Toll-like receptor modulators: a patent review (2006 – 2010)

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Introduction: The immune response is mediated via two parallel immune components, innate and adaptive, whose effector functions are highly integrated and coordinated for the protection of the human body against invading pathogens and transformed cells. The discovery of pathogen recognition receptors (PRRs), most notably toll-like receptors (TLRs), in innate immunity has evoked increased interest in the therapeutic handling of the innate immune system. TLRs are germ line-encoded receptors that play a potent role in the recognition of a diverse variety of ligands ranging from hydrophilic nucleic acids to lipopolysaccharide (LPS) or peptidoglycan (PGN) structures in pathogens.

Areas covered: This review discusses recent updates (2006 – 2010) in completed, ongoing and planned clinical trials of TLR immunomodulator-based therapies for the treatment of infectious diseases, inflammatory disorders and cancer.

Expert opinion: Since the discovery of human TLRs, modulating immune responses using TLR agonists or antagonists for therapeutic purposes has provoked intense activity in the pharmaceutical industry. The ability of TLRs to initiate and propagate inflammation makes them attractive therapeutic targets. We are now at the stage of evaluating such molecules in human diseases. Additionally, there is also extensive literature available on TLRs in diseased states. These data provide a basis for the identification of novel immunomodulators (agonists and antagonists) for the therapeutic targeting of TLRs.

Keywords: agonist, antagonist, clinical trial, immunomodulator, infection, inflammation, innate immunity, pathogen recognition receptor, toll-like receptor

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1. Introduction

The past decade has seen a rebirth of interest in innate immunity and in the regulation of subsequent adaptive responses [1-3]. The foot soldiers of the innate immune system, namely, dendritic cells and macrophages, ingest pathogens and release cytokines drawing secondary, active and defensive cells from the blood. These active immune cells, mainly antigen-specific T- and B-cell clones, are selected during an adaptive immune response and the subsets of these clonal cells become long living memory cells that can be readily reactivated on re-exposure to antigens. Since being first described in the fruitfly, *Drosophila melanogaster*, the toll-like receptor (TLR) family of pathogen recognition receptors (PRRs) has become a major component in innate immunity, innate-adaptive crosstalk, infectious diseases and inflammatory conditions. In 1997, Medzhitov *et al.* were the first to report the cloning of a mammalian TLR homologue (now identified as TLR4) [4,5]. More than a decade has passed since the discovery of the first human TLR. During this period, this field of research has exploded so rapidly that all TLRs (i.e., 10 human TLRs) have now been cloned, many of their ligands discovered and their associated main

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Article highlights.

- The primary selling point of toll-like receptor (TLR)-targeting drugs is that they have fewer side effects compared to drugs with alternative mechanisms of action.
- Therapeutic targeting of several TLRs using synthetic TLR immunomodulators has achieved immense success in both clinical and advanced preclinical programs.
- Both TLR4 and TLR9-based immunotherapies have progressed well into clinical development.
- A potent role for the use of microRNAs in the regulation of genes involved in immune defense is currently being uncovered.
- There has been a slow progress in the development of TLR antagonists and only a few antagonists have entered into clinical phase.
- TLR immunotherapy field has re-emerged in terms of both research and commercial interest.

This box summarizes key points contained in the article.

signaling pathways identified. Moreover, much research work has been done on PRRs (TLRs), starting from the discovery of the *Toll* gene (**Figure 1**). As our understanding of innate immunity has developed, our interest in applying this knowledge to clinical problems has also increased in a parallel manner. Most of these translational efforts have been centered on TLRs, which is one of the largest and most well-studied families of PRRs.

The initial discovery of TLRs heralded a renaissance of interest in innate immunity for immunologists. TLRs are of interest to immunologists and other investigators due to their front-line role in the initiation of innate immunity against invading pathogens. These receptors are prototypical PRRs that play essential roles in the innate immune responses to microbial pathogens based on their ability to recognize conserved pathogen-associated molecular patterns (PAMPs). PAMPs recognized by TLRs include lipids, lipoproteins, proteins and nucleic acids derived from a wide range of microbes such as bacteria, viruses, parasites and fungi [2]. TLRs that recognize bacterial and fungal components are localized to the cell surface, whereas TLRs that recognize viral or microbial nucleic acids are localized to intracellular membranes such as endosomes or phagosomes.

TLRs are type 1 integral membrane glycoproteins comprising leucine-rich repeat motifs (ectodomain) which mediate the recognition of PAMPs and a conserved cytoplasmic toll/IL-1 receptor (TIR) endodomain which is required for downstream signal transduction that are joined by a single transmembrane helix [6]. Database searches have led to the identification of TLR homologues in many other species, with vertebrates typically having a repertoire of 10 – 12 TLRs [6,7] and sea urchins having > 200 [8]. To date, 10 and 12 functional TLRs have been identified in humans and mice, respectively, with TLR1-9 being conserved in both species. Mouse TLR10 is not functional due to retrovirus insertion, and TLR11, TLR12 and TLR13 have been lost from the human genome [9,10].

Innate immune responses begin with TLR recognition of specific microbial components that are widely expressed by bacteria, fungi, protozoa and viruses. Pathogen-encoded TLR ligands fall into three broad categories: lipids and lipopeptides (TLR2/1; TLR2/6 and TLR4), proteins (TLR5 and TLR11) and nucleic acids (TLR3, 7, 8 and 9) [2,11]. Thus, different TLRs are amenable to targeting by different types of agents. The specificities and origins of TLR exogenous and endogenous ligands are summarized in **Table 1**. Cell surface TLRs can be targeted by small molecules and antibodies, whereas intracellular nucleic-acid sensing TLRs require targeting by modified oligonucleotides [12]. Recently, there has been tremendous progress in the elucidation of the crystal structures of several TLR ectodomains with their ligands [13-15]. These solved structures provide several key molecular insights, which can be utilized in the design of TLR therapeutics.

The initial step in signal transduction involves dimerization of two receptor chains induced by the binding of a specific ligand [16]. Alternatively, in the case of TLR7, 8 and 9, the receptor may be present in the cell as a preformed but inactive dimer, and ligand binding may cause reorientation of the TIR domains [17]. In either case, association of the TLR TIR domains would provide a new scaffold that allows the recruitment of specific adaptor proteins for the formation of a post-receptor signaling complex, ultimately leading to the activation of nuclear factors and the production of pro-inflammatory cytokines. MyD88 (myeloid differentiation primary response gene 88), Mal (MyD88 adaptor-like; also known as TIRAP, TIR domain-containing adapter protein), TRIF (TIR domain containing adaptor inducing IFN-β; also known as TICAM1, TIR domain-containing adapter molecule 1), TRAM (TRIF-related adaptor molecule; also known as TICAM-2), and sterile α- and armadillo-motif containing protein are the five adaptor proteins which contain TIR domains that function in TLR signaling [10]. All TLRs activate a classical/canonical inflammatory signaling pathway via the MyD88 adapter protein (which binds to all TLRs, with the possible exception of TLR3 and certain signals of TLR4), leading to the activation of NF-κB and MAPK. Activation of IFN regulatory factors (IRFs) by TLR7, 8 and 9 also occurs via MyD88-dependent pathway [18,19]. Conversely, there are alternative/noncanonical pathways of NF-κB activation that do not require MyD88. TLR3 and certain signals of TLR4 can signal independent of MyD88 via TRIF pathway which induces the activation of IRFs and production of type 1 IFNs [20].

