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Trial Watch

Toll-like receptor agonists in oncological indications

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Abbreviations: BCG, bacillus Calmette-Guérin; CI, confidence interval; DAMP, damage-associated molecular pattern; DC, dendritic cell; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; GLA, glucopyranosyl lipid adjuvant; GM-CSF, granulocyte macrophage colony-stimulating factor; HR, hazard ratio; HPV, human papillomavirus; IFN, interferon; LGG, low-grade glioma; LPS, lipopolysaccharide; MAGEA3, melanoma antigen family A3; MPL, monophosphoryl lipid A; MYD88, myeloid differentiation primary response 88; NHL, non-Hodgkin's lymphoma; NK, natural killer; NSCLC, non-small cell lung carcinoma; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; SNP, single nucleotide polymorphism; TICAM1, toll-like receptor adaptor molecule 1; TIRAP, toll-interleukin 1 receptor domain containing adaptor protein; TLR, toll-like receptor

Toll-like receptors (TLRs) are an evolutionarily conserved group of enzymatically inactive, single membrane-spanning proteins that recognize a wide panel of exogenous and endogenous danger signals. Besides constituting a crucial component of the innate immune response to bacterial and viral pathogens, TLRs appear to play a major role in anticancer immunosurveillance. In line with this notion, several natural and synthetic TLR ligands have been intensively investigated for their ability to boost tumor-targeting immune responses elicited by a variety of immunotherapeutic and chemotherapeutic interventions. Three of these agents are currently approved by the US Food and Drug Administration (FDA) or equivalent regulatory agencies for use in cancer patients: the so-called bacillus Calmette-Guérin, monophosphoryl lipid A, and imiquimod. However, the number of clinical trials testing the therapeutic potential of both FDA-approved and experimental TLR agonists in cancer patients is stably decreasing, suggesting that drug developers and oncologists are refocusing their interest on alternative immunostimulatory agents. Here, we summarize recent findings on the use of TLR agonists in cancer patients and discuss how the clinical evaluation of FDA-approved and experimental TLR ligands has evolved since the publication of our first Trial Watch dealing with this topic.

Introduction

Organisms as evolutionarily distant as plants, flies, fish, and mammals detect invading pathogens via a panel of conserved, enzymatically inactive, single membrane-spanning proteins commonly known as Toll-like receptors (TLRs),[1-3] owing to their high degree of homology with *Drosophila melanogaster* Toll.[4-6] At present, 13 distinct TLRs have been identified in mice (Tlr1-Tlr13), 10 of which are also encoded by the human genome (TLR1-TLR10).[7,8] Conversely, *TLR11* is a pseudogene and human cells are devoid of *Tlr12* and *Tlr13* homologs.[7-9]

TLRs are mainly expressed by immune cells including monocytes, mature macrophages, mast cells and dendritic cells (DCs) as well as by other cells involved in the first-line defense against infection (e.g., intestinal epithelial cells).[10,11] TLRs sense indeed a wide panel of conserved microbial components that

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are cumulatively referred to as "microbe-associated molecular patterns" (MAMPs), including specific nucleic acids and peculiar proteo-lipidic structures like lipopolysaccharide (LPS).[12-28] Moreover, several TLRs have been shown to respond to so-called "damage-associated molecular patterns" (DAMPs), i.e., endogenous molecules that are released by stressed, dying or dead cells as a signal of danger.[29-34] This is for instance the case of TLR2 and TLR4,[35-40] both of which are expressed on the cell surface (similar to other TLRs that recognize proteo-lipidic components),[8,41] as well as of TLR9,[42,43] which is mainly expressed in the endosomal compartment (hence resembling other TLRs that detect nucleic acids).[8,41]

