Review

TOLL-LIKE RECEPTORS AND THEIR ROLE IN CARCINOGENESIS AND ANTI-TUMOR TREATMENT

ANNA WOLSKA, EWA LECH-MARAŃDA and TADEUSZ ROBAK*
Department of Hematology, Medical University of Łódź, Ciołkowskiego 2,
93-513 Łódź, Poland

Abstract: Toll-like receptors (TLRs) have been described as major components of the innate immune system, recognizing the conserved molecular structures found in the large groups of pathogens called pathogen-associated molecular patterns (PAMPs). TLR expression is ubiquitous, from epithelial to immunocompetent cells. TLR ligation triggers several adapter proteins and downstream kinases, leading to the induction of key pro-inflammatory mediators but also anti-inflammatory and anti-tumor cytokines. The result of this activation goes beyond innate immunity to shape the adaptive responses against pathogens and tumor cells, and maintains host homeostasis via cell debris utilization. TLRs have already become potent targets in infectious disease treatment and vaccine therapy and in neoplastic disease treatment, due to their ability to enhance antigen presentation. However, some studies show the dual effect of TLR stimulation on malignant cells: they can be proapoptotic or promote survival

* Author for correspondence; e-mail: robaktad@csk.umed.lodz.pl, tel.: +48 42 689 51 91, fax: +48 42 689 51 92

under different conditions. It is therefore crucial to design further studies assessing the biology of these receptors in normal and transformed cells. The established role of TLRs in human disease therapy is based on TLR7 and TLR4 agonists, respectively for the novel treatment of some types of skin cancer and for the anti-hepatitis B virus vaccine. Some clinical trials involving TLR agonists as potent enhancers of the anti-tumor response in solid tumors have begun.

Key words: Toll-like receptors, Innate immunity, Treatment, Carcinogenesis, Tumor, Vaccine, Dendritic cells

INTRODUCTION

Innate immunity is based on receptors that are able to recognize and respond to pathogens within minutes of an invasion, providing efficient protection against infectious agents. Among them are the Toll-like receptors (TLRs). These are activated by conserved pathogen-associated molecular patterns (PAMPs), which are essential for pathogen survival (e.g. the components of the cell wall, nucleic acids), and therefore do not mutate among large groups of microbes [1]. They take their name from the Toll protein first found in the 1990s in the *Drosophila* fruit fly; that protein is implicated in the dorso-ventral polarization of the embryo [2] but is also responsible for the antifungal immunity of the adult fly [3]. It was observed that the intracellular domains of the *Drosophila* Toll and mammalian Interleukin-1 receptor (IL-1R) had similar structures [4]. The Toll-like protein was shown to induce pro-inflammatory gene expression after ligation with specific PAMPs [5]. There are 11 human TLRs described out of a total of 13 mammalian TLRs, but ligands for some have yet to be discovered [6].

STRUCTURE AND ACTIVATION

TLRs belong to the type I transmembrane receptor family. The intracellular Toll/Interleukin-1R domains (TIR) required for downstream signaling have a striking similarity to IL-1R. The three-dimensional structure of the extracellular domain is responsible for ligand discrimination, eliciting an adequate immune reaction to a broad range of agonists attributed to their respective TLRs. According to an X-ray analysis of the extracellular domain of TLR, there is a capacity for direct but not exact engagement of PAMPs with TLRs [7]. Dimerization between TLRs enables further tailoring of the agonist recognition: e.g. the TLR1/TLR2 heterodimer responds to triacyl peptides, whereas TLR2/TLR6 recognizes diacyl peptides [8, 9]. There are six major families of TLRs, based on their phylogenetic backgrounds [10]. Each family is attributed to a general class of PAMPs. TLR2 belongs to the TLR2 family, with TLR1, TLR6 and TLR10, and it responds to various bacterial (lipopeptides, peptidoglycan, lipoteichoic acid), fungal (zymosan) and viral (viral core proteins) PAMPs [11-13]. TLR2 also cooperates with lectin receptors, enabling

recognition of the fungal cell wall component zymosan [14, 15]. Double-stranded RNA and some synthetic RNA derivatives (polyinosilic-polycytidylic acid) are ligands for TLR3 [16, 17]. The ultimate lipopolysaccharide receptor is TLR4 [18]. It functions in a complex composed of the soluble LPS-binding protein (LBP), the membrane CD14 molecule, and the MD-2 glycoprotein that binds to the extracellular domain of TLR4 [19]. TLR5 has been shown to recognize an evolutionarily conserved domain of flagellin (a monomeric constituent of bacterial flagella) [20]. It has also been found on the basolateral side of intestinal epithelial cells and in the subepithelial compartment, indicating its relevant role in microbial recognition and homeostasis at the mucosal surface [21]. The sixth family contains TLR7, TLR8 and TLR9. TLR7 and TLR8 are both predicted to recognize a nucleic acid-like structure, guanosine- or uridine-rich single-stranded RNA (ssRNA), from viruses such as the human immunodeficiency virus and influenza virus [22]. ssRNA is abundant in the host. However, it is not detected by TLR7/8 due to the subcellular expression of these TLRs in the endosomes. Human TLR7 and TLR8 also recognize synthetic compounds: imidazoquinolines, which are currently approved in the treatment of genital warts, and loxoribine [23, 24]. TLR9 is a receptor for unmethylated CpG DNA motifs characteristic to bacterial DNA [25]. The function of this receptor has also been studied using synthetically designed oligodeoxynucleotides (ODNs) [26].

Some of the host molecules have also been found to contribute to TLR activation. They play an essential role in tissue repair, being released from necrotic but not apoptotic cells [27]. The so-called endogenous TLR agonists are capable of promoting the inflammatory response with maturation of dendritic cells into professional antigen-presenting cells (APC) [28, 29]. This allows for the immune system to be involved in responding to danger signals such as tissue damage caused by trauma or infection. However, induction of the inflammatory reaction to heat shock protein 60 or 70 may contribute to the development of autoimmune diseases like type I diabetes [30] and Crohn's disease [31]. Some researchers question the existence of such endogenous ligands. The reason is that TLR ligands may be contaminated with LPS during the process of production or purification, especially when manufactured by genetically modified *E. coli* strains [32, 33]. So far, only mRNA seems to be the true endogenous ligand for TLR3 [34]. Nevertheless, it is plausible that such reactions to minute amounts of endotoxin, not detectable using commercial testing kits, are due to the increased sensitivity of the studied cells to TLR stimulation. Kowalski *et al.* observed an excessive (more than 100-fold) responsiveness of peripheral blood mononuclear cells from patients with recent onset rheumatoid arthritis to LPS stimulation, compared to healthy controls and subjects diagnosed with degenerative arthritis [35]. That might also be the case in experiments studying the influence of endogenous ligand preparations contaminated with minute amounts of LPS.

TLR SIGNALING

The downstream pathway of TLR signaling requires two major adapter proteins, MyD88 and TRIF (TIR domain-containing adapter inducing IFNβ), which are exploited by all of the TLRs, and others that are exclusively utilized by TLR2 and TLR4: TIRAP (TIR domain-containing adapter protein or Mal) and TRAM (TRIF-related adapter molecule). Interaction of the TIR domains on TLRs and adapter molecules initiates a kinase cascade that induces transcription factors like nuclear factor-κB (NF-κB), AP-1, IRF3 and IRF7. Stimulating some TLRs results in the activation of specific adapter proteins, whereas others activate multiple adapter molecules. This enables the tailoring of the immune responses to different ligands, e.g. rapid antiviral response after IRF-3 activation through TRIF following TLR3 stimulation, and two-phase NF-κB activation subsequent to TLR4 ligation [for a review, see 36].

THE RESULTS OF TLR ACTIVATION – TLRs IN CARCINOGENESIS

TLRs are very potent activators of gene transcription and translation. Murine macrophages initiate about 1000 LPS-responsive genes following TLR4 stimulation [37]. The activity of the transcription factor NF-κB is enhanced by TLR stimulation as early as 15-30 minutes after TLR ligation, with subsequent cytokine production occurring 2 hours later [38, 39]. The most evident consequence of TLR stimulation is the induction of the inflammatory response. TLR ligation results in the production of pro- and anti-inflammatory cytokines (IL-1, IL-6, IL-8, IL-10, TNFα) [40-42], chemokines (IL-8, MCP-1) [43, 44], defensins [45], and antiviral type-I interferons [46, 47]. Other mechanisms attributed to TLR signaling are processes including apoptosis, tissue repair and angiogenesis.

It has long been observed that chronic infection and inflammation are important factors contributing to the development of malignant tumors. For example, gastric cancer is linked to chronic *Helicobacter pylori* inflammation, and there is an association between chronic inflammatory bowel diseases and colon cancer [48, 49]. The chronic inflammatory process promotes the proliferation of cells and may promote the development of cancer. What's more, the results of several studies imply that using non-steroidal anti-inflammatory drugs may lower the risk of some types of malignancies developing [50, 51]. The role of TLRs in mammalian cell biology seems overwhelming, and reaches far beyond the simple antimicrobial immune response of the innate immunity. Therefore, it has become even more tempting to scrutinize the Toll-like pathway in human diseases, especially in malignancies. TLRs are believed to be involved in tumor development and growth, with several possible mechanisms, but they have also been found in reactions directed against tumor cells (Tab. 1).