Since the discovery of TLRs, there has been much interest in modulating the activity of this signaling pathway for the development of drugs and vaccines that treat cancers and inflammatory diseases. Indeed, some naturally occurring positive and negative regulators of TLR signaling have been

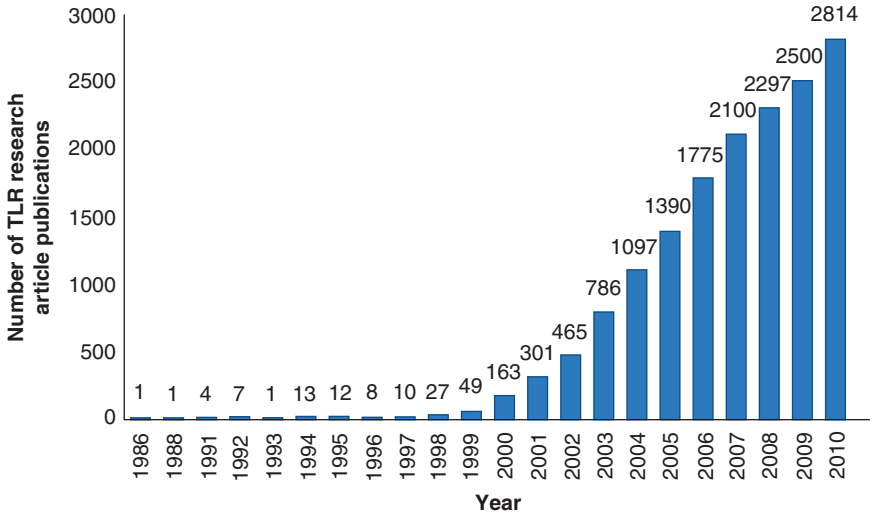

Figure 1. Research article publications related to TLRs. The number of TLR research articles published since 1986, grouped by year of publication. The total number of TLR related research articles published since the discovery of *Toll* gene is 15,821.

Table 1. Exogenous and endogenous ligands of TLRs.

Receptor	Exogenous and endogenous ligands	Ligand origin
TLR1	Triacyl lipopeptides*	Bacteria, mycobacteria
	Soluble factors	*Neisseria meningitidis*
TLR2	Lipoproteins/lipopeptides	Various pathogens
	Peptidoglycan	Gram-positive bacteria
	Lipoarabinomannan	Mycobacteria
	Lipoteichoic acid	Gram-positive bacteria
	Zymosan	Fungi
	Lipopolysaccharides (atypical)	*Leptospira interrogans* and *Porphyromonas gingivalis*
	Porins	*Neisseria* sp.
	Glycolipids	*Treponema maltophilum*
	Glycoinositol phospholipids	*Trypanosoma cruzi*
	Hsp70	Host
	HCV and non-structural 3 protein	HCV
TLR3	Double-stranded RNA	Viruses
TLR4	Lipopolysaccharides	Gram-negative bacteria
	Taxol	Plants
	Viral proteins	RSV, MMTV
	Hsp60 and Hsp70	*Chlamydia pneumoniae* and host
	Hyaluronic acid, type 3 repeat extradomain A of fibronectin, heparan sulfate (fragments) and fibrinogen	Host
	Envelope protein	MMTV
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides‡ such as MALP-2	Mycoplasma
	Zymosan	Fungi
	Lipoteichoic acid	Gram-positive bacteria
	Phenol-soluble modulin‡	*Staphylococcus epidermidis*
TLR7 and TLR8	Single-stranded RNA	Viruses
TLR9	CpG-containing DNA	Bacteria, malaria and viruses
TLR10	Not determined	Not determined
TLR11	Profilin-like molecule	*Toxoplasma gondii*

A limited list of known TLR ligands along with their specificities and origins.

*Ligands recognized by TLR1 and TLR2.

‡Ligands recognized by TLR2 and TLR6.

Hsp: Heat-shock protein; MMTV: Mouse mammary-tumor virus; RSV: Respiratory syncytial virus.

shown to be effective agents for the regulation of innate immunity [21]. Hence, manipulating the immune response by using TLR agonists or antagonists might be of therapeutic value. Here, in **Figure 2**, we summarize a number of worldwide patent applications filed in the past 5 years (2006 – 2010), while in **Table 2**, we highlight patent applications filed in the last 6 months (July – December 2010) that are related to the modulation of TLR pathways. Data were obtained from the European Patent Office database. TLR agonists are small molecular mimics of natural ligands that have improved pharmacodynamic and pharmacokinetic properties when compared to their natural large polymorphic parent moieties. They are often used as vaccine adjuvants and for the treatment of type 1 allergy, cancer and infectious diseases. Besides, TLR antagonists play a therapeutic role in suppressing overactive immune responses, as observed in chronic inflammatory and autoimmune diseases. Therefore, TLR agonists or antagonists may direct a cell-mediated or humoral response and thereby modulate diseases.

Recent advances in our understanding of TLR signaling pathways and the inducing and inhibitory effects of naturally occurring TLR modulators, discoveries related to exogenous and endogenous TLR ligands, developments in the inhibition of TLRs and structural insights into TLRs bound to their ligands, including antibodies, peptides and small molecules, have provided the possible means to interfere with TLRs clinically. In this review, we discuss the recent advances seen in the therapeutic targeting of both TLR agonists and antagonists, which are portrayed as emerging targets of immunomodulation.

2. Toll-like receptor agonists and their clinical applications

TLR agonists are immune system enhancers that rectify innate immune reactions and have been proposed to be useful in the treatment of cancer, allergies and viral infections. They are seen as adjuvants for potent new vaccines that prevent or treat cancer and infectious diseases. Recent investigations have demonstrated the presence of several host-derived molecules capable of binding TLR, in addition to PAMPs. It is a well-known fact that natural ligands are microbial components; hence, the search for synthetic agonists has increased. Natural TLR ligands are often the basis for first generation agonist molecules that can be developed for use in proof-of-concept experiments with preclinical models or in early clinical trials [22]. This schema has been applied to the development of synthetic TLR agonists. Generally, novel agonists are designed through variations of structures and synthetic stimulatory motifs can be used to modulate the immune system through TLR signaling pathways and develop novel drugs that are quite distinct from natural ligands [23]. The ability to modulate immune responses in a desired and optimal fashion may allow the targeting of a broad range of diseases. The safety and efficacy of some TLR agonists have been proven in humans as vaccine adjuvants and are currently in use in Europe [24,25]. Recent main findings in clinical trials related to the therapeutic effects of TLR agonists are summarized in **Table 3**.

2.1 TLR1, 2 and 6

TLR1, 2 and 6 are highly similar in their primary sequences and arose from an evolutionary gene duplication event [26]. Dimerization of these TLRs allows the recognition of a more specific and wider array of microbial components [27]. TLR2 is a cell surface receptor that senses a remarkable variety of bacterial, fungal and viral products as well as inflammatory self-components. TLR2 can form a heterodimeric complex with either TLR1, for recognition of triacylated lipopeptides such as Pam_3CSK_4, or TLR6, for recognition of diacylated lipopeptides such as macrophage-activating lipopeptide 2 kDa.

