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Full Length Research Paper

Potential therapeutic interventions on toll like receptors for clinical applications

Zhengwu Lu

1006 S De Anza Blvd#K104, San Jose, CA 95129, USA. E-mail address: Zhengwu.Lu@ieee.org.

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Toll like receptors (TLRs) function as pattern-recognition receptors (PRRs) and play key roles in the recognition of microbial components or endogenous ligands induced during inflammatory response. Studies on TLR-deficient mice have indicated TLRs' involvement in multiple pathologic conditions, and targeting of either the TLRs themselves or the signals they generate is proving to be of great interest to researchers as evidenced by increased research findings among immunologists, pharmacologist, and pharmaceutical scientists on TLRs. As animal models, cellular and molecular mechanisms on TLR mediated disease pathogenesis are made available, drug intervention strategies and early stage clinical studies can be planned and initiated to seek clinical proof for justifying TLRs and their associated signaling pathway/molecules as therapeutic targets. Moreover, a key functional output from TLRs is the generation of inflammatory cytokines such as tumor necrosis factor (TNF) and IL-6, which are excellent targets for inflammatory diseases such as rheumatoid arthritis.

Key words: TLR, PRR, NF-κB, agonist, antagonist, drug discovery.

INTRODUCTION

It is well established that the innate immune system is essential to human survival, offering the first line of defense by recognizing and responding to pathogenic threats when microorganisms invade an organism's barriers. Recent research using positional cloning and knockout animal models has provided us with insight of the powerful Toll like receptors (TLRs) involved in innate immunity. TLRs are pattern-recognition receptors (PRRs) and play a key role in the innate immune system (Akira and Hemmi, 2003). Once microbes have breached physical barriers such as the skin or intestinal tract mucosa, TLRs recognize specific components of microbial invaders and activate an immune response to these pathogens. A downstream signaling cascade is activated to stimulate the release of inflammatory cytokines and chemokines as well as to upregulate the expression of immune cells. All TLRs have a Toll-IL-1 receptor (TIR) domain that initiates the signaling cascade through TIR adapters. Adapters are platforms that organize downstream signaling cascades leading to a specific cellular response after exposure to a given pathogen (Guo and Cheng, 2007).

There are ten TLRs in humans; and they recognize different microbial ligands during infection (Janssens and Beyaert, 2003). It is recognized that TLRs bind and become activated by different ligands located on different types of organisms or structures. They also have different adapters to respond to activation located either at the cell surface or in internal cell compartments. Additionally, TLRs are expressed by different types of leucocytes or other cell types (Waltenbaugh et al., 2008). Also of great interest are the different signaling pathways activated by TLRs. These pathways lead to the activation of the respective transcription factors, nuclear factor kB (NF-kB) and interferon regulatory factor 3 (IRF3), which in turn induce various immune and inflammatory genes. A greater understanding of the TLRs and their roles in immunity holds potential for the development of therapeutics for bacterial and viral infections, allergies and cancer, and also to limit the damage caused by autoimmune disorders. Moreover, the role of TLRs in tissue repair and regeneration provides a further avenue for drug targeting (Coyne, 2008). However, the fine balance of cell signaling these receptors participate in has wide-ranging and powerful effects on phenotype expression owing to the impact on thousands of individual genes; clearly, the issue of adverse effects could be quite a challenge to address. It is recognized that TLRs bind to specific ligands, distribute on different cell types, and play key roles in the pathophysiology of various disorders involving both the innate and adaptive immunity (O'Neill et al., 2009). Additionally, NF-kB

governs the expression of numerous genes that are important for various cellular responses. Its activation is induced by a wide variety of stimuli including stress, cigarette smoke, viral and bacterial products, cytokines, free radicals, carcinogens and tumor promoters (Li et al., 2005). Deregulation of the NF-kB pathway has been observed in and attributed to the development of a variety of human ailments including cancers, autoimmune disorders, pulmonary, cardiovascular, neurodegenerative and skin diseases. Efforts to develop modulators of NF-kB have yielded several candidates, some of which are currently in Phase I/II of clinical trials such as NF-kB inhibitors CH828 from Leo Pharma to treat solid tumors and AS602868 from Serono International to treat acute myeloid leukemia (Sethi and Tergaonkar, 2009). On pharmacokinetic perspective, the wide tissue distribution of TLRs indicates complexity in deciding whether an agonist or an antagonist will be most effective therapeutically in humans for specified indications and disease types. This will need both pre-clinical and clinical data support to push any drug candidates further in the study phases. In this review, we focus on recent development of novel therapeutics that target TLRs or their pathways in various diseases.