The factor that links chronic inflammation and tumor development is NF-κB [52]. It was found activated in a number of human malignancies, including acute

Tab. 1. TLRs and tumor development and growth.

Tumor-promoting	TLR	References	Anti-tumor	TLR	Reference
Pro-angiogenic	2, 9	58	Anti-angiogenic	7, 9	[59-61]
Proliferation	3, 4	64-67	Apoptosis	3, 4, 7, 9	[68, 70-72, 74]
Chemoresistance	4	81, 82	Chemosensitivity	2, 4, 7	[83-86]
Treg activation	4, 5	93, 94	Treg inhibition Antigen presentation	4, 5, 7, 8, 9	[94, 95, 98-102]
			Cytotoxicity	9	[103, 106-109]

and chronic myelogenous leukemia, prostate cancer, multiple myeloma, and hepatocellular carcinoma [53-56]. Therefore, the agents that lead to the activation of NF-κB may be directly implicated in the development and progression of tumors. Toll-like receptors are potent activators of the NF-κB pathway. Their role in carcinogenesis depends on the activation of NF-κB and related genes, resulting in the production of cytokines such as IL-1, IL-2, IL-6, IL-10 and TNF-α; the recruitment of cells of the immune system by chemokines; the maintenance of chronic inflammation; and suppression in the tumor microenvironment. This complex net of chemical and direct cell-to-cell interactions may contribute to tumor survival and progression through an anti-apoptotic effect, the direct inhibition of cell cytotoxicity or angiogenesis [57, 58]. There is still controversy regarding TLRs in angiogenesis, as to whether they promote or inhibit the formation of new vessels. Pro-inflammatory cytokines and growth factors produced after TLR ligation favor angiogenesis, but at the same time, the synthetic TLR7 ligand (imiquimod) is found to halt this process and is a potent and approved topical anti-tumor agent in dermatological malignancies [59-61].

TLR expression has also been studied using tumor cells. The cells of several tumors display increased and changed TLR expression, and mice deficient in such receptors may be protected or develop fewer inducible tumors in experimental models [62, 63]. Moreover, the induction of TLRs on tumor cells may directly promote proliferation, such as in human prostate cancer and in head and neck cancer. This reaction is time- and dose-dependent and is reflected by NF-κB induction and the subsequent upregulation of oncoprotein c-Myc [64, 65]. However, there are studies suggesting a protective role of TLR stimulation and possible therapeutic options exploiting TLR ligation. A study of Paone *et al.* showed increased apoptosis of prostate cancer cells stimulated with the TLR3 agonist. This reaction may be due to the established balance favoring apoptosis rather than the proliferation of cancer cells under TLR ligation [68]. This balance may depend on the concentration of certain cytokines and the time of stimulation [69]. Direct TLR3 stimulation of melanoma cells leads to increased apoptosis. Moreover, TLR3-mediated cell death involves the activation of caspases and engages both the extrinsic and intrinsic apoptotic pathways. The

results suggest that TLR3 agonists may be very promising adjuvants for cancer vaccines thanks to their newly identified direct cytostatic and cytotoxic effects on tumor cells [73]. Apoptosis may be a potent mechanism of eliminating acute myeloid leukemia cells (AML). Monocytic AML cell lines were found to be sensitive to IFN-α with LPS-induced apoptosis, which is partially dependent on caspase-8 recruitment and associated with enhanced Fas/CD95 expression. A few samples of primary blast lines obtained from patients with AML also responded to the additive effects of LPS and IFN-α, indicating that sensitivity to TLR4-mediated inducible apoptosis is found in a fraction of AML samples with no correlation with FAB phenotypes [74]. It has been observed that cells stimulated with TLR ligands udergo programmed cell death after NF-κB activation by Fas-associated death domain protein (FADD) and subsequent caspase recruitment [75-78]. This phenomenon reflects the regulatory capacity of TLR signaling protecting the host from excessive proliferation of immunecompetent cells being activated during inflammatory reactions, and may be implicated in an anti-tumor response enhanced with TLR stimulation. However, some studies have had completely contrary outcomes. Fas-FasL interaction with murine and human macrophages has been shown to enhance activation via TLR4 signaling. This surprising outcome is associated with the progression of chronic collagen-induced inflammation in a murine model [79]. Even more astounding is the observation that intact FADD protein is essential for TLR3- and TLR4-induced proliferation of B lymphocytes [80]. This dual role of TLRs in apoptosis is still indistinct, and reflects the complex associations between different signaling pathways in cells expressing TLRs.

The Toll-like pathway favoring tumor growth may also be triggered by chemotherapeutic agents themselves. Chemotherapeutics such as doxorubicin or paclitaxel can activate NF-κB and promote cell survival rather than apoptosis, which may explain the increased tumor growth despite the treatment reflecting some kind of chemoresistance [81, 82]. It may then be appropriate to assess the unique TLR expression pattern in tumor cells and direct the treatment according to the actual sensitivity to TLR stimulation. This would be plausible to overcome the undesirable NF-kB activation in prone individuals. On the other hand, studies show enhanced chemosensitivity upon TLR stimulation; for example, TLR7 ligation results in sensitization of the B cells of chronic lymphocytic leukemia to chemotherapeutic agents *in vitro*, presumably by impaired signal transduction through a stress-activated protein kinase pathway [83, 85].

In spite of these controversies, the potential of TLR agonists to drive and enhance the adaptive immune response against certain antigens has been investigated in anti-tumour therapy. Effective anti-tumor therapy has some major aspects that must be addressed. As tumor cells are capable of modulating the immune system, favouring survival and inhibiting anti-tumor responses, it is important to induce effective immunogenicity of tumor cells by augmenting antigen presentation. The other concern is the immuneparesis and tolerance towards malignant cells that accompany weak immunogenicity and are reflected

by anti-inflammatory cytokine environment and naturally occurring regulatory T cells (Treg) that mediate antigen-specific local immune suppression. Not only do cells infiltrating the tumor seem to be reluctant to evoke anti-tumor reactions, but they also create an inflammatory microenvironment that contributes to cancer growth and supports the immune suppression to tumor cells [87]. Regulatory T cells occur naturally during any immune response and function as inhibitors of excessive immunological reactions, but also act as suppressors of autoreactive effector T cells [88, 89]. Animals depleted of regulatory T cells suffer from lethal infections when exposed to infectious agents [90, 91]. They exert their suppressive function with a delay to effector T cells, allowing for an effective immune response against non-self antigens, and limiting its extent [92]. DCs play an important role in stimulating Tregs, but Tregs have also been described as being able to directly respond to TLR ligands. In murine models, Tregs have been found to express mRNA for TLR4, 5, 7 and 8. Ligation of TLR4 with LPS results in the augmentation of their suppressive function on cytotoxic T lymphocytes without support from DCs. Stimulated Tregs are fully functional and suppress cytotoxic T cells *in vivo* in LPS-challenged alymphoid animals [93]. However, another study demonstrates the dual action of TLR stimulation on the anti-tumor effects of Tregs. Tregs stimulated with flagellin at the time of experimental tumor implantation exert their suppressive function and allow for tumor growth and progression, whereas delayed TLR5 ligation enhances the anti-tumor response reflected by the inhibition of tumor growth and a decrease in Treg number. Contemporaneous administration of the TLR5 agonist favors a Th2 polarized response, in contrast to the increased IFNγ and Il-4 production characteristic for a Th1 response. Moreover, the addition of CpG oligodeoxynucleotides with early flagellin treatment was associated with complete tumor suppression, which demonstrates an as-yet unknown synergy between the TLR agonists against the tumor [94]. Other TLR agonists are capable of reversing the suppressive function of Tregs. In the study of Peng *et al.*, restoration of tumor growth inhibition was observed after the adoptive transfer of Tregs treated with poly-G oligodeoxynucleotides and CD8+ immunized T cells 3 days after tumor cell inoculation [95]. There is a lot of interest in Treg and TLR roles in anti-tumor immune responses. However, the kinetics of these reactions remain unknown and further studies need to be conducted.

