071–82.
3 Bell JK, Botos I, Hall PR, Askins J, Shiloach J, Segal DM, et al. The molecular structure of the toll-like receptor 3 ligand-binding domain. Proc Natl Acad Sci U S A. 2005;102(31):10976–80.
4 Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, et al. Recognition of lipopeptide patterns by toll-like receptor 2-toll-like receptor 6 heterodimer. Immunity. 2009;31(6):873–84.
5 Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, Segal DM, et al. Structural basis of toll-like receptor 3 signaling with double-stranded RNA. Science. 2008;320(5874):379–81.
6 Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature. 2009;458(7242):1191–5.
7 Yoon SI, Kurnasov O, Natarajan V, Hong M, Gudkov AV, Osterman AL, et al. Structural basis of TLR5-flagellin recognition and signaling. Science. 2012;335(6070):859–64.
8 Zhang Z, Ohto U, Shibata T, Krayukhina E, Taoka M, Yamauchi Y, et al. Structural analysis reveals that toll-like receptor 7 is a dual receptor for guanosine and single-stranded RNA. Immunity. 2016;45(4):737–48.
9 Tanji H, Ohto U, Shibata T, Miyake K, Shimizu T. Structural reorganization of the toll-like receptor 8 dimer induced by agonistic ligands. Science. 2013;339(6126):1426–9.
10 Ohto U, Shibata T, Tanji H, Ishida H, Krayukhina E, Uchiyama S, et al. Structural basis of CpG and inhibitory DNA recognition by toll-like receptor 9. Nature. 2015;520(7549):702–5.
11 Lushpa VA, Goncharuk MV, Lin C, Zalevsky AO, Talyzina IA, Luginina AP, et al. Modulation of toll-like receptor 1 intracellular domain structure and activity by Zn. Commun Biol. 2021;4(1):1003.
12 Xu Y, Tao X, Shen B, Horng T, Medzhitov R, Manley JL, et al. Structural basis for signal transduction by the toll/interleukin-1 receptor domains. Nature. 2000;408(6808):111–5.
13 Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol. 2005;17(1):1–14.
14 Tsujimoto H, Ono S, Efron PA, Scumpia PO, Moldawer LL, Mochizuki H. Role of toll-like receptors in the development of sepsis. Shock. 2008;29(3):315–21.
15 Drexler SK, Foxwell BM. The role of toll-like receptors in chronic inflammation. Int J Biochem Cell Biol. 2010;42(4):506–18.
16 Marshak-Rothstein A. Toll-like receptors in systemic autoimmune disease. Nat Rev Immunol. 2006;6(11):823–35.
17 Bartels YL, van Lent PLEM, van der Kraan PM, Blom AB, Bonger KM, van den Bosch MHJ. Inhibition of TLR4 signalling to dampen joint inflammation in osteoarthritis. Rheumatology (Oxford). 2024;63(3):608–18.
18 Gambuzza ME, Sofo V, Salmeri FM, Soraci L, Marino S, Bramanti P. Toll-like receptors in Alzheimer's disease: A therapeutic perspective. CNS Neurol Disord Drug Targets. 2014;13(9):1542–58.
19 Urban-Wojciuk Z, Khan MM, Oyler BL, Fåhraeus R, Marek-Trzonkowska N, Nita-Lazar A, et al. The role of TLRs in anti-cancer immunity and tumor rejection. Front Immunol. 2019;10:2388.
20 Kline JN, Krieg AM. Toll-like receptor 9 activation with CpG oligodeoxynucleotides for asthma therapy. Drug News Perspect. 2008;21(8):434–9.
21 Jackson Hoffman BA, Pumford EA, Enueme AI, Fetah KL, Friedl OM, Kasko AM. Engineered macromolecular toll-like receptor agents and assemblies. Trends Biotechnol. 2023;41(9):1139–54.
22 Janku F, Han SW, Doi T, Amatu A, Ajani JA, Kuboki Y, et al. Preclinical characterization and phase I study of an anti-HER2-TLR7 immune-stimulator antibody conjugate in patients with HER2+ malignancies. Cancer Immunol Res. 2022;10(12):1441–61.
23 Monnet E, Choy EH, McInnes I, Kobakhidze T, de Graaf K, Jacqmin P, et al. Efficacy and safety of NI-0101, an anti-toll-like receptor 4 monoclonal antibody, in patients with rheumatoid arthritis after inadequate response to methotrexate: A phase II study. Ann Rheum Dis. 2020;79(3):316–23.
24 Takeuchi O, Kawai T, Mühlradt PF, Morr M, Radolf JD, Zychlinsky A, et al. Discrimination of bacterial lipoproteins by toll-like receptor 6. Int Immunol. 2001;13(7):933–40.
25 Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, et al. Cutting edge: Role of toll-like receptor 1 in mediating immune response to microbial lipoproteins. J Immunol. 2002;169(1):10–4.
26 Gao W, Xiong Y, Li Q, Yang H. Inhibition of toll-like receptor signaling as a promising therapy for inflammatory diseases: A journey from molecular to nano therapeutics. Front Physiol. 2017;8:508.