Drugs/candidates stimulating toll like receptors

Therapeutic development targeting TLRs is at early clinical stages. There are currently approximately twenty drugs in pre-clinical development, with a further dozen or so in clinical trials (Coyne, 2008). Innate Pharma is developing IPH-3201, a series of TLR7/8 modulators to treat cancer, autoimmune and infectious diseases. Also in Innate's pipeline is IPH-3102, a double-stranded RNA and natural ligand of TLR3. Activation of the TLR3 pathway leads to the activation of NF-kB and the production of type I interferons to elicit antiviral defenses, and it is hoped that this may be an effective method of destroying cancerous cells. TLR3 detects virus invasion and initiates the antiviral immune response via TRIF/IKK signaling in the activation and maturation of dendritic cells (DCs) and monocytes, allowing for the regulated processing and presentation of antigens, the up-regulation of major histocompatibility complex, and co-stimulatory molecules and secretion of pro-inflammatory chemokines and cytokines (Kawai & Akira, 2009). These events then mediate the activation of antigen-specific T- and B-cell responses. Both of Innate's TLR candidates are in the early stages of development, it remains to be seen how they perform in the clinical subjects.

Similarly, the development of safe and efficacious vaccines remains a major challenging goal in global public health. For these reasons, TLR ligands have become a focus for their potential use as adjuvants in vaccine formulations (Pulendran, 2007). By physically linking the TLR ligand and antigen, each antigen would be delivered to a vesicle with an activated TLR in a host antigen-presenting cell, potentially achieving optimal antigen processing and presentation (Blander, 2007). Monophosphoryl lipid A (MPL) derived from detoxifying Salmonella minnesota lipid A, produced its adjuvant effect through the stimulation of the TRAM/TRIF signal transduction pathway of TLR4 and deactivation of Mal/MyD88 signaling (Mata-Haro et al., 2007), thereby acting as a partial rather than full agonist at the receptor and has been licensed for use as a vaccine adjuvant (Casella and Mitchell, 2008). It is also recognized that the major use for compounds that activate TLR2 are as adjuvant. The synthetic compounds, such as Pam3CSK4 and mycoplasma-derived lipopeptide (MALP)-2, may be developed for adjuvant usage (Lombardi et al., 2008; Ishii and Akira, 2007).

TLR5 is the receptor for bacterial flagellin monomers and is the only TLR that recognizes a protein ligand (Andersen-Nissen et al., 2007). CBLB502, an engineered flagellin derivative was found to have potent NF_κB activation and reduced immunogenic characteristics. A single injection of CBLB502 before lethal total body irradiation protected mice and rhesus monkeys from both gastrointestinal (GI) and hematopoietic acute radiation symptoms and resulted in improved survival and yet, importantly, did not decrease tumor radio sensitivity (Burdelya et al., 2008). These results imply that TLR5 agonists may be valuable as adjuvants for cancer radiotherapy. The activation of TLR5 has also been recently reported to be an efficient adjuvant for influenza A vaccine. A recombinant protein containing a consensus extracellular domain of M2 protein (M2e) sequence linked to the TLR5 ligand provides an effective approach to developing vaccines against wide-spread epidemic and pandemic influenza (Huleatt et al., 2008). The findings suggest that TLR5 agonist may have broad therapeutic applications, not only in its role as a linker adjuvant for vaccines, but also as a stopper of excessive apoptosis in acute radiation syndromes, degenerative diseases, or myocardial infarction as well (O'Neill, 2009). Such potential application needs to be verified and confirmed by animal models, preclinical, and clinical studies.

Investigators have focused on developing TLR7/8 agonists as antiviral agents against virus such as human papillomavirus (HPV). Imidazoquinolines were originally developed as such antiviral agents, and many such small molecule compounds have been tested for their ability to induce TLR7/TLR8-mediated cytokine induction. Imiquimod is the first approved topically active TLR7 agonist. It is prescribed for treatment of external virus induced skin lesions, such as the genital and perianal warts resulting from papillomavirus infections (Gupta et al., 2004). There is also a growing evidence to indicate therapeutic interest in TLR7/TLR8 agonists for cancer treatment. As such, imiquimod is now also used as a treatment for cancer and has shown itself to be efficacious against primary skin tumors and cutaneous metastases (Schon and