Another possible way to overcome tumor-related immuneparesis is to augment the immunogenicity of tumor cells and make them visible to the host immune system. Dendritic cells possess the ability to drive and 'guide' the adaptive immune responses. Their role involves presenting antigens and providing additional stimulating signals to naïve T cells, thus enhancing effector reactions against given antigens. They also express TLRs and thus respond to a vast array of microbial derivatives linking the innate and adaptive immunities. TLR ligation results in maturation and gaining the potential to activate naïve T cells. Distinct PAMPs can have opposite effects regarding Th balance. For example,

dsRNA, the TLR3 ligand, induces a strong Th1 reaction by releasing type I IFNs [96], and LPS from *P. gingivalis* (TLR2 agonist) induces DCs to prime a Th2-type response, whereas LPS from *E. coli* (TLR4 agonist) promotes the development of DC with Th1-polarizing activities through IL-12 [96]. DCs are also capable of reversing the Treg cell-mediated suppression after being stimulated with LPS and CpG oligodeoxynucleotides *in vitro*. This effect is accompanied by near to normal proliferation of effector T cells, and is independent of costimulation, but requires IL-6 [98]. Malignant cells may also become antigen-presenting cells. One of the most thoroughly studied hematological malignancies is chronic lymphocytic leukemia (CLL). Leukemic B cells are not capable of effective antigen presentation to T lymphocytes, and so are weakly immunogenic. However, as with normal B lymphocytes, CLL B cells express mRNA for TLR7 and TLR9, and are responsive to stimulation with TLR7 and TLR9 agonists. TLR7 ligation induces proliferation, increased expression of costimulatory molecules and TNF-α and IL-10 production. Additional IL-2 stimulation leads to inhibition of proliferation and IL-10 production. CLL B cells become more immunogenic to cytotoxic T lymphocytes in a mixed *in vitro* culture [99, 100]. Synthetic agonists of TLR9 also enhance proliferation, proinflammatory cytokine production and the transformation of CLL B cells to APCs *in vitro* [101]. They also induce up-regulation of the high-affinity IL-2 receptor in malignant compared to normal B cells. The addition of IL-2 to CpG-ODN-stimulated cells augmented proliferation in both normal B cells and B-CLL cells, but no costimulatory effect on cytokine production or surface molecule expression could be observed in normal B lymphocytes. By contrast, TNF-α and IL-6 production was increased in CLL cells, and the expression of CD80 and CD86 was further augmented when IL-2 was used as a costimulus. Autologous and allogeneic immune recognition of CLL B cells stimulated with CpG-ODN and IL-2 was increased compared with CLL B cells stimulated with CpG-ODN alone [102]. Furthermore, synthetic TLR9 ligands (oligodeoxynucleotides, ODN) bear the potential to enhance the immunogenicity of B-CLL cells by up-regulation of CD25, a target for immunotoxin LMB-2. LMB-2 was found to be much less toxic to normal B and T lymphocytes compared with B-CLL cells, and immunostimulatory CpG-ODNs efficiently sensitized B-CLL cells to a recombinant immunotoxin via modulation of its target [103].

However, *in vivo* studies of neoplastic diseases on animal models are most important, as the microenvironment of the tumor consists of interacting cells rather than individual reactions. In a study of an animal model of melanoma, continuous TLR2, 3, 4, or 7 stimulation elicits cytotoxic T-cell generation, but no *in vivo* effect on anti-tumor responses reflected by uninterrupted tumor growth and no signs of autoimmunity (vitiligo) in treated animals can be observed. Additional modification of the TLR signaling pathway including SOCS1 inhibition in DCs induces effective anti-melanoma immunity with marked IL-12 secretion and visible tumor growth suppression [104]. Moreover, genetic immunization along with TLR ligation is not efficient enough in the

treatment of melanoma in mice, suggesting the existence of active tumor immunotolerance. Hence, the treatment may be sufficient to slow the progression of the primary tumor and reduce the number of metastases [105].

Last but not least is the phenomenon of tolerance, which is still controversial, and is undermined by some authors. It is an effect of prolonged TLR stimulation, which is supposed to protect the host from the detrimental activation of the innate immune reaction following a microbial challenge. Animals exposed to sub-lethal doses of LPS resist subsequent lethal doses. This is achieved by entering a refractory state which is either expressed by a decrease in surface TLR4 and impaired signal transduction (inhibited phosphorylation of the kinase pathway, suppression of LPS-induced chemokine expression), or mediated by anti-inflammatory cytokines [110-113]. Tolerance may be undesirable for anti-tumor therapy when it is directed to increase tumor immunogenicity and antigen presentation, but it would be of benefit in the case of inhibiting the regulatory functions of Tregs. Several studies have investigated the phenomenon in human cells using *in vitro* and *ex vivo* models. The results are quite surprising, as cells stimulated with TLR2, TLR4 and TLR9 agonists in a 24-hour culture show a sustained activation state rather than tolerance. Macrophages exposed to low doses of LPS rapidly secrete TNFα, but when exposed to higher doses of LPS, they progressively produce more cytokines (represented here by IL-1 and IL-12 promoter activation) [115]. The decreased responsiveness reflected by low levels of proinflammatory cytokines appears to be due to the induction of the anti-inflammatory cytokine milieu (IL-10 and Transforming Growth Factor-β; TGF-β) [110, 115].

The role of TLRs in mammalian cell biology seems overwhelming and reaches far beyond the simple anti-microbial immune response of the innate immunity. Therefore, it has become even more tempting to scrutinize the Toll-like pathway in the treatment of human diseases, especially in malignancies.

TLRs AS ANTI-TUMOR VACCINES

The potential of TLR ligands to induce appropriate and effective immune reactions against a given antigen has been exploited in the vaccine therapy of human melanoma (Tab. 2).

Tab. 2. TLRs in anti-tumor vaccine studies for melanoma.

TLR	Vaccine	Reference
7	Cancer/testis antigen + imiquimod	[120]
Poly	Melanoma antigen + Ribomunyl	[121]
9	Melanoma antigen + CpG7909	[118]
7	Melanoma antigen + Flt3 + imiquimod	[119]

TLR agonists have been shown to induce DC maturation and antigen presentation, which is one of the issues to be addressed in the effective anti-

tumor treatment. The rationale for designing studies with human tumors comes from animal models. Numerous studies have already been conducted on the use of TLR agonists along with tumor-antigen vaccination. The results are quite promising, showing the potential of TLR ligands to enhance and improve the efficacy of tumor vaccines. Adding TLR3, TLR4, TLR7 or TLR9 ligands potently activates CD8+ cytotoxic T cells with increased IFN-γ production and the leukocyte-rich immunostimulatory cytokine milieu at the tumor microenvironment [116-118]. Moreover, the most important evidence for the success of the combined treatment is the improved survival of tumor-bearing animals. However, studies in human malignancies reveal the great potential of TLR ligands but still mostly in *in vitro* models, which do not reflect the whole net of relationships between immunecompetent cells [119].

A pilot single-arm study was performed on 9 patients with malignant melanoma at stages II and III according to the AJCC criteria. The treatment included topical administration of imiquimod cream followed by intradermal injection with cancer/testis antigen formulation NY-ESO-1. The treatment was well tolerated, with only minor side effects at the site of administration and grade I systemic effects. In 4 of 9 patients, seropositivity for the tested antigen was observed. No CD4+ or CD8+ responses were detected, either by ELLISPOT or Delayed Type Hypersensitivity. However, CD4+ reactions were observed in *in vitro* sensitized cell cultures in 7 of the 9 study objects. No correlation between the clinical response and *in vitro* results was determined [120].

A phase I/II trial was conducted to evaluate clinical and immunological responses after intralymphatic and intranodal injections of mature dendritic cells loaded with specific tumor antigens and stimulated with a TLR ligand along with IFN-γ in patients with a metastatic melanoma. Fourteen patients enrolled in the study received 1 intralymphatic and 4 intranodal injections of DCs pulsed with melanoma antigens and matured *in vitro* with Ribomunyl (TLR ligand) and IFN-γ. Neither a complete nor partial response was observed. However, in 2 subjects, the lesions remained stable for up to 10 months and the longest survival was 48+ months. The median survival was 10.5 months. The most common adverse events included local reactions at the site of injection, and only 1 grade 4 lymphopenia occurred [121].

A study of Speiser *et al.* exploited another synthetic TLR ligand, CpG 7909. Eight patients enrolled in the study received vaccinations that consisted of the TLR9 agonist, melanoma antigen and incomplete Freund's adjuvant. All the subjects exhibited strong antigen-specific T-cell responses, as assessed by the *ex vivo* secretion of IFN-γ and expression of granzyme B and perforin. Furthermore, the frequency of Melan-A-specific T cells was one order of magnitude higher than the frequency seen in the 8 control patients treated similarly but without CpG. Moreover, T-cell clones recognized and killed melanoma cells in an antigen-specific manner *in vitro* [122].

In a study of Shackleton *et al.*, 27 patients with metastatic or high-risk resected melanoma received the Flt3 ligand (FL); eighteen of them were treated with

vaccination with melanoma antigens and 8 of the vaccinated subjects received imiquimod topically [123]. The treatment was generally safe and well tolerated, although some patients developed clinically significant toxicities related to FL. FL induced increases in immature CD11c+ and CD123+ peripheral blood (PB) DCs. Cutaneous reactions to peptide vaccination and circulating peptide-specific CD8+ T-cells were more frequent in the imiquimod-treated patients. FL treatment resulted in increased levels of circulating DCs, and stimulation with tumor antigens augmented with the TLR ligand enhanced the maturation of antigen-specific DCs.

TLRs IN CLINICAL TRIALS AGAINST HUMAN MALIGNANCIES

The promising role of the TLR pathway in the treatment of human malignancies was studied in several clinical trials (Tab. 3).

Tab. 3. TLRs in clinical trials.