Among TLR2 agonists, bacterial lipoproteins are the most potent [28,29]. Additionally, TLR2 agonists are comprised of diverse structures, including bacterial and fungal lipids, acylated sugars and proteins, unmodified protein complexes, as well as certain polysaccharides [30,31]. Synthetic lipopeptide agonists for TLR2 exhibit strong adjuvant activity when either mixed with or directly conjugated to various antigens [32,33]. Besides lipopeptides, a variety of other natural TLR2 agonists exhibit adjuvant activity, including zwitterionic polysaccharides from Group B *Streptococcus* [34], type 2b heat-labile enterotoxin from enteropathogenic *Escherichia coli* [35] and porin B from pathogenic *Neisseriae* sp. [36]. TLR2 agonist, SMP-105, consists of cell-wall skeleton components such as mycolic acids and peptidoglycans from *Mycobacterium bovis* (BCG Tokyo) and has been approved for the treatment of bladder cancer [37]. Moreover, this compound has shown strong adjuvant and antitumor activities. In mice, SMP-105 activates NF-κB in a TLR2-dependent and TLR4-independent manner. On administration of the compound, TLR2 knockout mice show impairment of TNF-α and IL-6 production as well as reduced tumor growth [38]. Recently, Guan *et al.* identified three novel synthetic small molecule TLR2-1-agonists by chemical library screening that are able to activate cells in the nanomolar range and stimulate cytokine production in human peripheral blood monocytes, suggesting further clinical development [39].

2.2 TLR3

Activation of TLR3 is potentially promising in anticancer therapy. TLR3 recognizes viral double-stranded RNA (dsRNA) and polyriboinosinic-polyribocytidylic acid (poly I:C), a synthetic analog of dsRNA [40,41]. Additionally, polyriboadenylic-polyribouridylic acid (poly A:U) is also an agonist of both TLR3 and TLR7 [42]. Several clinical trials have reported that injection of dsRNA (TLR3 agonist) is associated with survival in cancer patients, as functional TLR3 has been shown to be expressed in breast cancer cells and to a very high extent in both primary and metastatic clear-cell renal and breast carcinoma cells [43].

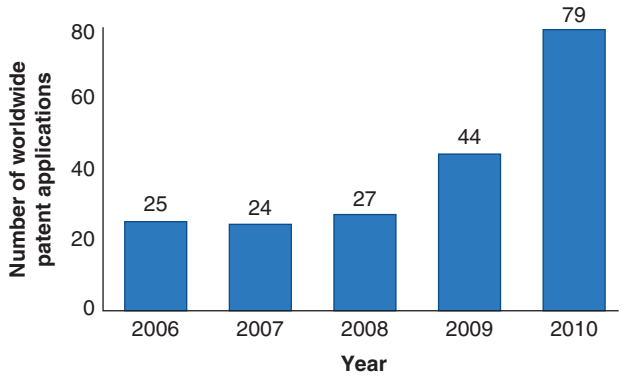

Figure 2. Worldwide patent applications related to TLRs. The number of worldwide patent applications published from the year 2006 to 2010.
Data were obtained from the European Patent Office database.

IPH 3102 is a high molecular mass synthetic dsRNA TLR3-specific agonist that activates NF-κB signaling and type 1 IFN responses *in vitro* and destroys melanoma and breast cancer cells. Additionally, IPH 3102 acts as a potent immunostimulator *in vivo* in mice [44]. This RNA-based drug candidate is being developed by Innate Pharma for the treatment of breast cancer and as a vaccine adjuvant. It is currently under the preclinical validation development phase. Ampligen (AMP-516, generic name: rintatolimod), a synthetic mismatched poly I:poly C dsRNA (polyI:polyC12U), is an experimental immunomodulatory drug being developed by HemiSpheRx for the treatment of chronic fatigue syndrome, hepatitis B and C infection, HIV, influenza, severe acute respiratory syndrome and cancer [45]. Poly I:C is known to induce T_H1-type immune responses, including induction of IFN-β [46,47], and has been studied as an adjuvant with a number of vaccines such as *Neisseria meningitides* serogroup B, HIV gag, *Plasmodium falciparum* circumsporozoite protein, *Mycobacterium tuberculosis* and tumor-associated proteins [48-51] in preclinical models. This drug is currently undergoing Phase III clinical testing.

2.3 TLR4

TLR4 is a critical component involved in the recognition of bacterial lipopolysaccharide (LPS) and is important to the host response when combating against Gram-negative bacterial infections [52]. TLR4 agonists have immunoregulatory applications as adjuvants for vaccines and in the treatment of chronic viral infections and cancer therapy. Monophosphoryl lipid A (MPLA), a TLR4 agonist, is an immunomodulatory product that is less toxic than LPS and specifically activates the TRAM/TRIF (MyD88-independent) pathway in TLR4 signaling, leading to the induction of IFN-β and regulation of CD80/86, which is a key aspect of adjuvancy [53]. MPLA has been approved as a component in an improved vaccine for hepatitis B in Europe (Fendrix) and is a component in a number of GSK (GlaxoSmithKline) biological vaccines for HPV, herpes simplex virus, malaria and tuberculosis, which are currently in clinical testing [54]. MPLA is also used in GSK's Cervarix, a cervical cancer vaccine. This vaccine is used to prevent early stage pre-cancerous lesions, pap smear abnormalities and cervical cancer caused by HPV types 16 and 18 [55]. It has recently been approved by FDA for the treatment of cervical cancer.

OM-174 is a diphosphorylated glucosamine disaccharide bearing three fatty acid chains and acts as a TLR4 agonist. This compound reduces tumor growth, increases IFN-γ production and prolongs the survival of mice [56,57]. It also has a good safety profile for use in humans and is currently under development by OM Pharma as a cancer immmunotherapeutic agent (Phase Ib clinical trial) and vaccine adjuvant. Pollinex Quattro is a novel vaccine containing ragweed pollen extract (chemically modified by glutaraldehyde) adsorbed onto L-tyrosine with added immunostimulatory adjuvant, MPL [58,59]. This drug has been developed for the prevention or relief of allergic symptoms caused by pollen from *Ambrosia* sp. (ragweed) and is currently in a Phase III clinical trial. Stimuvax (BLP25 liposome vaccine) is an innovative cancer vaccine designed to induce an immune response to cancer cells expressing MUC1, a protein antigen that is widely expressed in common cancers [60]. Stimuvax is thought to work by stimulating the body's immune system to identify and destroy cancer cells expressing MUC1. Stimuvax is being developed by Merck and is currently undergoing two Phase III clinical trials.

2.4 TLR5

TLR5 receptor recognizes flagellin, a principal protein component of bacterial flagella [61]. Vaccine adjuvants that act as TLR5 agonists are currently under investigation for the treatment of viral infections. VaxInnate is conducting Phase I clinical trials (NCT00603811) using VAX102 (Flagellin.HuM2e), a TLR5-targeting flagellin derivative conjugated to the M2e protein of influenza [62], and flagellin.HuHA and flagellin.AvHA fusion proteins (flagellin linked with the most immunoprotective domain of viral hemagglutinin, the globular head which is derived from human and avian influenza virus). These vaccine adjuvants could potentially protect against all strains of seasonal and pandemic influenza and are used in the treatment of various diseases, including bacterial, viral and parasitic infections.

CBLB502 [63] is a bio-engineered derivative of a microbial protein flagellin that potentially reduces injury from acute stresses, such as radiation and chemotherapy, by mobilizing several natural cell protective mechanisms, including inhibition of programmed cell death (apoptosis), reduction of oxidative damage and induction of regeneration promoting cytokines. This suggests that TLR5 agonists may be valuable as adjuvants for cancer radiotherapy. CBLB502 is currently being developed by Cleveland BioLabs under the FDA's Animal Efficacy Rule. The above findings demonstrate that

Table 2. Recent patent applications filed in the last 6 months (July – December 2010) related to the modulation of TLR activity.