Upon binding to their ligands, most TLRs form dimers that recruit the adaptor proteins myeloid differentiation primary response 88 (MYD88) and toll-interleukin 1 receptor domain containing adaptor protein (TIRAP, also known as MAL), hence initiating the assembly of plasma-membrane proximal multiprotein signaling complex.[44,45] Among several effects, such a supramolecular complex promotes the activation of the transcription factor NF-κB, hence driving the synthesis of multiple pro-inflammatory cytokines.[44,45] At odds with other TLRs, all of which employ MYD88 as an obligate or facultative adaptor, TLR3 dimers trigger a MYD88-independent signaling pathway that critically relies on toll-like receptor adaptor molecule 1 (TICAM1, also known as TRIF) and does not trigger NF-κB activation.[46-48] Rather, TICAM1-transduced signals activate interferon regulatory factor 7 (IRF7),[49,50] hence regulating the expression of type I interferons (IFNs) and other IFN-responsive mediators.[46-48] Of note, *Ticam1*$^{-/-}$ mice exhibit defects in both TLR3 and TLR4 signaling,[47] suggesting that TLR4 can also employ TICAM1 as an adaptor, at least under some circumstances. Moreover, MYD88 and TIRAP have been shown to actively inhibit TLR3 signaling,[51-53] lending further support to the notion that the functional profile of TLR3 is rather dissimilar from that of other members of the TLR family.[8,41,54-56]

Reflecting their ligand-binding profile, TLRs are not only crucial for the initiation of an innate immune response against bacterial and viral pathogens,[7,8] but also participate in the organismal reaction to inflammatory conditions that usually do not involve a microbial component, including a wide array of neoplasms.[43,57,58] In line with this notion, single nucleotide polymorphisms (SNPs) that alter the functionality of multiple TLRs have been shown to influence the natural progression of several tumors of non-viral etiology.[59-77] Of note, TLR signaling is impaired in many neoplasms of viral etiology, including hepatitis B virus-associated hepatocellular carcinoma, human papillomavirus (HPV)-associated cervical carcinoma and Merkel cell carcinoma.[78] At least in part, this reflects the ability of several viruses to avoid the activation of TLR9 by their own nucleic acids.[78]

Some TLRs have also been shown to play a critical role in the (re)activation of tumor-specific immune responses by a diverse array of chemo-, radio- and immunotherapeutic interventions.[79-85] Accordingly, SNPs that reduce the activity of various TLRs have been shown to negatively impact the response of cancer patients to therapy in multiple scenarios.[83,86-88] At least in part, this stems from the key role that some TLRs play in the perception of cell death as immunogenic.[79,80] Indeed, cancer cells subjected to specific therapeutic regimens, including irradiation, some forms of photodynamic therapy as well as doxorubicin-, mitoxantrone-, and oxaliplatin-based chemotherapy, die while emitting a spatiotemporally defined combination of DAMPs that renders them capable of triggering a therapeutically relevant adaptive immune response.[79,80]

Three TLR agonists are currently licensed by the US Food and Drug Administration and equivalent regulatory agencies for use in cancer patients. First, the so-called bacillus Calmette-Guérin (BCG): an attenuated variant of *Mycobacterium bovis* originally conceived as an anti-tuberculosis vaccine that is nowadays approved as a standalone immunotherapeutic intervention in patients with non-invasive transitional cell carcinoma of the bladder.[89] BCG appears to operate as a mixed TLR2/TLR4 agonist.[90,91] Second, monophosphoryl lipid A (MPL): a derivative of *Salmonella minnesota* LPS that is currently employed as adjuvant in Cervarix®, a vaccine specific for HPV-16 and -18.[92,93] MPL resembles BCG in its ability to trigger both TLR2 and TLR4 signaling.[14] Third, imiquimod: an imidazoquinoline derivative and guanosine analog that is nowadays used for the topical therapy of actinic keratosis, superficial basal cell carcinoma and external genital/perianal warts (*condylomata acuminata*).[89] At odds with BCG and MPL, imiquimod (formerly known as R837) mostly operates through TLR7.[94-96] This said, Aldara® (imiquimod 5% cream as commercialized by 3M Pharmaceuticals) has been shown to mediate TLR7-independent immunostimulatory effects, perhaps owing to the pro-inflammatory activity of isostearic acid.[97,98] Of note, picibanil – a lyophilized preparation of *Streptococcus pyogenes* that activates TLR2 and TLR4 – is not licensed by the US FDA but has been approved for use in cancer patients by the Japanese Ministry of Health and Welfare as early as in 1975.[57,99]

Along the lines of our monthly Trial Watch series,[100,101] here we summarize recent key discoveries on the biological activity of TLRs and discuss the latest developments on the use of natural and synthetic TLR agonists as therapeutic agents in cancer patients.