Schon, 2008). In fact, imiquimod has been approved for the treatment of external genital and perianal warts, but has also been found to be effective for a host of other virus-associated dermatologic lesions, including common and flat warts, molluscum contagiosum and herpes simplex. Oncological lesions showing improvement with 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 (Berman et al., 2002; Miller et al., 2008). A number of studies suggest that activation of TLR7 would be beneficial in patients infected with hepatitis C virus (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 (Lee et al., 2006). An oral prodrug of isatoribine, ANA975 was developed as an antiviral HCV treatment but clinical studies for this TLR7 agonist were discontinued by Anadys Pharmaceuticals due to indicated unacceptable toxicity via long term animal studies (Fletcher et al., 2006). Though the drug candidate produced intense immune stimulation, its chronic administration would have been inadvisable. Further studies with different dosing strategy are necessary to determine whether there will be any advantage of TLR therapy over the current option.

Cytosine-phosphate-guanosine oligodinucleotide (CpG-ODN), the common TLR9 agonist has shown substantial potential as vaccine adjuvants, and as mono- or combination therapies for the treatment of cancer, infectious and allergic diseases (Vollmer and Krieg, 2009). Phase I and II clinical trials have indicated that CpG-ODNs have antitumor activity as single agents and enhance the development of antitumor T-cell responses when used as therapeutic vaccine adjuvants. CpG-ODNs have shown benefit in multiple rodent and primate models of asthma and other allergic diseases, with encouraging results in some early human clinical trials. Though their potential clinical contributions are enormous, the safety and efficacy of these TLR9 agonists in humans remain to be determined. Chikh et al. (2009) reported that both methylated and unmethylated CpG ODN acts through a common receptor signaling pathway, specifically via TLR9 to initiate potent immune responses. It seems that CpG ODN holds great potential in further clinical development. Pfizer's agatolimod, a CpG oligonucleotide, selectively targets TLR9, thereby activating dendritic and B cells and stimulating cytotoxic T cell and antibody responses against tumor cells bearing tumor antigens. This product is in Phase II trials in breast and renal cancers, asthma, allergies and hepatitis-B virus infection. One of the Phase II trials "Agatolimod and Trastuzumab in Treating Patients With Locally Advanced or Metastatic Breast Cancer" registered through the http://clinicaltrials.gov website is currently in enrollment stage and will evaluate if monoclonal antibodies, such as trastuzumab, can block tumor growth and kill more tumor cells via concurrent administration of agatolimod for the locally advanced or metastatic breast cancer. However, the drug's clinical development for advanced non-small cell lung carcinoma (NSCLC) was discontinued after an independent Data Safety Monitoring Board (DSMB) found that trial data did not show increased efficacy over standard chemotherapy alone. This is not the only CpG oligonucleotide targeting TLR9 that has failed to live up to expectations for Pfizer; CpG-10101 was suspended at Phase II, when it failed to show efficacy in treating hepatitis C (Coyne, 2008). It seems important to realize that TLR agonist may not work equally well on different clinical indications. Pfizer has had more success using oligonucleotide TLR9 agonists as vaccine adjuvants; its vaccine adjuvant CpGTLR9 is currently in Phase III trials with GlaxoSmithKline's MAGE-A3 cancer vaccine. It is therefore proposed that use as adjuvants is the most promising avenue for TLR agonists due to low dosing requirement.

Drugs or antibodies inhibiting toll like receptors

Antagonists of lipid A have been under clinical development before the discovery of TLRs as treatments for Gram-negative sepsis and endotoxemia (Leon et al., 2008). The following analogs or natural molecules E5564 (eritoran), curcumin, auranofin (an antirheumatic gold compound), cinnamaldehyde, and acrolein are just a few of the sample candidates currently under investigation. For instance, Acrolein with an alpha, beta-unsaturated carbonyl group inhibits LPS-induced homodimerization of TLR4 (Lee et al., 2008). Small molecules that inhibit MyD88 binding to TLR4 are also emerging. Cell-penetrating peptides fused with the BB loop (a highly conserved sequence in the TIR that is situated between the second -strand and the second helix) sequences of TLR2 and TLR4 also inhibit LPS-induced signaling, probably by interfering with either receptor dimerization or adapter recruitment (Toshchakov et al., 2007). Treatment of patients with sepsis with anti-inflammatory therapies has so far not been beneficial (Rittirsch et al., 2008); therefore, it will be of interest to ascertain the clinical efficacy of inhibiting TLR4/MD-2 activity in sepsis. Numerous diseases such as sepsis, diabetes, rheumatoid arthritis, and cardiovascular diseases, seem to be associated with both TLR2 and TLR4; therefore, Mal seems to be an attractive therapeutic target for these diseases (O'Neill et al., 2009). Blocking TLR2 or TLR4 with a neutralizing antibody seems to be another promising route of drug discoveries. One such antibody, T2.5, has been shown to prevent sepsis induced by TLR2 ligands (Meng et al., 2004); furthermore, when T2.5 is used in combination with an anti-TLR4/MD-2 antibody, it protects mice against sepsis induced by Salmonella enterica or *Escherichia coli* when given with antibiotics (Spiller et al., 2008). 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. It is well known that combination medication in clinical practice is not an uncommon application. Another TLR4 antagonist, Eisai's eritoran tetrasodium, has reached Phase III trials for the treatment of sepsis and septic shock. In Phase I trials it proved its ability to dose-dependently inhibit TNFα production. Though the results from Phase II trials were not outstanding, it remains to be seen how the drug will perform in the larger scale Phase III trial (Coyne, 2008).