Malignancy	TLR	Reference
Advanced-stage non-small-cell lung cancer	TLR9	[124]
IV stage melanoma	TLR7	[125]
IIIb/c or IV stage melanoma	TLR9	[126]
Incompletely resectable pancreatic carcinoma	TLR2/6	[127]
Recurrent non-Hodgkin lymphoma	TLR9	[128]
Recurrent glioblastoma	TLR9	[129]
Recurrent non-Hodgkin lymphoma	TLR9	[130]
Recurrent non-Hodgkin lymphoma	TLR9	[131]
CLL, skin deposits	TLR7	[132]

A randomized phase II trial of combination therapy with taxane and platinum plus an additional synthetic TLR9 agonist was performed with the aim of increasing the efficacy of a first-line treatment for the III and IV stage non-small-cell lung carcinoma. One hundred and eleven patients were included in the study. The major side effects concerning subcutaneous administration of ODN were flu-like symptoms and local reactions, although grade 3 and 4 cytopenias were more common in the TLR arm. The objective response rates (complete and partial response) were 38% in the TLR9 agonist group and 19% in the conventional treatment arm. Blinded independent radiological reviews of CT or MRI scans from 90 patients with available scans in the study database revealed similar results with respect to the confirmed objective response rate (19% in the ODN plus chemotherapy arm, and 11% in the chemotherapy-alone arm; $p = 0.323$). Statistical analysis suggests a trend toward improved survival for patients treated with ODN plus chemotherapy. The median survival for patients in the ODN plus chemotherapy arm was 12.3 months compared with 6.8 months for patients in the chemotherapy-alone group [124].

A phase II, multicenter, open-label study in patients with chemotherapy-refractory metastatic melanoma studied the anti-tumor activity of a systemically administered TLR7 agonist, 852A. Twenty one patients were enrolled and 13 completed the whole 12-week treatment cycle, with two discontinuing for adverse events considered to be possibly related to the study drug. A systemic, intravenously administered TLR7 agonist was well tolerated and was capable of inducing activation of the anti-tumor immune response assessed by serum IFN-γ concentration. Four (19%) patients had disease stabilization for > 100 days [125].

A phase II, open-label, single-arm study was designed for the evaluation of the safety and efficacy of a subcutaneous injection of the TLR9 agonist in patients with unresectable stage IIIb/c or stage IV melanoma. Twenty patients were enrolled in the study, and they received a mean of 10.7 injections of the TLR9 ligand. Adverse events included mostly flu-like symptoms of 1/2 grade, but in 3 patients, a slight increase in the anti-dsDNA antibody titre was observed with no clinical evidence of autoimmune disease. Two patients experienced a clinical response: metastatic leasion regression. One of these responses was ongoing at more than 140 weeks after TLR9 agonist therapy, whereas the other patient developed new leasions with regression of previous target ones. Three patients experienced stabilization of the disease, although all presented with progression at week 24. The markers of immunological activation showed increased DC activation with elevated levels of type I IFN, and induction of NK cell cytotoxicity [126].

A phase I/II trial was designed to assess the safety and efficacy of the Toll-like receptor 2/6 agonist MALP-2 in combination with gemcitabine in patients with incompletely resectable pancreatic carcinoma. Ten patients were included in the study, receiving an intratumoral injection of MALP-2 during surgery plus subsequent chemotherapy. The TLR2/6 agonist was well tolerated and induced clear local effects reflected by the influx of lymphocytes and monocytes, and the enhancement of NK activity. The most important effect was a 17.1 +/- 4.2-month increase in the mean survival, with 2 patients alive after 31 months, which is rarely seen with pancreatic cancer [127].

A phase I trial of the TLR9 agonist in patients with previously treated non-Hodgkin lymphoma (NHL) evaluated safety across a range of doses, and assessed immunomodulatory and clinical effects. Twenty three patients received up to 3 weekly infusions of ODN. Serious adverse events included cytopenias of grade 3/4, probably due to progression of the disease. There were no clinical responses at day 42, as assessed by imaging techniques [128].

A phase I trial of CpG-28, a TLR9 agonist, administered intratumorally by an intracranial catheter in patients with recurrent glioblastoma was designed to evaluate safety and clinical efficacy of the studied immunomodulator. Twenty four patients, refractory to previous therapies (chemo- and radiotherapy) were observed for at least 4 months. The main side effects were limited to worsening of neurological conditions, fever and transient grade 3 lymphopenia. A minor response was observed in 2 patients with a reduced mass effect and

decreased surrounding oedema, and the overall median survival was 7.2 months from the time of enrollment. At the time of analysis, 20 patients had died [129].

A phase I trial to investigate the safety, tolerability, and preliminary anti-tumor activity of PF-3512676 in combination with rituximab was conducted on patients with relapsed or refractory CD20 positive B-cell lymphoma. Fifty patients were included in the study. The main side effects and adverse events were mild to moderate systemic flu-like symptoms and injection-site reactions. Four patients experienced grade 3/4 neutropenia. Objective responses occurred in 24% of patients overall [130].

Friedberg et al. investigated the influence of systemic administration of ODNs concurrently with rituximab on the disease course in 20 patients with non-Hodgkin lymphoma not responding to conventional chemotherapy. The regimen included once weekly administration of ODN with rituximab for 4 consecutive weeks. Drug tolerance and clinical efficacy were assessed. The most common side effects observed during the study were allergic reactions and skin irritation at the site of administration, a cough, airway infection and a headache. A dose-related increase was measured in the expression of several interferon-inducible genes after CpG administration in peripheral blood mononuclear cells. Partial remission was achieved in 6 patients, while in 13 patients, the disease remained stable with a median progression-free survival of 12 months (from 5 to 23.5 months) [131].

Spaner et al. observed the regression of lymphomatous skin deposits in a CLL patient treated with a topically administered synthetic TLR7/8 agonist, imiquimod, which is approved for use against genital warts and basal-cell skin carcinoma [132]. Further clinical trials studying the efficacy of TLR ligands in the treatment of human malignancies are ongoing.

CONCLUSION

Toll-like receptors comprise a family of pattern recognition receptors. The effects of their activation reach beyond innate immune reactions. Activating dendritic cells and modulating specific immune reactions at the levels of T and B cells link mechanisms of innate and adaptive immunity to elicit an efficient response against the invading pathogen or maintenance of homeostasis. However, TLR involvement in the pathogenesis of neoplastic diseases has also been observed. Some issues concerning the exploitation of TLRs in the treatment of neoplastic diseases still need to be addressed. It is crucial to adequately assess the expression of TLRs on transformed cells and study the biology of TLR signaling, as there are still some discrepancies among researchers about the functionality of TLRs in malignant cells. The functionality of TLRs in transformed cells goes far beyond simple NFκB activation, but should be studied in the context of the tumor environment with its suppressive nature and weak immunogenicity.

REFERENCES

1. Myeong, S.L. and Young-Joon, K. Pattern-recognition receptor signaling initiated from extracellular, membrane, and cytoplasmic space. **Mol. Cells** 23 (2007) 1-10.
2. Anderson, K.V., Jurgens, G. and Nusslein-Volhard, C. Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product. **Cell** 42 (1995) 779-789.
3. Rosetto, M., Engström, Y., Baldari, C.T., Telford, J.L. and Hultmark, D. Signals from the IL-1 receptor homolog, Toll, can activate an immune response in a Drosophila hemocyte cell line. **Biochem. Biophys. Res. Commun.** 209 (1995) 111-116.
4. Gay, N.J. and Keith, F.J. Drosophila Toll and IL-1 receptor. **Nature** 351 (1991) 355-356.
5. Medzhitov, R., Preston-Hurlburt, P. and Janeway, C.A. Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. **Nature** 388 (1997) 394.
6. Hopkins, P.A. and Sriskandan, S. Mammalian Toll-like receptors: to immunity and beyond. **Clin. Exp. Immunol.** 140 (2005) 395-407.
7. Bell, J.K., Mullen, G.E., Leifer, C.A., Mazzoni, A., Davies, D.R. and Segal, D.M. Leucine-rich repeats and pathogen recognition in Toll-like receptors. **Trends Immunol.** 24 (2003) 528-533.
8. Ozinsky, A., Underhill, D.M., Fontenot, J.D., Hajjar, A.M., Smith, K.D., Wilson, C.B., Schroeder, L. and Aderem, A. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. **Proc. Natl. Acad. Sci. USA** 97 (2000) 13766-13771.
9. Meng, G., Grabiec, A., Vallon, M., Ebe, B., Hampel, S., Bessler, W., Wagner, H. and Kirschning, C.J. Cellular recognition of tri-/di-palmitoylated peptides is independent from a domain encompassing the N-terminal seven leucine-rich repeat (LRR)/LRR-like motifs of TLR2. **J. Biol. Chem.** 278 (2003) 39822-39829.
10. Roach, J.C., Glusman, G., Rowen, L., Kaur, A., Purcell, M.K., Smith, K.D., Hood, L.E. and Aderem, A. The evolution of vertebrate Toll-like receptors. **Proc. Natl. Acad. Sci. USA** 102 (2005) 9577-9582.
11. Takeuchi, O., Kawai, T., Mühlradt, P.F., Morr, M., Radolf, J.D., Zychlinsky, A., Takeda, K. and Akira, S. Discrimination of bacterial lipoproteins by Toll-like receptor 6. **Int. Immunol.** 13 (2001) 933-940.
12. Takeuchi, O., Sato, S., Horiuchi, T., Hoshino, K., Takeda, K., Dong, Z., Modlin, R.L. and Akira, S. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. **J. Immunol.** 169 (2002) 10-14.