Patent	Applicant	Invention
US2010168058	Stichting Katholieke Universiteit	Covers the usage of lipopolysaccharide isolated from the bacterium *Bartonella quintana* as novel antagonist of TLR4
US2010166778	Centocor Ortho Biotech, Inc.	Preparation and usage of TLR3 antibody antagonists and polynucleotides encoding TLR3 antibody antagonists or fragments are disclosed
WO2010077613	Gilead Sciences, Inc.	Generation of modulators of TLRs of formula (II): pharmaceutically acceptable salts, compositions containing such compounds and therapeutic methods that include the administration of such compounds
WO2010080007	Pusan National University	Covers the usage of lipoproteins derived from Gram-positive bacteria as TLR2 ligand
HK1112018	Schering Corp.	Generation of nucleic acids encoding nine human receptors designated DNAX TLR2–10(, mono-/polyclonal antibodies against these receptors, and their diagnostic and therapeutic use
CN101784548	Gilead Sciences	Includes compositions, methods and compounds of purine derivatives for the modulation of TLR7 pathways
US2010183638	HemiSpheRx Biopharma	*In vitro* or *in vivo* usage of mismatched double-stranded ribonucleic acid TLR3 agonist as antiviral agent, antiproliferative agent and immunostimulant
EP2209476	University of Pittsburgh	Describes the use of a TLR9 agonist and/or a TLR4 antagonist and/or a NOD2 agonist for treatment or prevention of disorders involving TLR4 activation, such as systemic sepsis and necrotizing enterocolitis
CN101790380	University of California	Covers the usage of conjugates of synthetic TLR agonists in vaccines to prevent, inhibit or treat a variety of disorders including pathogen infection and asthma
US2010189772	Coley Pharma	Methods and products for the treatment of viral infection using a combination of anti-viral agents and TLR ligands
WO2010088395	Idera Pharma	Generation of novel synthetic agonists of TLR9 that produce unique cytokine and chemokine profiles
EP2214692	Centocor Ortho Biotech	Describes the methods for the treatment or prevention of osteoarthritic conditions by using TLR4 antagonists
EP2216047	National University Corp. Tokyo Medical and Dental University; Nippon Chemiphar Co., Ltd	Generation of medicament containing cathepsin inhibitor as an active ingredient, which is used as a modulator for TLR signaling
WO2010093436	Carson Dennis A	Generation of small molecule conjugates that are agonists or antagonists of one or more TLRs
PT1830881	NovImmune	Describes the use of multiple neutralizing antibodies that immunospecifically bind to one or more TLRs in the treatment of inflammatory diseases
US2010247557	Sanofi Pasteur	Immunostimulant composition comprising at least one TLR7 or TLR8 agonist and a TLR4 agonist. The inventive composition can also comprise a vaccine antigen
MX2010009738	IRM LLC	Generation of novel class of compounds to treat or prevent diseases or disorders associated with TLR7 and TLR8
US2010255040	BioLeaders Corp.	Includes methods and compositions of poly-γ-glutamic acid for inducing the enhancement of TLR-mediated T_H1 cellular immunity
US2010256085	University of Yale	Includes methods, compositions and compounds of TLR agonists for the regulation of a VEGF-induced tissue response
EP2238155	Centocor Ortho Biotech	Generation of isolated polynucleotides encoding cynomolgus monkey TLR3
WO2010124226	University Of Massachusetts	TLR-based biosensors for detecting TLR binding to ligands and test compounds
WO2010127113	Centocor Ortho Biotech	Generation of TLR3 antagonists
US2010291577	University Of Massachusetts	Generation of TLR9 modulators that affect translocation and activity of TLR9 and MyD88
US2010291109	University of Colorado	Covers the usage of fusion proteins and DNA conjugates as immune adjuvants and vaccines for the treatment of several chronic diseases

Data were obtained from the European Patent Office database.

Table 2. Recent patent applications filed in the last 6 months (July – December 2010) related to the modulation of TLR activity (continued).

Patent	Applicant	Invention
WO2010131009	Medical Research Council	Treatment of IL-25 mediated diseases with TLR antagonists.
US2010297165	Office of Technology Transfer	Describes the methods and use of immunostimulatory combinations of TLR ligands
WO2010135054	USA: represented by the Secretary of Agriculture	Covers the identification of NS4B-VGIv using functional genetics (motif resembles TLR proteins) that efficiently protects swine from challenge
NZ571010	Vaxart, Inc.	Covers the usage of chimeric adenoviral vectors with TLR3 agonists for eliciting immune responses against the heterologous peptide
US2010303847	VaxInnate	Compositions of TLR agonists and papillomavirus antigens.
EP2257313	University Of Kentucky Research Foundation	Includes methods and compositions for the treatment or prevention of macular degeneration or other diseases or disorders associated with activation of TLR3 using ultra-small RNAs
WO2010141619	Harvard College	Methods and compositions for modulating the activity of XBP-I protein, or a protein in a signal transduction pathway involving XBP-I to modulate the TLR-mediated activation of cells of the innate immune system and also to identify compounds that modulate TLR-mediated signaling
US2010310606	Los Angeles Biomedical Research Institute	Claims the usage of a vaccine with killed but metabolically active (KBMA) protozoans with TLR agonists for induction of immune response

Data were obtained from the European Patent Office database.

TLR5 agonist may have broad therapeutic applications, as it acts as a linker adjuvant for vaccines and also halts excessive apoptosis in acute radiation syndromes, degenerative diseases and myocardial infarction [64].

2.5 TLR7 and 8

TLR7 and 8 are found in endosomes of monocytes and macrophages. Both of these receptors recognize single-stranded RNA from viruses. TLR7 and 8 are the only receptors where new chemical entities have been defined as agonists. Synthetic ligands have structures reminiscent of DNA or RNA oligonucleotides, such as guanosine-containing compounds and imidazoquinolines [22,65]. Investigators have focused on developing TLR7/8 agonists as antiviral agents against viruses such as HPV. Imidazoquinolines were originally developed as antiviral agents and many such small compounds have been tested for their ability to induce TLR7/8-mediated cytokine production.

Imiquimod (Aldara) is the first approved topically active TLR7 agonist. It is used for the treatment of cancer and has shown itself to be efficacious against primary skin tumors and cutaneous metastases [66]. Oncological lesions showing improvement on the use of Imiquimod include basal cell carcinoma, actinic keratosis, squamous cell carcinoma *in situ*, malignant melanoma, cutaneous T-cell lymphoma and cutaneous extramammary Paget's disease [67]. This drug has been approved for the treatment of external genital and perianal warts, but is also effective for the treatment of a host of other virus-associated dermatologic lesions, including common and flat warts, molluscum contagiosum and herpes simplex. 852A is a small molecule TLR7 agonist that is structurally related to Imiquimod and is currently being evaluated in a Phase II clinical trial by 3M Pharma for the treatment of melanoma [68]. This compound stimulates dendritic cells to produce multiple cytokines, including IFN-α *in vitro* and *in vivo* [69]. Another structurally related compound, R-848 (Resiquimod, 3M Pharma), a dual TLR7/TLR8 agonist inducing IFN-α, IL-12 and TNF-α, is currently undergoing a Phase II study for the treatment of HCV and other viral infections [70]. However, Phase III clinical trials of the drug for treatment of herpes simplex virus were suspended due to lack of efficacy [70].