TLR3 antagonist may be beneficial in treating West Nile virus (WNV) infection. Infection of macrophages or DCs by WNV in peripheral lymphoid tissue induces TLR3-dependent secretion of TNFα and results in a transient increase in the permeability of the blood-brain barrier (BBB), facilitating the penetration of WNV across the BBB and into the CNS. It is clear that TLR3 activation is vital to the passage of the virus into the CNS (Wang et al., 2004). Therefore, inhibition of TLR3 signaling and the subsequent reduction in TNFα may be effective treating persons infected with WNV. However, this needs to be confirmed by further animal model, pre-clinical, or clinical studies.

Current therapies for systemic lupus erythematosus (SLE) are general immunosuppressants often leading to a host of serious adverse effects. TLR-targeted therapy may represent a more targeted approach. TLR9 and/or TLR7 antagonist may provide therapeutic benefits to SLE by inhibiting the production of anti-nuclear immune complex, interferon-α, and TLR activation (Kim et al., 2009; Kalia and Dutz, 2007). Recent data supports that several suppressive oligodeoxynucleotides (ODNs) block IFNα and reduce symptoms in SLE murine models, therefore representing a promising therapeutic agent for SLE (Barrat et al., 2007). Dynavax Technologies' TLR7/9 antagonist, IRS954, has shown early signs of efficacy; in a murine model of lupus, it reduced serum levels of nucleic acid-specific antibodies and decreased proteinurea, glomerularnephritis and end-organ damage, demonstrating an overall survival benefit.

Asthma is a human disease characterized by a massive accumulation of eosinophils that release an array of tissue-damaging mediators. Respiratory viral infections are thought to be a leading cause of exacerbations of asthma. One possible explanation might be a direct activation of viral components through TLRs. The virus-recognizing TLRs are TLR3, TLR7/8 and TLR9, which respond to viral dsRNA, ssRNA and CpG-DNA. Mansson and Cardell (2008) investigated the expression of these TLRs and their functions in human eosinophils and showed that Poly (I:C), R-837 and CpG directly activate eosinophils through their TLRs pointing this system represents a clinical target for the resolution of asthmatic disease. A recent interesting finding in relation to TLR4 and disease concerns allergy caused by airborne allergens. Derp2, the key allergen from the house dust mite, has been shown to be structurally similar to MD-2 and acts to deliver LPS to TLR4 in airways, thereby provoking inflammation (Trompette et al., 2009). This might be a common mechanism, because several airborne allergens are lipid-binding proteins and might act analogously. This makes TLR4 an interesting target for allergy in the airways. Any potential interventional success in this area will generate huge benefits to the allergic population.

Inhibitors of NF-kB function

Given that hyperactivation of NF-kB has a central role in the development and progression of cancer and chronic inflammatory disorders, a substantial amount of effort has been put in to developing strategies that block NF-kB signaling (Sethi and Tergaonkar, 2009). More than 700 compounds have been reported to inhibit NF-kB activation (Gilmore and Herscovitch, 2006). These NF-kB inhibitors include small molecules, biologics, inhibitory peptides, antisense RNAs, and natural agents blocking various steps leading to NF-kB activation. They may be classified further depending where in the signaling steps the inhibitory effect is exerted.