13. Iwaki, D., Mitsuazawa, H. and Murakami, S. The extracellular toll-like receptor 2 domain directly binds peptidoglycan derived from *Staphylococcus aureus*. **J. Biol. Chem.** <u>277</u> (2002) 24315-24320.
14. Means, T.K., Lien, E., Yoshimura, A., Wang, S., Golenbock, D.T. and Fenton, M.J. The CD14 ligands lipoarabinomannan and lipopolysaccharide differ in their requirement for Toll-like receptors. **J. Immunol.** <u>163</u> (1999) 6748-6755.
15. Gantner, B.N., Simmons, R.M., Canavera, S.J., Akira, S. and Underhill, D.M. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. **J. Exp. Med.** <u>197</u> (2003) 1107-1117.
16. Alexopoulou, L., Holt, A.C., Medzhitov, R. and Flavell, R.A. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. **Nature** <u>413</u> (2001) 732-738.
17. Matsukura, S., Kokubu, F., Kurokawa, M., Kawaguchi, M., Ieki, K., Kuga, H., Odaka, M., Suzuki, S., Watanabe, S., Takeuchi, H., Kasama, T. and Adachi, M. Synthetic double-stranded RNA induces multiple genes related to inflammation through Toll-like receptor 3 depending on NF-kappaB and/or IRF-3 in airway epithelial cells. **Clin. Exp. Allergy** <u>36</u> (2006) 1049-1062.
18. Yang, H., Young, D.W., Gusovsky, F. and Chow, J.C. Cellular events mediated by lipopolysaccharide-stimulated toll-like receptor 4. MD-2 is required for activation of mitogen-activated protein kinases and Elk-1. **J. Biol. Chem.** <u>275</u> (2002) 20861-20866.
19. Rallabhandi, P., Bell, J., Boukhvalova, M.S., Medvedev, A., Lorenz, E., Arditi, M., Hemming, V.G., Blanco, J.C., Segal, D.M. and Vogel, S.N. Analysis of TLR4 polymorphic variants: new insights into TLR4/MD-2/CD14 stoichiometry, structure, and signaling. **J. Immunol.** <u>177</u> (2006) 322-332.
20. Hayashi, F., Smith, K.D., Ozinsky, A., Hawn, T.R., Yi, E.C., Goodlett, D.R., Eng, J.K., Akira, S., Underhill, D.M. and Aderem, A. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. **Nature** <u>410</u> (2001) 1099-1103.
21. Smith, K.D., Andersen-Nissen, E., Hayashi, F., Strobe, K., Bergman, M.A., Barrett, S.L., Cookson, B.T. and Aderem, A. Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. **Nat. Immunol.** <u>4</u> (2003) 1247-1253.
22. Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H. and Bauer, S. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. **Science** <u>303</u> (2004) 1526-1529.
23. Jurk, M., Heil, F., Vollmer, J., Schetter, C., Krieg, A.M., Wagner, H., Lipford, G. and Bauer, S. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. **Nat. Immunol.** <u>3</u> (2002) 499.

24. Heil, F., Ahmad-Nejad, P., Hemmi, H., Hochrein, H., Ampenberger, F., Gellert, T., Dietrich, H., Lipford, G., Takeda, K., Akira, S., Wagner, H. and Bauer, S. The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. **Eur. J. Immunol.** 33 (2003) 2987-2997.

25. Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., Sato, S., Sanjo, H., Matsumoto, M., Hoshino, K., Wagner, H., Takeda, K. and Akira, S. A Toll-like receptor recognizes bacterial DNA. **Nature** 408 (2000) 740-745.

26. Jurk, M. and Vollmer, J. Therapeutic applications of synthetic CpG oligodeoxynucleotides as TLR9 agonists for immune modulation. **BioDrugs.** 21 (2007) 387-401.

27. Li, M., Carpio, D.F., Zheng, Y., Bruzzo, P., Singh,V., Ouaaz, F., Medzhitov, R.M. and Beg,A.A. An essential role of the NF-kappa B/Toll-like receptor pathway in induction of inflammatory and tissue-repair gene expression by necrotic cells. **J. Immunol.** 166 (2001) 7128-7135.

28. Basu, S., Binder, R.J., Suto, R., Anderson,K.M. and Srivastava, P.K. Necrotic but not apoptotic cell death releases heat Shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. **Int. Immunol.** 12 (2000) 1539-1546.

29. Wang, Y., Kelly, C.G., Singh, M., McGowan, E.G., Carrara, A.S., Bergmeier, L.A. and Lehner, T. Stimulation of Th1-polarizing cytokines, C-C chemokines, maturation of dendritic cells, and adjuvant function by the peptide binding fragment of heat Shock protein 70. **J. Immunol.** 169 (2002) 2422-2429.

30. Abulafia-Lapid, R., Elias, D., Raz, I., Keren-Zur, Y., Atlan, H. and Cohen, I.R. T-cell proliferative responses of type 1 diabetes patients and healthy individuals to human hsp60 and its peptides. **J. Autoimmun.** 12 (1999) 121-129.

31. Szewczuk, M.R. and Depew, W.T. Evidence for T lymphocyte reactivity to the 65 kilodalton heat Shock protein of mycobacterium in active Crohn's disease. **Clin. Invest. Med.** 15 (1992) 494-505.

32. Bausinger, H., Lipsker,D., Ziylan, U., Manié, S., Briand, J.P., Cazenave, J.P., Muller, S., Haeuw, J.F., Ravanat, C., de la Salle, H. and Hanau, D. Endotoxin-free heat-Shock protein 70 fails to induce APC activation. **Eur. J. Immunol.** 32 (2002) 3708-3713.

33. Gao, B. and Tsan, M.F. Recombinant human heat Shock protein 60 does not induce the release of tumor necrosis factor alpha from murine macrophages. **J. Biol. Chem.** 278 (2003) 22523-22529.

34. Kariko, K., Ni, H., Capodici, J., Lamphier, M. and Weissman, D. mRNA is an endogenous ligand for toll-like receptor 3. **J. Biol. Chem.** 279 (2004) 12542-12550.

35. Kowalski, M.L., Wolska, A., Grzegorczyk, J., Hilt, J., Jarzebska, M., Drobniewski, M., Synder, M. and Kurowski, M. Increased responsiveness to toll-like receptor 4 stimulation in peripheral blood mononuclear cells

from patients with recent onset rheumatoid arthritis. **Mediators Inflamm.** (2008) 132732.

36. Kawai, T. and Akira, S. TLR signaling. **Cell Death Differ.** <u>13</u> (2006) 816-825.

37. Björkbacka, H., Fitzgerald, K.A., Huet, F., Li X., Gregory, J.A., Lee, M.A., Ordija, C.M., Dowley, N.E., Golenbock, D.T. and Freeman, M.W. The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades. **Physiol. Genomics** <u>19</u> (2004) 319-330.

38. Zhao, J. and Wu, X.Y. Triggering of toll-like receptors 2 and 4 by *Aspergillus fumigatus* conidia in immortalized human corneal epithelial cells to induce inflammatory cytokines. **Chin. Med. J. (Engl).** <u>121</u> (2008) 450-454.

39. Covert, M.W., Leung, T.H., Gaston, J.E. and Baltimore, D. Achieving stability of lipopolysaccharide-induced NF-kappaB activation. **Science** <u>309</u> (2005) 1854-1857.

40. Wang, J.E., Jørgensen, P.F., Almlöf, M., Thiemermann, C., Foster, S.J., Aasen, A.O. and Solberg, R. Peptidoglycan and lipoteichoic acid from Staphylococcus aureus induce tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-10 production in both T cells and monocytes in a human whole blood model. **Infect. Immun**. <u>68</u> (2000) 3965-3670.

41. Ellingsen, E., Morath, S., Flo, T., Schromm, A., Hartung, T., Thiemermann, C., Espevik, T., Golenbock, D., Foster, D., Solberg, R., Aasen, A. and Wang, J. Induction of cytokine production in human T cells and monocytes by highly purified lipoteichoic acid: involvement of Toll-like receptors and CD14. **Med. Sci. Monit.** <u>8</u> (2002) BR149-156.

42. Wang, J.P., Kurt-Jones, E.A., Shin, O.S., Manchak, M.D., Levin, M.J. and Finberg, R.W. Varicella-zoster virus activates inflammatory cytokines in human monocytes and macrophages via Toll-like receptor 2. **J. Virol.** <u>79</u> (2005) 12658-12666.

43. Smith, M.F. Jr, Mitchell, A., Li, G., Ding, S., Fitzmaurice, A.M., Ryan, K., Crowe, S. and Goldberg, J.B. Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells. **J. Biol. Chem.** <u>278</u> (2003) 32552-32560.

44. Gaudreault, E., Fiola, S., Olivier, M. and Gosselin, J. Epstein-Barr virus induces MCP-1 secretion by human monocytes via TLR2. **J. Virol.** <u>81</u> (2007) 8016-8024.