Literature Update

To the best of our knowledge, the results of no more than 6 studies assessing the clinical activity of TLR agonists in oncological indications have been published in the peer-reviewed scientific literature since the submission of our latest Trial Watch dealing with this topic (May 2013)[102] (source http://www.ncbi.nlm.nih.gov/pubmed).

Belani and colleagues investigated the ability of agatolimod (CpG-7909, PF-3512676, Promune®), an unmethylated CpG oligodeoxynucleotide that activates TLR9,[103] to improve the therapeutic profile of the FDA-approved epidermal growth factor receptor (EGFR) inhibitor erlotinib[104] in patients with

advanced recurrent EGFR+ non-small cell lung carcinoma (NSCLC). In this Phase II clinical trial, patients were randomized 1:1 to receive 150 mg erlotinib per day, alone or combined with subcutaneous agatolimod (0.20 mg/kg once weekly). The study was terminated upon the enrollment of 43 patients as an unplanned interim analysis suggested that agatolimod was unlikely to significantly ameliorate the therapeutic activity of erlotinib. Median progression free-survival (PFS) in patients receiving erlotinib alone or erlotinib plus agatolimod was 1.7 and 1.6 mo, respectively (HR, 1.00; 95% CI, 0.5–2.0; P = 0.9335). The incidence of Grade 3–4 toxicities was similar in both study arms, the most adverse events being diarrhea, fatigue, decreased appetite and rash. Thus, agatolimod appears to be unable to improve PFS among EGFR+ NSCLC patients treated with erlotinib.[105]

Witzig and collaborators tested whether agatolimod would potentiate the therapeutic profile of ^{111}In-ibritumomab tiuxetan and ^{90}Y-ibritumomab tiuxetan (two FDA-approved, radionuclide-conjugated monoclonal antibodies targeting CD20),[106,107] in patients with relapsed B-cell non-Hodgkin lymphoma (NHL). Thirty patients affected by relapsing, biopsy-proven CD20+ B-cell NHL and eligible were enrolled. These subjects received 250 mg/m^2 rituximab (an FDA-approved, naked monoclonal antibody specific for CD20)[108,109] on days 1, 8 and 15; ^{111}In-ibritumomab tiuxetan on days 1 and 8; agatolimod (0.08, 0.16, 0.32 or 0.48 mg/kg) on days 6, 13, 20, 27; and ^{90}Y-ibritumomab tiuxetan on day 15, all as intravenous injections. No dose-limiting toxicity was associated with the administration of agatolimod. Moreover, the authors observed an overall response rate (ORR) of 93% (28/30), including 63% (19/30) complete remissions, a median PFS of 42.7 mo and a median duration of response of 35 mo.[110] These encouraging results warrant further investigation in large, randomized clinical studies.