A number of studies suggest that activation of TLR7 has benefits in patients infected with HCV. One study has shown that TLR7 is expressed in normal and HCV-infected hepatocytes, and activation of TLR7 alone reduces HCV mRNA and protein levels [71]. ANA975 (oral prodrug of isatoribine) was developed as an antiviral HCV treatment, but clinical studies for this TLR7 agonist were discontinued by Anadys Pharma due to indicated unacceptable toxicity via long-term animal studies [72]. Another small molecule TLR7 agonist, ANA773 (NCT01211626: an oral inducer of endogenous IFNs), is currently under investigation (Phase I clinical trial) for the potential treatment of HCV infection and cancer [73]. AZD8848/DSP-3025 is a potent TLR7 agonist currently undergoing a Phase IIa clinical trial (NCT00999466) to investigate its efficacy, tolerability, safety and therapeutic effect in allergic asthma patients administered inhaled allergen. This drug is being tested for the treatment of asthma and allergic rhinitis (hay fever).

Table 3. Clinical development status of TLR agonists.

Compound	Targeting TLR	Developing company/institute	Indications	Clinical status	Ref.
SMP-105	TLR2	Dainippon Sumitomo Pharma	Bladder cancer	Preclinical	[37,38,121]
AMP-516	TLR3	HemiSpheRx Biopharma	Cancer	Preclinical	[45]
			Viral infection	Phase II	
IPH 3102	TLR3	Innate Pharma	Breast cancer, melanoma and other cancers	Preclinical	[44]
Poly I:C	TLR3	Idera Pharma	Vaccine adjuvants	Phase III	[48,49]
Fendrix	TLR4	GSK (GlaxoSmithKline)	HBV infection	Marketed (in Europe)	[122]
Cervarix	TLR4	GSK	HBV infection, cervical cancer	Approved	[55]
OM-174	TLR4	OM Pharma	Cancer, vaccine adjuvant	Phase Ib	[56,57]
Pollinex Quattro	TLR4	Allergy Therapeutics	Allergy	Phase III	[59]
Stimuvax	TLR4	Merck	Cancer	Phase III	[60]
VAX102, flagellin.HuHA and flagellin.AvHA fusion proteins	TLR5	VaxInnate	Vaccine adjuvants: bacterial, viral and parasitic infections	Phase I	[62]
CBLB502	TLR5	Cleveland Biolabs, Inc.	Acute radiation syndrome	Phase Ib	[63]
Imiquimod (Aldara)	TLR7	3M Pharma	Virus-associated dermatologic lesions, papillomavirus infection	Approved	[66,67]
852A	TLR7	3M Pharma	Melanoma	Phase II	[69]
R-848 (resiquimod)	TLR7/TLR8	3M Pharma	HCV infection	Phase II	[70]
			Herpes simplex virus infection	Phase III: suspended (lack of efficacy)	
ANA975	TLR7	Anadys Pharma	HCV infection	Discontinued (unacceptable toxicity)	[72]
ANA773	TLR7	Anadys Pharma	HCV infection and cancer	Phase I	[73]
AZD8848	TLR7	AstraZeneca	Asthma and allergic rhinitis	Phase IIa	
VTX-1463	TLR8	VentiRx Pharma	Allergic rhinitis	Phase Ib	
VTX-2337	TLR8	VentiRx Pharma	Advanced solid tumors, lymphoma	Phase I	
IMO-2055	TLR9	Idera Pharma	Lung carcinoma	Phase Ib	
			Colorectal cancer	Phase Ib	
MGN-1703	TLR9	MOLOGEN AG	Colorectal cancer	Phase II – III	[81]
MGN-1706			Prostate cancer	Phase II	
ISS1018	TLR9	Dynavax Technologies	Non-Hodgkin's lymphoma, hepatitis vaccine adjuvant, allergy	Phase II	[80]
Agatolimod	TLR9	National Cancer Institute	T-cell lymphoma	Phase I – II	[82]
			Non-Hodgkin's lymphoma	Phase I – II	
			Breast cancer	Phase II	
SD-101	TLR9	Dynavax Technologies	HCV infection	Phase Ib	[79]
IMO-2125	TLR9	Idera Pharma	HCV infection	Phase I	[23]
NuThrax	TLR9	Emergent BioSolutions	Anthrax	Phase I	[82]
HEPLISAV	TLR9	Dynavax Technologies	HBV infection	Phase III	[79]
AVE0675	TLR9	Sanofi-aventis/Coley Pharma	Asthma and allergic rhinitis	Phase I	[108]
IMO-2134	TLR9	Idera Pharma	Allergy, asthma	Phase I	[123]
SAR-21609	TLR9	Sanofi-aventis/Coley Pharma	Asthma	Phase I	[83]
DIMS 0150	TLR9	InDex Pharma	Ulcerative colitis	Phase II	[84]

VTX-1463 is the first novel TLR8 agonist being developed intranasally by VentiRx Pharma for the treatment of allergic rhinitis (Phase Ib clinical trial). Preclinical studies of this drug have shown reduced allergic responses through the suppression of the T_H2-mediated allergic response. When administered intranasally, it resulted in a decreased number of eosinophils and less congestion. VTX-2337 is another small molecule TLR8 agonist that stimulates myeloid dendritic cells and monocytes and enhances NK cell responses. It is administered subcutaneously on a weekly basis and is currently under Phase I clinical development in oncology (NCT00688415). The VTX-1463 data combined with recent data generated from VTX-2337 in oncology validate TLR8 as an important and relevant target in human diseases.

2.6 TLR9

TLR9 acts as a receptor for unmethylated cytosine-phosphate-guanine (CpG) motifs in bacterial and DNA viruses [30]. CpG motifs contain adjacent cytosine and guanine nucleotides and are found to commonly occur in unmethylated form in bacterial and viral DNA, whereas in vertebrate DNA, these regions are rare and are usually in methylated form. CpG-containing oligo-deoxynucleotides (ODNs) targeting TLR9 have been widely studied for the treatment of cancer and other diseases. Synthetic TLR9 agonists resembling bacterial DNA consist of short ODNs bearing an unmethylated CpG motif with a phosphorothioate backbone that increases *in vivo* stability [74]. Depending on their nucleotide sequence and length, CpGs are classified into class A, class B and class C and either oligomerize or form duplexes, leading to the activation of different cell types and ultimately the production of different cytokine profiles [75]. CpG ODNs have shown substantial potential as vaccine adjuvants and as mono- or combination therapies for the treatment of cancer, infectious and allergic diseases [76].

Immune modulatory oligonucleotides (IMOs) that stimulate TLR9 signaling are being developed. The TLR9 agonist IMO-2125 is in clinical development for the treatment of chronic HCV infection. IMO-2125 induces high levels of IFN-α as well as other immune system proteins that have potent activity in HCV replicon cell-based assays. Currently, Idera is evaluating IMO-2125 in two ongoing clinical trials (NCT00990938: Phase I clinical trial with ribavirin in treatment-naive patients with HCV; NCT00728936: Phase I clinical trial in null-responder patients with HCV) featuring two different HCV patient populations. IMO-2055 became the company's first IMO drug candidate to enter into clinical development. This drug has anticancer activity in a mouse model when used as a monotherapy, and its activity was found to be amplified when used in combination with chemotherapeutic agents [77]. In a Phase I trial that evaluated the safety and immunological activity of IMO-2055 alone and in combination with chemotherapy agents, IMO was found to be much more efficacious when used in combination. This compound is currently being analyzed in two Phase Ib trials, the first trial being in patients with non-small cell lung carcinoma in combination with Avastin and Tarceva (NCT00633529) and the second in patients with colorectal cancer in combination with Erbitux and chemotherapy (NCT00719199) [78]. During Idera's collaboration with Novartis, IMO-2134 was identified as a lead compound for asthma and allergy indications, and Novartis initiated a Phase I clinical trial using IMO-2134, also known as QAX935. On termination of the research collaboration and option agreement in February 2010, Idera regained the rights to IMO-2134. The company is currently evaluating the next steps in developing IMO-2134 for the treatment of respiratory diseases.