45. Hertz, C.J., Wu, Q., Porter, E.M., Zhang, Y.J., Weismüller, K.H., Godowski, P.J., Ganz, T., Randell, S.H. and Modlin, R.L. Activation of Toll-like receptor 2 on human tracheobronchial epithelial cells induces the antimicrobial peptide human beta defensin-2. **J. Immunol.** <u>171</u> (2003) 6820-6826.

46. Alexopolou, L., Holt, A.C., Medzhitov, R. and Flavell, R.A. Recognition of double-stranded RNA and activation of NF-κB by toll-like receptor 3. **Nature** <u>413</u> (2001) 732-738.

47. Guillot, L., Le Goffic, R., Bloch, S., Escriou, N., Akira, S., Chignard, M. and Si-Tahar, M. Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus. **J. Biol. Chem.** 280 (2005) 5571-5580.

48. Li, Q., Withoff, S. and Verma, I.M. Inflammation-associated cancer: NF-kappaB is the lynchpin. **Trends Immunol.** 26 (2005) 318-325.

49. Balkwill, F. and Coussens, L.M. Cancer: an inflammatory link. **Nature** 431 (2004) 405-406.

50. Gupta, R.A. and Dubois, R.N. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. **Nat. Rev. Cancer** 1 (2001) 11-21.

51. Robak, P., Smolewski, P. and Robak, T. The role of non-steroidal anti-inflammatory drugs in the risk of development and treatment of hematologic malignancies. **Leuk. Lymphoma** 49 (2008) 1452-1462.

52. Pikarsky, E., Porat, R.M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., Gutkovich-Pyest, E., Urieli-Shoval, S., Galun, E. and Ben-Neriah, Y. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. **Nature** 431 (2004) 461-466.

53. Palayoor, S.T., Youmell, M.Y., Calderwood, S.K., Coleman, C.N. and Price, B.D. Constitutive activation of IkappaB kinase alpha and NF-kappaB in prostate cancer cells is inhibited by ibuprofen. **Oncogene** 18 (1999) 7389-7394.

54. Baron, F., Turhan, A.G., Giron-Michel, J., Azzarone, B., Bentires-Alj, M., Bours, V., Bourhis, J.H., Chouaib, S. and Caignard, A. Leukemic target susceptibility to natural killer cytotoxicity: relationship with BCR-ABL expression. **Blood** 99 (2002) 2107-2113.

55. Griffin, J.D. Leukemia stem cells and constitutive activation of NF-kappaB. **Blood** 98 (2001) 2291.

56. Feinman, R., Koury, J., Thames, M., Barlogie, B., Epstein, J. and Siegel, D.S. Role of NF-kappaB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2. **Blood** 93 (1999) 3044-3052.

57. Philip, M., Rowley, D.A. and Schreiber, H. Inflammation as a tumor promoter in cancer induction. **Semin. Cancer Biol.** 14 (2004) 433-439.

58. Chang, Y.J., Wu, M.S., Lin, J.T. and Chen, C.C. *Helicobacter pylori*-induced invasion and angiogenesis of gastric cells is mediated by cyclooxygenase-2 induction through TLR2/TLR9 and promoter regulation. **J. Immunol.** 175 (2005) 8242-8252.

59. Li, V.W., Li, W.W., Talcott, K.E. and Zhai, A.W. Imiquimod as an antiangiogenic agent. **J. Drugs Dermatol.** 4 (2005) 708-717.

60. Majewski, S., Marczak, M., Mlynarczyk, B., Benninghoff, B. and Jablonska, S. Imiquimod is a strong inhibitor of tumor cell-induced angiogenesis. **Int. J. Dermatol.** 44 (2005) 14-19.

61. Damiano, V., Caputo, R., Bianco, R., D'Armiento, F.P., Leonardi, A., De Placido, S., Bianco, A.R., Agrawal, S., Ciardiello, F. and Tortora, G. Novel toll-like receptor 9 agonist induces epidermal growth factor receptor

(EGFR) inhibition and synergistic antitumor activity with EGFR inhibitors. **Clin. Cancer Res.** <u>12</u> (2006) 577-583.

62. Fukata, M., Chen, A., Vamadevan, A.S., Cohen, J., Breglio, K., Krishnareddy, S., Hsu, D., Xu, R., Harpaz, N., Dannenberg, A.J., Subbaramaiah, K., Cooper, H.S., Itzkowitz, S.H. and Abreu, M.T. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. **Gastroenterology** <u>133</u> (2007) 1869-1881.

63. Swann, J.B., Vesely, M.D., Silva, A., Sharkey, J., Akira, S., Schreiber, R.D. and Smyth, M.J. Demonstration of inflammation-induced cancer and cancer immunoediting during primary tumorigenesis. **Proc. Natl. Acad. Sci. USA** <u>105</u> (2008) 652-656.

64. Kundu, S.D., Leem, C., Billips, B.K., Habermacher, G.M., Zhang, Q., Liu, V., Wong, L.Y., Klumpp, D.J. and Thumbikat, P. The toll-like receptor pathway: a novel mechanism of infection-induced carcinogenesis of prostate epithelial cells. **Prostate** <u>68</u> (2008) 223-229.

65. Pries, R., Hogrefe, L., Xie, L., Frenzel, H., Brocks, C., Ditz, C. and Wollenberg, B. Induction of c-Myc-dependent cell proliferation through toll-like receptor 3 in head and neck cancer. **Int. J. Mol. Med.** <u>21</u> (2008) 209-215.

66. Jego, G., Bataille, R., Geffroy-Luseau, A., Descamps, G. and Pellat-Deceunynck, C. Pathogen-associated molecular patterns are growth and survival factors for human myeloma cells through Toll-like receptors. **Leukemia** <u>20</u> (2006) 1130-1137.

67. Chochi, K., Ichikura, T., Kinoshita, M., Majima, T., Shinomiya, N., Tsujimoto, H., Kawabata, T., Sugasawa, H., Ono, S., Seki, S. and Mochizuki, H. *Helicobacter pylori* augments growth of gastric cancers via the lipopolysaccharide-toll-like receptor 4 pathway whereas its lipopolysaccharide attenuates antitumor activities of human mononuclear cells. **Clin. Cancer Res.** <u>14</u> (2008) 2909-2917.

68. Paone, A., Starace, D., Galli, R., Padula, F., De Cesaris, P., Filippini, A., Ziparo, E. and Riccioli, A. Toll-like receptor 3 triggers apoptosis of human prostate cancer cells through a PKC-alpha-dependent mechanism. **Carcinogenesis** <u>29</u> (2008) 1334-1342.

69. Barnhart, B.C. and Peter, M.E. The TNF receptor 1: a split personality complex. **Cell** <u>114</u> (2003) 148-150.

70. Jahrsdörfer, B., Wooldridge, J.E., Blackwell, S.E., Taylor, C.M., Griffith, T.S., Link, B.K. and Weiner, G.J. Immunostimulatory oligodeoxy-nucleotides induce apoptosis of B cell chronic lymphocytic leukemia cells. **J. Leukoc. Biol.** <u>77</u> (2005) 378-387.

71. Jahrsdörfer, B., Jox, R., Mühlenhoff, L., Tschoep, K., Krug, A., Rothenfusser, S., Meinhardt, G., Emmerich, B., Endres, S. and Hartmann, G. Modulation of malignant B cell activation and apoptosis by bcl-2 antisense ODN and immunostimulatory CpG ODN. **J. Leukoc. Biol.** <u>72</u> (2002) 83-92.

72. Smits, E.L., Ponsaerts, P., Van de Velde, A.L., Van Driessche, A., Cools, N., Lenjou, M., Nijs, G., Van Bockstaele, D.R., Berneman, Z.N. and Van Tendeloo, V.F. Proinflammatory response of human leukemic cells to dsRNA transfection linked to activation of dendritic cells. **Leukemia** 21 (2007) 1691-1699.

73. Salaun, B., Lebecque, S., Matikainen, S., Rimoldi, D. and Romero, P. Toll-like receptor 3 expressed by melanoma cells as a target for therapy? **Clin. Cancer Res.** 13 (2007) 4565-4574.

74. Lehner, M., Bailo, M., Stachel, D., Roesler, W., Parolini, O. and Holter, W. Caspase-8 dependent apoptosis induction in malignant myeloid cells by TLR stimulation in the presence of IFN-alpha. **Leuk. Res.** 31 (2007) 1729-1735.

75. Haase, R., Kirschning, C.J., Sing, A., Schröttner, P., Fukase, K., Kusumoto, S., Wagner, H., Heesemann, J. and Ruckdeschel, K. A dominant role of Toll-like receptor 4 in the signaling of apoptosis in bacteria-faced macrophages. **J. Immunol.** 171 (2003) 4294-4303.

76. Hsu, L.C., Park, J.M., Zhang, K., Luo, J.L., Maeda,S., Kaufman,R.J., Eckmann, L., Guiney, D.G. and Karin, M. The protein kinase PKR is required for macrophage apoptosis after activation of Toll-like receptor 4. **Nature** 428 (2004) 341-345.

77. Into, T., Kiura, K., Yasuda, M., Kataoka, H., Inoue, N., Hasebe, A., Takeda, K., Akira, S. and Shibata, K. Stimulation of human Toll-like receptor (TLR) 2 and TLR6 with membrane lipoproteins of Mycoplasma fermentans induces apoptotic cell death after NF-kappa B activation. **Cell. Microbiol.** 6 (2004) 187-199.