One of the drugs currently used for the treatment of chronic myelogenous leukemia is arsenic trioxide (ATO) as Fowler's solution. A multicenter trial in the USA in patients with relapsed acute promyelocytic leukemia found a complete response rate of 85% (Niu et al., 1999); and, ATO has been approved by the FDA for the indication since 2001. ATO is being evaluated in ongoing clinical trials in patients with other hematological and solid tumors. Preliminary evidence of some activity in patients with multiple myeloma has been reported (Hussein et al., 2004). Several newer classes of chemotherapeutics have been developed that were intended, at least in part, to target NF-kB. The best example is the proteasome inhibitor bortezomib (Velcade, Millenium Pharmaceuticals), approved by the USA-FDA for clinical use in 2003 for relapsed multiple myeloma refractory to conventional therapy (Kane et al., 2003). Because proteasome inhibition impacts many signaling pathways, it is not clear whether the therapeutic effects of bortezomib are mediated by inhibition of NF-kB activation. However, numerous preclinical studies with bortezomib have shown that proteasome inhibition blocks activation of NF-kB and enhances the effects of chemotherapeutic drugs, including Camptothecin-11 (Adams, 2002). Bortezomib is currently undergoing further clinical development in hematological malignancies and solid tumors, as a single agent and in combination with conventional chemotherapeutic drugs and new agents. Anti-TNF antibodies (e.g. Humira) and a soluble TNF receptor (e.g. Enbrel), approved by the FDA, have also been shown to suppress NF-kB activation in patients with arthritis and inflammatory bowel disease

(Gaddy and Robbins, 2008). Drugs like Humira have helped many arthritis patients by relieving pain, improving joint function, and slowing disease progression. Humira is a fully human monoclonal antibody, meaning it is manufactured in a laboratory using human proteins, and no animal proteins thus pointing another drug targeting strategies in TLRs. Because NF-kB is an important target in the IL-1β signal transduction pathway, inhibition of IL-1β also inhibits NF-kB activation. Additionally, ongoing research has also prompted new signal pathways and furthered our understanding in disease pathogenesis, pharmacotherapeutic targeting, and systematic balancing mechanisms. A study by Koga et al (2008) shows that *Streptococcus pneumoniae* (*S. pneumoniae*) activates nuclear factor of activated T cells (NFAT) transcription factor independently of TLR2 and 4, brings new insights into the molecular pathogenesis of *S. pneumoniae* infections through the NFAT-dependent mechanism and further identify gene tumor suppressor cylindromatosis as a negative regulator for NFAT signaling, thereby opening up new therapeutic targets for these diseases.

Directions in TLRs therapeutic development

Many attempts to use TLR manipulation for the treatment of infectious, allergic and autoimmune diseases, as well as cancer, are in the early clinical phases, and results have not been always positive. One successful TLR candidate is Ampligen, a mismatched, double-stranded RNA which activates TLR3 and is currently awaiting registration in the US for the treatment of chronic fatigue syndrome (CFS), an illness that is not fully understood, but often seems to be associated with viral infection (Gowen et al., 2007). Ampligen new drug application (NDA) was filed, but marketing for the treatment of CFS has not yet been approved. Ampligen is received intravenously. It is generally administered twice weekly for periods of one year or greater. Two toxicology studies were recently completed that establish the safety of intranasal and intramucosal methods of Ampligen administration. Hemispherx Biopharma reports that it is currently researching an oral drug that uses nucleic acid technology related to Ampligen (Hemispherx Biopharma Inc R&D. Drug candidates - Ampligen®). Aditionally, Ampligen is in Phase II development for HIV and hepatitis infections, as well as for cancer treatment indicating the sponsor company's active development interest for multiple challenging indications.

While TLRs are able to recognize viral PAMPs, the exact action mechanism is complex and involves multiple key signal pathways and participant molecules/genes. Moreover, an increasing number of host breakdown products from the extracellular matrix such as hyaluron, intracellular components released when cells rupture, and products of proteolytic cascades are all able to stimulate TLRs, suggesting TLRs' function in sensing tissue damage signals caused by disease, stimuli or injury. Recognition of these products by TLRs leads to the activation/recruitment of immune cells and cytokines that repair the tissue damage. There are already drugs in the pipeline for tissue regeneration applications, such as Clinquest's TLR3 agonist CQ-07001, an endogenous human protein, currently in clinical development for anti-inflammatory and tissue regeneration applications (Clinquest Group BV openPR. Clinquest obtains exclusive worldwide rights to clinical development and commercialization - Amsterdam, the Netherlands, 06-03-2007). Progress in this relative new area may provide hint on developing new treatment strategies for several challenging diseases such as coronary myocardial infarction. Great strides are being made worldwide in our ability to synthesize and assemble nanoscale building blocks to create advanced materials with novel properties and functionalities. The novel properties of nanostructures are derived from their confined sizes and their very large surface-to-volume ratios. A fundamental issue in much of nanomedicine, and especially tissue regeneration, is to understand and to eventually control nanostructure-biomolecule interactions (Nuffer and Siegel, 2009).