27 Choteau L, Vancraeyneste H, Le Roy D, Dubuquoy L, Romani L, Jouault T, et al. Role of TLR1, TLR2 and TLR6 in the modulation of intestinal inflammation and. Gut Pathog. 2017;9:9.
28 Spyvee MR, Zhang H, Hawkins LD, Chow JC. Toll-like receptor 2 antagonists. Part 1: Preliminary SAR investigation of novel synthetic phospholipids. Bioorg Med Chem Lett. 2005;15(24):5494–8.
29 Agnihotri G, Crall BM, Lewis TC, Day TP, Balakrishna R, Warshakoon HJ, et al. Structure- activity relationships in toll-like receptor 2-agonists leading to simplified monoacyl lipopeptides. J Med Chem. 2011;54(23):8148–60.
30 Anwar MA, Shah M, Kim J, Choi S. Recent clinical trends in toll-like receptor targeting therapeutics. Med Res Rev. 2019;39(3):1053–90.
31 Tsukidate T, Hespen CW, Hang HC. Small molecule modulators of immune pattern recognition receptors. RSC Chem Biol. 2023;4(12):1014–36.
32 Clabbers MTB, Holmes S, Muusse TW, Vajjhala PR, Thygesen SJ, Malde AK, et al. MyD88 TIR domain higher-order assembly interactions revealed by microcrystal electron diffraction and serial femtosecond crystallography. Nat Commun. 2021;12(1):2578.
33 Brentano F, Schorr O, Gay RE, Gay S, Kyburz D. RNA released from necrotic synovial fluid cells activates rheumatoid arthritis

synovial fibroblasts via toll-like receptor 3. Arthritis Rheum. 2005;52(9):2656–65.
34 Basith S, Manavalan B, Lee G, Kim SG, Choi S. Toll-like receptor modulators: A patent review (2006–2010). Expert Opin Ther Pat. 2011;21(6):927–44.
35 Cen X, Wang B, Liang Y, Chen Y, Xiao Y, Du S, et al. Small molecule SMU-CX24 targeting toll-like receptor 3 counteracts inflammation: A novel approach to atherosclerosis therapy. Acta Pharm Sin B. 2022;12(9):3667–81.
36 Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. Science. 1998;282(5396):2085–8.
37 Pérez-Regidor L, Guzmán-Caldentey J, Oberhauser N, Punzón C, Balogh B, Pedro JR, et al. Small Molecules as Toll-like Receptor 4 Modulators Drug and in-house computational repurposing. Biomedicines. 2022;10(9):2326.
38 Chan M, Hayashi T, Mathewson RD, Nour A, Hayashi Y, Yao S, et al. Identification of substituted pyrimido[5,4-b] indoles as selective toll-like receptor 4 ligands. J Med Chem. 2013;56(11):4206–23.
39 Barochia A, Solomon S, Cui X, Natanson C, Eichacker PQ. Eritoran tetrasodium (E5564) treatment for sepsis: Review of preclinical and clinical studies. Expert Opin Drug Metab Toxicol. 2011;7(4):479–94.
40 Chan M, Kakitsubata Y, Hayashi T, Ahmadi A, Yao S, Shukla NM, et al. Structure-activity relationship studies of pyrimido[5,4-b] indoles as selective toll-like receptor 4 ligands. J Med Chem. 2017;60(22):9142–61.
41 Takashima K, Matsunaga N, Yoshimatsu M, Hazeki K, Kaisho T, Uekata M, et al. Analysis of binding site for the novel small-molecule TLR4 signal transduction inhibitor TAK-242 and its therapeutic effect on mouse sepsis model. Br J Pharmacol. 2009;157(7):1250–62.
42 Matsunaga N, Tsuchimori N, Matsumoto T, Ii M. TAK-242 (resatorvid), a small-molecule inhibitor of toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. Mol Pharmacol. 2011;79(1):34–41.
43 Rice TW, Wheeler AP, Bernard GR, Vincent JL, Angus DC, Aikawa N, et al. A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. Crit Care Med. 2010;38(8):1685–94.
44 Wang YC, Wang PF, Fang H, Chen J, Xiong XY, Yang QW. Toll-like receptor 4 antagonist attenuates intracerebral hemorrhage-induced brain injury. Stroke. 2013;44(9):2545–52.
45 Meijvis SC, van de Garde EM, Rijkers GT, Bos WJ. Treatment with anti-inflammatory drugs in community-acquired pneumonia. J Intern Med. 2012;272(1):25–35.
46 Samarpita S, Kim JY, Rasool MK, Kim KS. Investigation of toll-like receptor (TLR) 4 inhibitor TAK-242 as a new potential anti-rheumatoid arthritis drug. Arthritis Res Ther. 2020;22(1):16.
47 Feng S, Zhang C, Chen S, He R, Chao G, Zhang S. TLR5 signaling in the regulation of intestinal mucosal immunity. J Inflamm Res. 2023;16:2491–501.