78. Jung, D.Y., Lee, H., Jung, B.Y., Ock, J., Lee, M.S., Lee, W.H. and Suk, K. TLR4, but not TLR2, signals autoregulatory apoptosis of cultured microglia: a critical role of IFN-beta as a decision maker. **J. Immunol.** 174 (2005) 6467-6476.

79. Ma, Y., Liu, H., Tu-Rapp, H., Thiesen, H.J., Ibrahim, S.M., Cole, S.M. and Pope, R.M. Fas ligation on macrophages enhances IL-1R1-Toll-like receptor 4 signaling and promotes chronic inflammation. **Nat. Immunol.** 5 (2004) 380-387.

80. Imtiyaz, H.Z., Rosenberg, S., Zhang, Y., Rahman, Z.S., Hou, Y.J., Manser, T. and Zhang, J. The Fas-associated death domain protein is required in apoptosis and TLR-induced proliferative responses in B cells. **J. Immunol.** 176 (2006) 6852-6861.

81. Kelly, M.G., Alvero, A.B., Chen, R., Silasi, D.A., Abrahams, V.M., Chan, S., Visintin, I., Rutherford, T. and Mor, G. TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. **Cancer Res.** 66 (2006) 3859-3868.

82. Bottero, V., Busuttil, V., Loubat, A., Magné, N., Fischel, J.L., Milano, G. and Peyron, J.F. Activation of nuclear factor kappaB through the IKK complex by the topoisomerase poisons SN38 and doxorubicin: a brake to

apoptosis in HeLa human carcinoma cells. **Cancer Res.** <u>61</u> (2001) 7785-7791.

83. Tosi, P., Zinzani, P.L., Pellacani, A., Ottaviani, E., Magagnoli, M. and Tura, S. Loxoribine affects fludarabine activity on freshly isolated B-chronic lymphocytic leukemia cells. **Leuk. Lymphoma** <u>26</u> (1997) 343-348.

84. Pellacani, A., Tosi, P., Zinzani, P.L., Ottaviani, E., Albertini, P., Magagnoli, M. and Tura, S. Cytotoxic combination of loxoribine with fludarabine and mafosfamide on freshly isolated B-chronic lymphocytic leukemia cells. **Leuk. Lymphoma** <u>33</u> (1999) 147-153.

85. Shi, Y., White, D., He, L., Miller, R.L. and Spaner, D.E. Toll-like receptor-7 tolerizes malignant B cells and enhances killing by cytotoxic agents. **Cancer Res.** <u>67</u> (2007) 1823-1831.

86. Garay, R.P., Viens, P., Bauer, J., Normier, G., Bardou, M., Jeannin, J.F. and Chiavaroli, C. Cancer relapse under chemotherapy: why TLR2/4 receptor agonists can help. **Eur. J. Pharmacol.** <u>563</u> (2007) 1-17.

87. Coussens, L.M. and Werb, Z. Inflammation and cancer. **Nature.** <u>420</u> (2002) 860-867.

88. Sakaguchi, S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. **Annu. Rev. Immunol.** <u>22</u> (2004) 531-562.

89. Fisson, S., Darrasse-Jèze, G., Litvinova, E., Septier, F., Klatzmann, D., Liblau, R. and Salomon, B.L. Continuous activation of autoreactive CD4+ CD25+ regulatory T cells in the steady state. **J. Exp. Med.** <u>198</u> (2003) 737-746.

90. Singh, B., Read, S., Asseman, C., Malmström, V., Mottet, C., Stephens, L.A., Stepankova, R., Tlaskalova, H. and Powrie, F. Control of intestinal inflammation by regulatory T cells. **Immunol Rev.** <u>182</u> (2001) 190-200.

91. Hori, S., Carvalho, T.L. and Demengeot, J. CD25+CD4+ regulatory T cells suppress CD4+ T cell-mediated pulmonary hyperinflammation driven by Pneumocystis carinii in immunodeficient mice. **Eur. J. Immunol.** <u>32</u> (2002) 1282-1291.

92. Hänig, J. and Lutz, M.B. Suppression of mature dendritic cell function by regulatory T cells in vivo is abrogated by CD40 licensing. **J. Immunol.** <u>180</u> (2008) 1405-1413.

93. Caramalho, I., Lopes-Carvalho, T., Ostler, D., Zelenay, S., Haury, M. and Demengeot, J. Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. **J. Exp. Med.** <u>197</u> (2003) 403-411.

94. Sfondrini, L., Rossini, A., Besusso, D., Merlo, A., Tagliabue, E., Mènard, S. and Balsari, A. Antitumor activity of the TLR-5 ligand flagellin in mouse models of cancer. **J. Immunol.** <u>176</u> (2006) 6624-6630.

95. Peng, G., Guo, Z., Kiniwa, Y., Voo, K.S., Peng, W., Fu, T., Wang, D.Y., Li, Y., Wang, H.Y. and Wang, R.F. Toll-like receptor 8-mediated reversal of CD4+ regulatory T cell function. **Science** <u>309</u> (2005) 1380-1384.

96. Cella, M., Salio, M., Sakakibara, Y., Langen, H., Julkunen, I. and Lanzavecchia, A. Maturation, activation, and protection of dendritic cells induced by double-stranded RNA. **J. Exp. Med.** 189 (1999) 821-829.

97. Pulendran, B., Kumar, P., Cutler, W., Mohamadzadeh, M., Van Dyke, T. and Banchereau, J. Lipopolysaccharides from distinct pathogens induce different classes of immune responses in vivo. **J. Immunol.** 167 (2001) 5067-5076.

98. Pasare, C. and Medzhitov, R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. **Science** 299 (2003) 1033-1036.

99. Spaner, D.E., Shi, Y., White, D., Mena, J., Hammond, C., Tomic, J., He,L., Tomai, M.A., Miller, R.L., Booth, J. and Radvanyi, L. Immunomodulatory effects of Toll-like receptor-7 activation on chronic lymphocytic leukemia cells. **Leukemia** 20 (2006) 286-295.

100. Tomic, J., White, D., Shi, Y., Mena, J., Hammond, C., He, L., Miller, R.L. and Spaner, D.E. Sensitization of IL-2 signaling through TLR-7 enhances B lymphoma cell immunogenicity. **J. Immunol.** 176 (2006) 3830-3839.

101. Decker, T., Schneller, F., Sparwasser, T., Tretter, T., Lipford, G.B., Wagner, H. and Peschel, C. Immunostimulatory CpG-oligonucleotides cause proliferation, cytokine production, and an immunogenic phenotype in chronic lymphocytic leukemia B cells. **Blood** 95 (2000) 999-1006.

102. Decker, T., Schneller, F., Kronschnabl, M., Dechow, T., Lipford, G.B., Wagner, H. and Peschel, C. Immunostimulatory CpG-oligonucleotides induce functional high affinity IL-2 receptors on B-CLL cells: costimulation with IL-2 results in a highly immunogenic phenotype. **Exp. Hematol.** 28 (2000) 558-568.

103. Decker, T., Hipp, S., Kreitman, R.J., Pastan, I., Peschel, C., Licht, T. Sensitization of B-cell chronic lymphocytic leukemia cells to recombinant immunotoxin by immunostimulatory phosphorothioate oligodeoxy-nucleotides. **Blood** 99 (2002) 1320-1326.

104. Evel-Kabler, K., Song, X.T., Aldrich, M., Huang, X.F. and Chen, S.Y. SOCS1 restricts dendritic cells' ability to break self tolerance and induce antitumor immunity by regulating IL-12 production and signaling. **J. Clin. Invest.** 116 (2006) 90-100.

105. Tormo, D., Ferrer, A., Bosch, P., Gaffal, E., Basner-Tschakarjan, E., Wenzel, J. and Tüting, T. Therapeutic efficacy of antigen-specific vaccination and toll-like receptor stimulation against established transplanted and autochthonous melanoma in mice. **Cancer Res.** 66 (2006) 5427-5435.

106. Wysocka, M., Benoit, B.M., Newton, S., Azzoni, L., Montaner, L.J. and Rook, A.H. Enhancement of the host immune responses in cutaneous T-cell lymphoma by CpG oligodeoxynucleotides and IL-15. **Blood** 104 (2004) 4142-4149.

107. Mangsbo, S.M., Ninalga, C., Essand, M., Loskog, A. and Tötterman, T.H. CpG therapy is superior to BCG in an orthotopic bladder cancer model and generates CD4+ T-cell immunity. **J. Immunother.** 31 (2008) 34-42.

108. Ren, T., Wen, Z.K., Liu, Z.M., Qian, C., Liang, Y.J., Jin, M.L., Cai, Y.Y. and Xu, L. Targeting toll-like receptor 9 with CpG oligodeoxynucleotides enhances anti-tumor responses of peripheral blood mononuclear cells from human lung cancer patients. **Cancer Invest.** 26 (2008) 448-455.