SD-101 is a novel C type TLR9 agonist [79] being developed by Dynavax for the treatment of chronic HCV infections and may offer a more effective therapeutic option. *In vitro* data studies of the drug in human blood cells demonstrate that compared to first-generation TLR9 agonists, SD-101 stimulates 20-fold higher levels of both IFN-α and IFN-λ, which are two classes of IFNs with potent activity against HCV. Another TLR9 agonist in development by Dynavax is ISS1018. This IMO is currently being used in clinical trials alone or in combination with antigens to combat non-Hodgkin's lymphoma and other cancers. It also acts as a hepatitis vaccine adjuvant (NCT00426712, NCT00435812 and NCT00511095) and in allergy indications (NCT00537355) [80]. ISS1018 has demonstrated efficacy in treating follicular lymphoma in combination with Rituxan in a Phase II clinical trial (NCT00251394) and in treating non-Hodgkin's lymphoma. This compound is in a Phase I clinical trial (NCT00403052) for the treatment of metastatic colorectal cancer. Dynavax's lead product candidate is HEPLISAV, a hepatitis B vaccine that met its primary end point in a Phase III trial and demonstrated the potential to provide more rapid and increased protection against hepatitis B viral infection. This vaccine combines hepatitis B surface antigen with a proprietary TLR9 agonist known as ISS to enhance the immune response. Recently, HEPLISAV also demonstrated superior seroprotection in diabetics when compared to Engerix-B.

MOLOGEN AG has developed two novel types of TLR9 agonists in the form of the DNA immunomodulator dSLIM (double stem loop immunomodulator). The use of dSLIM activates the immune system to protect against tumor-associated antigens by targeting the TLR9 receptor on certain immune cells [81]. As a result of chemotherapy and radiotherapy, tumor-associated antigens are released by cancer cells. The immune system activated by dSLIM is in a position to overcome its fatal tolerance towards cancer cells and tumor-associated antigens and attacks them selectively. The results of a completed Phase Ib study confirm an excellent safety profile for MGN1703. Treatment with the investigational drug was well tolerated and no dose-limiting or serious side effects were identified. This cancer medication, MGN1703, is currently being investigated in a Phase II – III clinical study for the treatment of metastasized colorectal cancer. Another DNA-based immunomodulator,

MGN1706, is currently in a Phase II clinical study for the treatment of metastasized, hormone-refractory prostate cancer.

Agatolimod is a CpG-based oligonucleotide therapy [82] that is being developed to treat cutaneous T-cell lymphoma (NCT00091208: Phase I – II), a non-Hodgkin's lymphoma, together with Rituximab and Yttrium Y 90 ibritumomab tiuxetan drugs (NCT00438880: Phase I – II) and metastatic breast cancer in combination with Trastuzumab (NCT00824733: Phase II). Emergent BioSolutions, Inc. initiated a Phase I clinical trial for NuThrax (anthrax vaccine adsorbed with CpG 7909 adjuvant), also known as AV7909. The product candidate, a third generation vaccine being developed as part of Emergent's anthrax franchise, consists of BioThrax (anthrax vaccine adsorbed) in combination with a novel immunostimulatory compound, CpG 7909 [82]. Preliminary data from a Phase I clinical trial are expected to be available in the third quarter of 2011. CpG DNA-based TLR9 agonists, AVE0675 and SAR-21609, are being examined for the treatment of asthma and viral respiratory tract infection, either alone or in combination with specific allergen immunotherapies. AVE0675 inhalation monotherapy has been approved for a Phase I clinical trial in patients with allergies [83]. InDex Pharmaceuticals AB's lead product DIMS 0150 has orphan drug designation in Europe and has completed its third clinical Phase II trial for the treatment of steroid-resistant/dependent ulcerative colitis [84]. It has currently been approved for use in clinics as a rectal, single-dose administration.

2.7 Poly-TLR agonists

Cadi-05 is a poly-TLR, poly-antigenic vaccine containing autoclaved mycobacteria with potential immunostimulating and antineoplastic activities. On administration, poly-TLR, poly-antigenic vaccine activates numerous TLRs. This vaccine is being evaluated for the treatment of advanced stage III or IV melanoma (NCT00675727). In a Phase I clinical trial, six out of nine patients responded to therapy. Four of the six patients were available for follow-up at 18 months following therapy and demonstrated stable disease. Three years after therapy, three of the six responders became asymptomatic and showed no disease occurrence. Cadi-05 used along with chemo-radiotherapy was shown to provide a durable response. However, a Phase I – II clinical trial examining safety in patients with stage III or IV melanoma was terminated due to safety concerns and lack of efficacy.

3. Toll-like receptor antagonists and their clinical applications

Although the most notable function of TLRs is in pathogen sensing, overactivation of TLRs to invading pathogens may lead to dysregulated systemic immune responses during the sepsis-development process [85]. It is a well-known fact that disease conditions are triggered by infections with ensuing inflammation. This condition subsequently leads to the production of endogenous ligands for TLR pathways, which further propagate the inflammation directly pivoting into severe chronicity. Increasing evidence has shown that TLRs recognize not only PAMPs but also endogenous ligands associated with cell stress [86]. These findings suggest that TLRs play a Janus role in disease development, acting as key molecules in immunity against microbes as well as playing a role in the development of autoimmune diseases, such as systemic lupus erythematosus (SLE) [87], arthritis [88], asthma [89] and arteriosclerosis [90]. Hence, targeting of TLRs by means of antagonists might lead to remission of these chronic diseases.

Development of TLR antagonists is recognized as a promising direction in suppressing associated inflammatory reactions. TLR antagonists are compounds that block immune system activation that is mediated through the targeted receptor. TLR antagonists have mostly been developed as structural analogs of agonists, which bind to the receptor but fail to induce signal transduction, thus, preventing the agonistic action of TLR ligands responsible for the induction of the inflammatory/autoimmune cascade [91]. Some of these antagonists actually stem from structure–activity relationships developed in TLR agonist programs [92]. Other TLR antagonists include anti-TLR antibodies and small molecule antagonists selected from compound libraries. The recent main findings in clinical trials related to the therapeutic effects of TLR antagonists are summarized in **Table 4**.

3.1 TLR2

Hyperstimulation of TLR2-expressing immune cells by microbial products can contribute to pathogen-induced chronic inflammatory joint disease, Gram-positive sepsis and other inflammatory disorders [93]. OPN-305, developed by Opsona, is a TLR2-specific mAb that inhibits TLR2-mediated pro-inflammatory cytokine production and is being tested for the potential treatment of inflammatory diseases. Positive preclinical data has been generated in multiple models of diseases, including cardiac and kidney ischemia/reperfusion injuries, sepsis and *ex vivo* models of human rheumatoid arthritis (RA) [94]. Currently, Opsona intends to develop the antibody through to the completion of Phase II human trials.