48 Lopetuso LR, Jia R, Wang XM, Jia LG, Petito V, Goodman WA, et al. Epithelial-specific toll- like receptor (TLR)5 activation mediates barrier dysfunction in experimental ileitis. Inflamm Bowel Dis. 2017;23(3):392–403.
49 Chamberlain ND, Vila OM, Volin MV, Volkov S, Pope RM, Swedler W, et al. TLR5, a novel and unidentified inflammatory mediator in rheumatoid arthritis that correlates with disease activity score and joint TNF-α levels. J Immunol. 2012;189(1):475–83.
50 Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science. 2008;320(5873):226–30.
51 Mizel SB, Bates JT. Flagellin as an adjuvant: Cellular mechanisms and potential. J Immunol. 2010;185(10):5677–82.
52 Cervantes JL, Weinerman B, Basole C, Salazar JC. TLR8: The forgotten relative revindicated. Cell Mol Immunol. 2012;9(6): 434–8.
53 Ishii N, Funami K, Tatematsu M, Seya T, Matsumoto M. Endosomal localization of TLR8 confers distinctive proteolytic processing on human myeloid cells. J Immunol. 2014;193(10):5118–28.
54 Mark KE, Corey L, Meng TC, Magaret AS, Huang ML, Selke S, et al. Topical resiquimod 0.01% gel decreases herpes simplex virus type 2 genital shedding: A randomized, controlled trial. J Infect Dis. 2007;195(9):1324–31.
55 Pockros PJ, Guyader D, Patton H, Tong MJ, Wright T, McHutchison JG, et al. Oral resiquimod in chronic HCV infection: safety and efficacy in 2 placebo-controlled, double-blind phase IIa studies. J Hepatol. 2007;47(2):174–82.
56 Vasilakos JP, Tomai MA. The use of toll-like receptor 7/8 agonists as vaccine adjuvants. Expert Rev Vaccines. 2013;12(7):809–19.
57 Bhagchandani S, Johnson JA, Irvine DJ. Evolution of toll-like receptor 7/8 agonist therapeutics and their delivery approaches: From antiviral formulations to vaccine adjuvants. Adv Drug Deliv Rev. 2021;175:113803.
58 Hawtin S, André C, Collignon-Zipfel G, Appenzeller S, Bannert B, Baumgartner L, et al. Preclinical characterization of the toll-like receptor 7/8 antagonist MHV370 for lupus therapy. Cell Rep Med. 2023;4(5):101036.
59 Zhang S, Hu Z, Tanji H, Jiang S, Das N, Li J, et al. Small-molecule inhibition of TLR8 through stabilization of its resting state. Nat Chem Biol. 2018;14(1):58–64.
60 Kou M, Wang L. Surface toll-like receptor 9 on immune cells and its immunomodulatory effect. Front Immunol. 2023;14:1259989.
61 Nishimoto S, Fukuda D, Sata M. Emerging roles of toll-like receptor 9 in cardiometabolic disorders. Inflamm Regen. 2020;40:18.
62 Nickerson KM, Christensen SR, Shupe J, Kashgarian M, Kim D, Elkon K, et al. TLR9 regulates TLR7- and MyD88-dependent autoantibody production and disease in a murine model of lupus. J Immunol. 2010;184(4):1840–8.
63 Psallidas I, Backer V, Kuna P, Palmér R, Necander S, Aurell M, et al. A phase 2a, double- blind, placebo-controlled randomized trial of inhaled TLR9 agonist AZD1419 in asthma. Am J Respir Crit Care Med. 2021;203(3):296–306.
64 Ruzsa A, Sen M, Evans M, Lee LW, Hideghety K, Rottey S, et al. Phase 2, open-label, 1:1 randomized controlled trial exploring the efficacy of EMD 1201081 in combination with cetuximab in second-line cetuximab-naïve patients with recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN). Invest New Drugs. 2014;32(6):1278–84.
65 Casale TB, Cole J, Beck E, Vogelmeier CF, Willers J, Lassen C, et al. CYT003, a TLR9 agonist, in persistent allergic asthma - A randomized placebo-controlled Phase 2b study. Allergy. 2015;70(9):1160–8.
66 Wu YW, Tang W, Zuo JP. Toll-like receptors: potential targets for lupus treatment. Acta Pharmacol Sin. 2015;36(12):1395–407.
67 Calore F, Londhe P, Fadda P, Nigita G, Casadei L, Marceca GP, et al. The TLR7/8/9 antagonist IMO-8503 inhibits cancer-induced cachexia. Cancer Res. 2018;78(23):6680–90.
68 Kim YJ, Schiopu E, Dankó K, Mozaffar T, Chunduru S, Lees K, et al. A phase 2, double- blinded, placebo-controlled trial of toll-like receptor 7/8/9 antagonist, IMO-8400, in dermatomyositis. J Am Acad Dermatol. 2021;84(4):1160–2.
69 Lin SC, Lo YC, Wu H. Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. Nature. 2010;465(7300):885–90.