109. Roda, J.M., Parihar, R. and Carson, W.E. 3rd. CpG-containing oligodeoxy-nucleotides act through TLR9 to enhance the NK cell cytokine response to antibody-coated tumor cells. **J. Immunol.** 175 (2005) 1619-1627.

110. Frankenberger, M., Pechumer, H. and Ziegler-Heitbrock, H.W. Interleukin-10 is upregulated in LPS tolerance. **J. Inflamm.** 45 (1995) 56-63.

111. Tominaga, K., Saito, S., Matsuura, M. and Nakano, M. Lipopolysaccharide tolerance in murine peritoneal macrophages induces downregulation of the lipopolysaccharide signal transduction pathway through mitogen-activated protein kinase and nuclear factor-kappaB cascades, but not lipopolysaccharide-incorporation steps. **Biochim. Biophys. Acta** 1450 (1999) 130-144.

112. Medvedev, A.E., Kopydlowski, K.M. and Vogel, S.N. Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. **J. Immunol.** 164 (2000) 5564-5574.

113. Nomura, F., Akashi, S., Sakao, Y., Sato, S., Kawai, T., Matsumoto, M., Nakanishi, K., Kimoto, M., Miyake, K., Takeda, K. and Akira, S. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. **J. Immunol.** 164 (2000) 3476-3479.

114. Hume, D.A., Underhill, D.M., Sweet, M.J., Ozinsky, A.O., Liew, F.Y. and Aderem, A. Macrophages exposed continuously to lipopolysaccharide and other agonists that act via toll-like receptors exhibit a sustained and additive activation state. **BMC Immunol.** 2 (2001) 11.

115. Randow, F., Syrbe, U., Meisel, C., Krausch, D., Zuckermann, H., Platzer, C. and Volk, H.D. Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor beta. **J. Exp. Med.** 181 (1995) 1887-1892.

116. Hamdy, S., Molavi, O., Ma, Z., Haddadi, A., Alshamsan, A., Gobti, Z., Elhasi, S., Samuel, J. and Lavasanifar, A. Co-delivery of cancer-associated antigen and Toll-like receptor 4 ligand in PLGA nanoparticles induces potent CD8(+) T cell-mediated anti-tumor immunity. **Vaccine** 26 (2008) 5046-5057.

117. Ramakrishna, V., Vasilakos, J.P., Tario, J.D. Jr, Berger, M.A., Wallace, P.K. and Keler, T. Toll-like receptor activation enhances cell-mediated immunity induced by an antibody vaccine targeting human dendritic cells. **J. Transl. Med.** 5 (2007) 5.

118. den Brok, M.H., Sutmuller, R.P., Nierkens, S., Bennink, E.J., Toonen, L.W., Figdor, C.G., Ruers, T.J. and Adema, G.J. Synergy between in situ cryoablation and TLR9 stimulation results in a highly effective in vivo dendritic cell vaccine. **Cancer Res.** <u>66</u> (2006) 7285-7292.

119. Koido, S., Hara, E., Homma, S., Torii, A., Toyama, Y., Kawahara, H., Watanabe, M., Yanaga, K., Fujise, K., Tajiri, H., Gong, J. and Toda, G. Dendritic cells fused with allogeneic colorectal cancer cell line present multiple colorectal cancer-specific antigens and induce antitumor immunity against autologous tumor cells. **Clin. Cancer Res.** <u>11</u> (2005) 7891-7900.

120. Adams, S., O'Neill, D.W., Nonaka, D., Hardin, E., Chiriboga, L., Siu, K., Cruz, C.M., Angiulli, A., Angiulli, F., Ritter, E., Holman, R.M., Shapiro, R.L., Berman, R.S., Berner, N., Shao. Y., Manches, O., Pan, L., Venhaus, R.R., Hoffman, E.W., Jungbluth, A., Gnjatic, S., Old, L., Pavlick, A.C. and Bhardwaj, N. Immunization of malignant melanoma patients with full-length NY-ESO-1 protein using TLR7 agonist imiquimod as vaccine adjuvant. **J. Immunol.** <u>181</u> (2008) 776-784.

121. Lesimple, T., Neidhard, E.M., Vignard, V., Lefeuvre, C., Adamski, H., Labarrière, N., Carsin, A., Monnier, D., Collet, B., Clapissonm G,, Birebent, B., Philip, I., Toujas, L., Chokri, M. and Quillien, V. Immunologic and clinical effects of injecting mature peptide-loaded dendritic cells by intralymphatic and intranodal routes in metastatic melanoma patients. **Clin. Cancer Res.** <u>12</u> (2006) 7380-7388.

122. Speiser, D.E., Liénard, D., Rufer, N., Rubio-Godoy, V., Rimoldi, D., Lejeune, F., Krieg, A.M., Cerottini, J.C. and Romero, P. Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. **J. Clin. Invest.** <u>115</u> (2005) 739-746.

123. Shackleton, M., Davis, I.D., Hopkins, W., Jackson, H., Dimopoulos, N., Tai, T., Chen, Q., Parente, P., Jefford, M., Masterman, K.A., Caron, D., Chen, W., Maraskovsky, E. and Cebon, J. The impact of imiquimod, a Toll-like receptor-7 ligand (TLR7L), on the immunogenicity of melanoma peptide vaccination with adjuvant Flt3 ligand. **Cancer Immun.** <u>4</u> (2004) 9.

124. Manegold, C., Gravenor, D., Woytowitz, D., Mezger, J., Hirsh, V., Albert, G., Al-Adhami, M., Readett, D., Krieg, A.M. and Leichman, C.G. Randomized phase II trial of a toll-like receptor 9 agonist oligodeoxynucleotide, PF-3512676, in combination with first-line taxane plus platinum chemotherapy for advanced-stage non-small-cell lung cancer. **J. Clin. Oncol.** <u>26</u> (2008) 3979-3986.

125. Dummer, R., Hauschild, A., Becker, J.C., Grob, J.J., Schadendorf, D., Tebbs, V., Skalsky, J., Kaehler, K.C., Moosbauer, S., Clark, R., Meng, T.C. and Urosevic, M. An exploratory study of systemic administration of the toll-like receptor-7 agonist 852A in patients with refractory metastatic melanoma. **Clin. Cancer Res.** <u>14</u> (2008) 856-864.

126. Pashenkov, M., Goëss, G., Wagner, C., Hörmann, M., Jandl, T., Moser, A., Britten, C.M., Smolle, J., Koller, S., Mauch, C., Tantcheva-Poor, I., Grabbe, S.,

Loquai, C., Esser, S., Franckson, T., Schneeberger, A., Haarmann, C., Krieg, A.M., Stingl, G. and Wagner, S.N. Phase II trial of a toll-like receptor 9-activating oligonucleotide in patients with metastatic melanoma. **J. Clin. Oncol.** 24 (2006) 5716-5724.

127. Schmidt, J., Welsch, T., Jäger, D, Mühlradt, P.F., Büchler, M.W., Märten, A. Intratumoural injection of the toll-like receptor-2/6 agonist 'macrophage-activating lipopeptide-2' in patients with pancreatic carcinoma: a phase I/II trial. **Br. J. Cancer** 97 (2007) 598-604.

128. Link, B.K., Ballas, Z.K., Weisdorf, D., Wooldridge, J.E., Bossler, A.D., Shannon, M., Rasmussen, W.L., Krieg, A.M. and Weiner, G.J. Oligodeoxy-nucleotide CpG 7909 delivered as intravenous infusion demonstrates immunologic modulation in patients with previously treated non-Hodgkin lymphoma. **J. Immunother.** 29 (2006) 558-568.

129. Carpentier, A., Laigle-Donadey, F., Zohar, S., Capelle, L., Behin, A., Tibi, A., Martin-Duverneuil, N., Sanson, M., Lacomblez, L., Taillibert, S., Puybasset. L,, Van Effenterre, R., Delattre, J.Y. and Carpentier, A.F. Phase 1 trial of a CpG oligodeoxynucleotide for patients with recurrent glioblastoma. **Neuro- Oncol.** 8 (2006) 60-66.

130. Leonard, J.P., Link, B.K., Emmanouilides, C., Gregory, S.A., Weisdorf, D., Andrey, J., Hainsworth, J., Sparano, J.A., Tsai, D.E., Horning, S., Krieg, A.M. and Weiner, G.J. Phase I trial of toll-like receptor 9 agonist PF-3512676 with and following rituximab in patients with recurrent indolent and aggressive non Hodgkin's lymphoma. **Clin. Cancer Res.** 13 (2007) 6168-6174.

131. Friedberg, J.W., Kim, H., McCauley, M., Hessel, E.M., Sims, P., Fisher, D.C., Nadler, L.M., Coffman, R.L. and Freedman, A.S. Combination immunotherapy with a CpG oligonucleotide (1018 ISS) and rituximab in patients with non-Hodgkin lymphoma: increased interferon-alpha/beta-inducible gene expression, without significant toxicity. **Blood** 105 (2005) 489-495.

132. Spaner, D.E., Miller, R.L., Mena, J., Grossman, L., Sorrenti, V. and Shi, Y. Regression of lymphomatous skin deposits in a chronic lymphocytic leukemia patient treated with the Toll-like receptor-7/8 agonist, imiquimod. **Leuk. Lymphoma** 46 (2005) 935-939.