Aptamers are single-stranded DNA or RNA molecules that possess the capacity to bind to specific target molecules. DNA aptamer, AP177, identified by SELEX (systemic evolution of ligands by exponential enrichment) screening binds to TLR and competitively antagonizes TLR2 ligand binding, thereby, inhibiting NF-κB and pro-inflammatory cytokine production [95]. This novel technique combines immunoprecipitation with SELEX to identify and characterize immune-regulating oligonucleotides to facilitate the screening of high-affinity DNA or RNA molecules that bind to TLRs. The results of functional assays have shown that AP177 acts as a TLR2 antagonist and may hold therapeutic

Table 4. Clinical development status of TLR antagonists.

Compound	Targeting TLR	Developing company	Indications	Clinical status	Ref.
OPN-305	TLR2	Opsona Therapeutics	Inflammation, autoimmunity, ischemia/reperfusion	Preclinical	[94]
OPN-401	TLR4	Opsona Therapeutics	Inflammatory bowel disease, RA	Preclinical	
Eritoran	TLR4	Eisai Pharma	Sepsis and septic shock	Phase III	[97,124]
TAK-242	TLR4	Takeda Pharma	Sepsis	Suspended in Phase III	[100]
Cpn10	TLR4	CBio Ltd	RA, MS, psoriasis	Phase II	[98]
NI-0101	TLR4	NovImmune	Acute and chronic inflammation	Preclinical	
1A6	TLR4	NovImmune	Colitis	Preclinical	[102]
AV411	TLR4	Avigen	Pain management and withdrawal	Phase II	[101,125]
IRS-954 (DV-1079)	TLR7 and 9	Dynavax Technologies	SLE, HIV	Preclinical	[106]
IMO-3100	TLR7 and 9	Idera Pharma	SLE, RA, MS	Phase I	
CPG-52364	PolyTLR	Pfizer	SLE	Phase I completed	[108]

RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus.

potential in the treatment of diseases related to dysregulated TLR2 immune responses.

Blocking TLR2 or TLR4 with a neutralizing antibody seems to be another promising route of drug discovery. One such mAb, T2.5, has been shown to prevent sepsis induced by TLR2 ligands. Additionally, when T2.5 is used in combination with an anti-TLR4/MD-2 antibody, it protects mice against sepsis induced by *Salmonella enterica* or *E. coli* when administered with antibiotics [96]. This latter finding suggests that a combination approach involving anti-TLR4 and anti-TLR2 might be an effective adjunct to antibiotics in the prevention or treatment of sepsis.

3.2 TLR4

TLR4 signaling has been demonstrated to play a potent role in the development of sepsis, RA, psoriasis, asthma and multiple sclerosis (MS). Hence, blocking TLR4 signaling with antagonists may prevent these diseases and, consequently, TLR4 antagonists are being developed for these indications. A more advanced TLR antagonist is Eisai's Eritoran (E5564), which targets TLR4 and has reached Phase III trials (NCT00334828) for the treatment of sepsis and septic shock. E5564 is a synthetic lipid A analog that inhibits LPS from activating TLR4 in patients with endotoxinemia [92]. In Phase I clinical trials, it inhibited TNF-α production in a dose-dependent manner, and in a Phase II trial, it reduced the mortality rate due to sepsis by 6.4% compared to the placebo group [97]. Moreover, the pharmacodynamic profile of E5564 requires administration as a continuous infusion or by repeated intravenous injections to maintain the levels of active drug high enough to elicit a therapeutic effect.

Cbio's Cpn10 (chaperonin 10) molecule has anti-inflammatory and immunomodulatory properties via inhibition of downstream events in TLR-activated pathways. It has also shown promising safety and efficacy in Phase II clinical trials for the treatment of RA [98], psoriasis and MS [99]. Cpn10 inhibits both the TLR4-mediated induction of NF-κB activation by LPS as well as the production of TNF-α and IL-6 in human PBMCs from healthy volunteers and patients with MS. Cpn10 is safe and well tolerated when administered to patients with MS for 3 months; however, an extended Phase II study primarily focused on efficacy is warranted. OPN-401 is a viral protein-derived peptide being developed by Opsona that inhibits TLR4-dependent signaling. This drug is currently in preclinical development phase.

TAK-242 (Resatorvid) also targets TLR4-dependent signaling, although the precise target is not known [100]. Development of this compound was discontinued during a Phase III sepsis clinical trial (NCT00633477) because the drug's profile did not meet the criteria required to support continued development, not due to drug safety issues. Ibudilast (AV411), another TLR4 antagonist, suppresses pro-inflammatory cytokines such as TNF-α and IL-6 and may induce the anti-inflammatory cytokine IL-10. This drug is currently undergoing Phase II trials (NCT00723177) for treatment of opioid dependence (chronic pain and addiction withdrawal) [101]. NovImmune's NI-0101 is a humanized mAb that binds specifically and selectively to human TLR4. It binds to an epitope on TLR4 and interferes with the dimerization required for intracellular signaling and induction of numerous pro-inflammatory pathways. A mAb, 1A6, targets the TLR4–MD-2 complex and has previously shown protective effects in a mouse model of sepsis [96]. When this mAb was administered to murine dextran sulfate sodium, it delayed the development of colitis and reduced the inflammatory response. However, on administration during the recovery stage of the disease, it impaired mucosal healing [102].

3.3 TLR7 and 9

Dysregulated activation of the immune system through TLR pathways is believed to drive many inflammatory and

autoimmune disorders. TLR7 and 9 have been shown to play a major role in the activation of autoreactive B cells [103] and subsequent development of systemic autoimmune disease such as SLE [104]. The production of both pathogenic auto-antibodies and type 1 IFNs, which are hallmarks of SLE pathogenesis [105], can be driven by RNA- and DNA-associated auto-antigens and immune complexes through TLR7 and 9 activation.

IMO-3100, an antagonist of TLR7 and 9, is a leading drug candidate in development by Idera for the treatment of autoimmune and inflammatory diseases. Independent research studies suggest that pro-inflammatory cytokines characteristic of autoimmune disease are induced through activation of TLR7 and 9. IMO-3100 is designed to block the production of multiple pro-inflammatory cytokines induced through TLR7 and 9. In contrast, many current autoimmune disease treatments aim to block the activity of individual cytokines. IMO-3100 has demonstrated potent activity in reducing pathologic and immunologic manifestations in preclinical mouse models of diseases such as SLE, RA, psoriasis and hyperlipidemia. IMO-3100 is currently being evaluated in a Phase I clinical program.

Dynavax has pioneered a new approach for the treatment of autoimmune and inflammatory diseases with its first-in-class oligonucleotide-based endosomal TLR inhibitors, called immunoregulatory sequences (IRS). Dynavax's lead inhibitor drug candidate, DV-1079 (IRS 954), is a bifunctional inhibitor of TLR7 and 9. This drug prevents SLE progression in SLE-prone mice and reduces serum-levels of nucleic acid specific antibodies [106]. Administration of this drug to HIV-stimulated peripheral blood monocyte cells also leads to a decrease in IFN-α production, suggesting a potential therapeutic opportunity for treating HIV infection [107]. Preclinical data from animal model studies show that Dynavax's TLR inhibitors block IFN-α and also reduce symptoms in multiple autoimmune disease models of SLE, inflammatory skin disorders and RA.

3.4 Poly-TLR antagonists

CPG-52364, a small molecule TLR7, 8 and 9 antagonist (orally available), has recently completed a Phase I clinical trial (Pfizer: NCT00547014). It is designed to inhibit disease development of SLE and other autoimmune disorders in which TLRs are inappropriately activated, such as in RA and psoriasis [108]. This drug is found to interfere at the early stage of immune cascade by blocking inappropriate immune activation of these three TLRs without causing general suppression of immune function.