Accumulating evidence indicates that TLRs seem to be implicated in many unmet medical conditions due to the fact that many TLR induced cytokines are well manifested in these diseases. A number of strategies may be considered to alter TLRs, including agonists, antagonists, neutralizing antibodies, and signal transduction inhibitors or regulators. Neutralizing antibodies to TLRs may be possible, but only for those on the cell surface, such as TLR2, TLR4, and TLR5. TLRs typically initiate pathologic conditions in the event of microbe invasion or stimuli with the ensuing inflammation leading to the production of endogenous ligands, further propagating inflammation. The two discreet signaling pathways (Mal/MyD88 and TRAM/TRIF) offer targets for selective modulation of TLR activity. The precise clinical goal of modifying TLR activity remains a challenging question (Beutler, 2004). We may have a partial agonist or a drug identified *in vitro* that selectively stimulates TRAM/TRIF signaling. But, such results are hard to be expected *in vivo* or in clinical studies when multiple other factors must be considered. Species differences in the response to different agonists at TLR4 suggest that caution needs to be exercised in developing safe new drugs with well planned preclinical and early stage clinical studies. It is critical to select objective study outcomes, analyze clinical end points, and drug safety from a contextual systems approach. It is imperative to run controlled randomized studies to minimize bias. Drug safety seems to cause a lot more damages in later study stages; and, it must be emphasized to conduct a full toxicity analysis in preclinical and phase 1 studies and ensure proper safety data monitoring, data collection, and independent adverse event adjudication. Other notable pointers for

running a clinical study such as therapeutic indication for the selected patient population, dosing (exposure), the measurable indicators (molecular, cellular, and *in vivo*), assessment of patients' prior medical history, baseline characteristics, functional modalities, and even patient reported outcome and quality of life, must all be considered to conduct evaluation of the efficacy and adverse effects associated with the medical regimens. All these details must be clearly planned, reviewed, defined, and deemed feasible in the clinical protocol prior to seeking institutional review board (IRB) approval to conduct a clinical study. In conducting clinical studies, the will to initiate and be creative will be primary, the study design secondary, and collecting and analyzing data critical to accept or reject the research hypothesis. The goal is to achieve the expected efficacy with acceptable adverse event profile.

Small-molecule antagonists seem to present a promising prospect, though these are not traditional "drug-like" molecules. One concern, however, is that such inhibitors might block multiple TLRs and therefore gives rise to unwanted immunosuppression. In addition, adjuvancy study seems to yield new agents. More adjuvants may be expected to improve vaccine efficacy or have antitumor effects. In terms of antagonism, we have data beyond phase II for only one TLR inhibitor - eritoran. As described, its effects were significant but somewhat marginal (Parkinson, 2008). Based on the literature reviewed, it reasons to state that manipulating the activity of TLRs to modulate immune responses for therapeutic intervention has created strong interest in the pharmaceutical industry. The focus has been largely in the areas of infectious diseases, cancer, allergic diseases and vaccine adjuvants. Though initial clinical trials for infectious diseases and cancer showed early promise, longer-term trials have not always been positive and more research is required to find dosing regimes that balance efficacy with acceptable side-effect profiles and suitable indications. So far, the clinical data indicates that TLR agonists as vaccine adjuvants seem to hold greater potential and have less safety concerns than for other applications.

Though it is hard to predict where therapeutic targeting TLRs is going, we have some promising data and late phase trials on the horizon, where the fundamental research and development have never been stopped. To further develop more effective TLR therapeutic targeting strategy, there are a few more tasks: further identifying and determining the pathogenesis of challenging medical conditions such as virus infection, allergy, cancer, and SLE; analysis of genetic sequence, molecular structure, epigenetic observations, and functional activities on both animal model and human clinical studies; design of clinical study based on study indication, dosing regimens, drug delivery route or format consideration, and pharma-cokinetics; timely and objective assessment of adverse events with details. With the insights of all these revealed, disease occurring mechanisms on genetic, molecular, functional and *in vivo* levels for challenging pathologic conditions may be defined (Chen et al., 2009). Target driven molecular, drug or biological interventions may further be designed and developed to act on specific receptor or sub-unit, cellular signaling or metabolic pathways to induce therapeutic cure.

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