Kruit and coworkers compared the immunostimulatory activity of two distinct preparations that trigger TLR signaling, namely AS02B and AS15, in 75 Stage III or IV M1a melanoma patients vaccinated with full-length melanoma antigen family A3 (MAGEA3). AS02B includes MPL, QS-21 (a water soluble saponin extracted from the South American tree *Quillaja saponaria* Molina),[111] and SB62 (a commercial oil-in-water emulsion), hence activating TLR2 and TLR4.[112-114] Conversely, AS15 contains MPL, QS-21 and agatolimod, thus operating as a mixed TLR2/TLR4/TLR9 agonist.[11,102] The co-administration of recombinant MAGEA3 with AS02B and AS15 was equally well tolerated. However, the use of AS15 was associated with 4 objective responses, a 6-mo PFS rate of 25%, and a median overall survival (OS) of 33 mo, while that of AS02B resulted in 1 objective response, a 6-mo PFS rate of 14% and an OS of 19.9 mo. All patients developed anti-MAGEA3 antibodies, yet their levels were 3-fold higher in the AS15 arm, corroborating the superior immunostimulatory and clinical activity of this preparation.[115,116] Interestingly, the authors also identified a 84-gene signature associated with clinical benefit among AS15-treated and less so AS02B-treated patients. The same signature, including several immunologically relevant genes (e.g., IFNγ-related genes, chemokine-coding genes), turned out to predict the likelihood of resected NSCLC patients to respond to full-length MAGEA3 plus AS15.[117]

Hartmann et al. conducted a prospective open-label Phase II trial to test the therapeutic profile of Hiltonol™, a particular formulation of polyriboinosinic polyribocytidylic acid (polyI:C, also known as Ampligen™) that includes carboxymethylcellulose and poly-L-lysine as stabilizing agents,[118,119] in pediatric patients with newly diagnosed or recurrent brain tumors. In this setting, 47 children affected by variety of brain neoplasms were treated with Hiltonol™ as a standalone therapeutic intervention. The authors observed no dose-limiting toxicities. Moreover, 3 out of 12 patients with progressive high-grade glioma manifested an objective response to treatment, while 2 out of 4 patient affected by progressive low-grade glioma (LGG) experienced disease stabilization for 18–24 mo. These results prompted the authors to initiate a second study focusing on LGG patients. In this follow-up Phase II clinical trial, 5 out of 10 patients responded to treatment, 2 of which exhibiting stable disease for over 18 mo.[120] These data indicate that Hiltonol™ may constitute a promising therapeutic option for pediatric LGG patients.

Dhodapkar and colleagues tested the safety, immunogenicity and clinical activity of CDX-1401, a DC-based vaccine targeting the tumor-associated antigen NY-ESO-1,[121,122] adjuvanted with Hiltonol™ and resiquimod, an imiquimod-like molecule formerly called R848 that operates as a mixed TLR7/TLR8 agonist.[96,123-125] This Phase I clinical trial enrolled a total of 45 patients with advanced malignancies refractory to available therapies. CDX-1401 promoted NY-ESO-1-specific immune responses in all patients bearing NY-ESO-1+ tumors,[126] and the treatment was not associated with dose-limiting or Grade 3–4 toxicities. Moreover, 13 patients experienced disease stabilization (median duration: 6.7 mo) and 2 subjects had objective tumor regression (shrinkage of the target lesion of approximately 20%). A similar response was achieved by 6 out of 8 patients who also received immune checkpoint-blocking antibodies[127] within 3 mo after vaccination. These results suggest that Hiltonol™ and resiquimod may adequately support the immunogenic potential of vaccines that target DCs in vivo, such as CDX-1401.

Paleja and collaborators investigated the expression levels of various TLRs on the peripheral blood lymphocytes of oral cancer patients, as well as the ability of these cells to respond to various TLR ligands. The TLR expression profile was altered in oral cancer patients as compared with healthy individuals. In particular, unconventional T-cell subsets including γδ T cells and natural killer (NK) T cells were found to express abnormally high levels of several TLRs. Moreover, the peripheral blood lymphocytes of oral cancer patients failed to respond normally to a panel of TLR agonists in terms of (1) TLR signaling, (2) proliferative burst, (3) IFNγ secretion, (4) upregulation of activation markers (e.g., CD25 and CD69), and (5) cytotoxic activity.[128] These findings suggest that a deregulation of the

TLR system may at least in part contribute to the systemic immunosuppression that often characterizes malignant conditions.