4. Conclusion

Recent pharmaceutical developments in the area of targeting TLR receptors with agonists or antagonists for the therapeutic treatment of several diseases hold great clinical promise, despite the pitfalls observed using some drugs, which have been suspended in late clinical stages. In fact, TLR research has come a long way, starting from the discovery of the first *Toll* gene in fruitfly. As more and more details of TLR signaling pathways continue to be uncovered, this area of research represents an interesting mining field for the discovery of novel therapies.

5. Expert opinion

In the past few years, there has been a tremendous progress towards deciphering the role and biology of TLRs. These innate immune sensors play pivotal roles in providing immunity to infection, chronic inflammation and adjuvanticity. TLRs have been implicated in the therapeutic targeting of several diseases due to their key functions in inducing cytokine production in disease states and in the early stages of TLR pathways. Hence, stimulating or inhibiting TLRs will prove more potent in the clinic. TLR agonists or antagonists have effects beyond infectious control and may represent new therapeutics for immunostimulation in vaccination, cancer, inflammatory disorders and allergies. Using solved crystal structures and molecular docking studies of TLRs [109-111], it is now possible to design varied immunomodulators. The solved structures of several TLRs may aid medicinal chemists in the rational design of small molecule agonists and antagonists. However, there is also likely to be significant potential of non-traditional approaches to drug discovery, such as the use of microRNAs (miRNAs) [12]. A key role for miRNAs in the regulation of genes involved in immune defense is currently being uncovered [112].

To date, therapeutic targeting of several TLRs using synthetic TLR immunomodulators has achieved immense success in clinical and advanced preclinical programs. Recent clinical data support our belief that targeted modulation of the innate immune response at the level of TLRs might prevent uncontrolled infection and limit inflammation in multiple diseases. The primary selling point of TLR-targeting drugs is that they have fewer side effects compared to drugs with alternative mechanisms of action. Moreover, TLR antagonists appear to be quite promising for the treatment of a number of inflammatory and autoimmune diseases. Thus, the immunotherapy field has re-emerged in terms of both research and commercial interest. From our current review, it can be witnessed that both TLR4 and TLR9-based immunotherapies have progressed well into clinical development (**Tables 3** and **4**). By exploiting pathogen-associated microbe-derived agonists to TLR4 and 9, it is anticipated that adaptive immunity will be accelerated and become more durable, and possibly render 'weak' antigens more immunogenic, eliciting a protective response to a variety of infectious agents. So far, many preclinical studies and clinical outcomes support superior vaccine performance on administration of TLR4 agonists as an adjuvant. For example, the cervical cancer vaccine, Cervarix, has recently been approved by the FDA and has proven to be safe and relatively effective. The underlying success for

Cervarix's protective effects is due to its adjuvant system, ASO4, which consists of the bacterial endotoxin derivative, MPLA, a TLR4 agonist [55]. This vaccine sensation has solidified the future of TLR agonists as vaccine adjuvants to be more optimistic. Additionally, endosomally-located TLRs, such as TLR3, 7, 8 and 9, and their ligands have created a great deal of interest in immunotherapeutic applications. TLR3 agonists such as poly I:C and small molecule agonists of TLR7/8 such as imidazoquinoline compounds could promote the development of T_H1 immune responses [113]. TLR7/8 agonist, Aldara (Imiquimod), has recently been approved for the topical treatment of actinic keratosis, genital warts and superficial basal cell carcinoma in humans. TLR9-based synthetic agonists are being evaluated for allergy, asthma and cancer immunotherapies and vaccine adjuvants [114].

Treatment of patients with sepsis using anti-inflammatory therapies has thus far not achieved any beneficial effects in improving clinical diseases [115]. Hence, there has been slow progress in the development of TLR antagonists, and only a few antagonists have entered into clinical phase. In terms of TLR synthetic antagonists, we have promising data beyond Phase III clinical trials for only one inhibitor, Eritoran. Most of the other synthetic TLR antagonists are still in their preclinical development stages. Clinical trials with TLR antagonists are currently focused on the treatment of septic shock and autoimmune disorders. Small molecules that inhibit MyD88 binding to TLR4 are also emerging [116]. Cell-penetrating peptides fused with the BB loop sequences of TLR2 and 4 also inhibit LPS-induced signaling, probably by interfering with either receptor dimerization or adaptor recruitment [117]. Despite recent successes in the development of therapeutics to treat autoimmunity, new therapeutic strategies are being developed to address remaining areas of a high unmet clinical need. Still, efforts are required to address certain problems in the utilization of TLR therapeutics. One major problem is that some antagonists might block multiple TLRs and, therefore, may give rise to unwanted immunosuppression. Another concern is that when TLRs are improperly routed, they may pave the way for chronic inflammation, as seen in the case of the TLR7 agonist Imiquimod, which results in aggravation of psoriatic plaques in patients [118]. Moreover, there is a question as to whether these new synthetic TLR immunomodulators should be designed to fight the host's mediators or improve anti-inflammatory protective responses. Additionally, long-term monitoring of the novel drug's immunogenic effect in patients must be undertaken.

The major drawback of utilizing immune-related therapies is that animal models may be insufficient for defining new therapeutic approaches. This has been a matter of debate regarding TLR targeting drugs due to the differences observed in expression and function of different TLRs, such as the key differences seen between the activation profiles of human and mouse TLR8 [119,120]. It is also important to note that animal studies are usually carried out using inbred strains, which have less genetic diversity compared to humans. Moreover, responses to TLR7 agonists result in widely varying induction of IFN-α and pro-inflammatory cytokines such as TNF-α in humans. Therefore, it is likely that cytokine induction in animal models will not reveal the true range of human responses; hence, there is a large margin of error that must be considered when predicting doses and safety windows for clinical trials. Another intriguing quest lies in the activation mechanism of TLR receptors by synthetic agonists, which remains unknown. For example, TLR4 receptor activation requires a co-receptor such as MD-2. Does this same mechanism hold true for TLR4 designed agonists? Do these synthetic agonists also need a co-receptor to bind with TLR receptor? Are the binding sites similar for different agonists? Unless and until there are some crystallographic evidences available showing the binding of agonists to TLRs, these queries cannot be solved.

Despite these challenges, harnessing the powerful stimulatory properties of TLRs has great potential to aid in the development of new strategies to fight infection. Therapeutic targeting of TLRs in several diseases and its associated clinical development (TLR immunomodulators) suggest that this area is more productive in the field of drug research. Although it is hard to predict where therapeutic targeting of TLRs will be in the future, we have some promising data and late clinical phase trials on the horizon in which the fundamental research and development have never been hitched. This steadily increasing number of newly discovered TLR immunomodulators will have a crucial impact on our understanding of the mechanisms of action of immune adjuvants as well as the pathogenesis of inflammatory disorders and infectious diseases and also help investigators in the direction of the development of new drugs in the near future.

Declaration of interest

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Databases

ClinicalTrials.gov: http://www.clinicaltrials.gov/
European Patent Office Database: http://ep.espacenet.com/

Further information

Phase Ib clinical trial of VTX-1463 in patients with allergy:
http://www.ventirx.com/product/allergy-vtx-1463.htm
IMO-2134 development:
http://www.iderapharma.com/development/imo-2134.php
Clinical development program of HEPLISAV:
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