A large body of preclinical literature on the use of TLR agonists as immunostimulatory agents against cancer has been published during the last 13 mo (source http://www.ncbi.nlm.nih.gov/pubmed). Among all these studies, we have found of particular interest the work of (1) Newton and collaborators, who demonstrated that a monoclonal antibody specific for IL-10 receptor α (IL10Rα) consistently improves the immunostimulatory and therapeutic potential of BCG, most likely as it supports BCG-induced T_H1 responses;[129,130] (2) Perret and colleagues, who reported that CpG oligodeoxynucleotides and Ampligen, but not imiquimod and Quil-A® (a saponin-type adjuvant),[131,132] are able to promote the accumulation of effector, rather than regulatory, T cells upon the subcutaneous administration of peptide-based anticancer vaccines, hence stimulating a therapeutically relevant T_H1 immune response against established neoplasms;[133] (3) Ali and coworkers, who not only identified the combination of granulocyte macrophage colony-stimulating factor (GM-CSF) and CpG oligodeoxynucleotides or Ampligen as the most efficient adjuvant for polymeric anticancer vaccines (in both prophylactic and therapeutic settings), but also elucidated the critical role played by basic leucine zipper transcription factor, ATF-like 3 (BATF3)-dependent DCs in this scenario;[134] (4) Yamazaki et al., who found that a chemically defined TLR4 agonist, dendrophilin, can be used to restore the immunogenic potential of tumors lacking the endogenous TLR4 ligand high mobility group box 1 (HMGB1);[135] (5) Tai and colleagues, who proved that the perioperative administration of Ampligen™ can limit the loss of NK cell functions that is generally associated with tumor resection, hence exerting a robust anti-metastatic effect;[136] and (6) Huang and collaborators, who showed that imiquimod can promote aerobic glycolysis in cancer cells in a TLR7- and TLR8-independent fashion, a protective response centered around the upregulation of hypoxia-inducible factor 1α (HIF1α).[137] In this setting, imiquimod and multiple pharmacological interventions that inhibit the glycolytic flux, including 2-deoxyglucose and 17-*N*-allylamino-17-demethoxygeldanamycin,[138] were found to exert synergistic antitumor effects, in vitro and in vivo.[137] These data indicate that the therapeutic activity of imiquimod may involve a cancer cell-intrinsic component.

Update on Ongoing Clinical Trials

When this Trial Watch was being prepared (May 2014), official sources listed no less than 14 clinical trials launched after May 1st, 2013 that would assess the immunostimulatory and therapeutic profile of TLR agonists in cancer patients (source http://www.clinicaltrials.gov). Seven of these studies involve FDA-approved molecules, i.e., imiquimod (6 trials) and BCG (1 trial), while the other 7 aim at investigating the safety and efficacy of hitherto experimental TLR ligands, i.e., Hiltonol™ (5 trials), the DNA-based double stem-loop immunomodulator[139] MGN1703 (1 trial), and glucopyranosyl lipid adjuvant (GLA), a synthetic TLR4 agonist that – when formulated as a stable emulsion – promotes potent poly-functional T_H1 responses upon intradermal administration[140-142] (**Table 1**).

In particular, imiquimod is being tested (1) as a standalone topical intervention, in HIV^+ patients with high-grade anal squamous skin lesions (NCT02059499); (2) in combination with ProCervix, a vaccine targeting HPV-16 and -18 (source http://www.genticel.com/products/procervix/),[143-145] in HPV^+ women who have not yet developed high-grade cervical lesions (NCT01957878); (3) together with GM-CSF as adjuvant to a synthetic peptide-based vaccine (SL-701),[146] in subjects with recurrent glioblastoma multiforme (NCT02078648); (4) in combination with cyclophosphamide (an immunogenic alkylating agent)[147,148] and GM-CSF to support an autophagosome-based vaccine derived from allogeneic cancer cells,[149-151] administered to NSCLC patients (NCT01909752); and (5) as adjuvant to DCs loaded ex vivo with autologous cancer cell lysates, in children with high-grade brain tumors (NCT01902771). On a slightly different note, NCT01926496 has recently been initiated to prospectively evaluate the risk of subjects with actinic keratosis treated with Aldara® or ingenol mebutate (an FDA-approved substance commercialized as 0.015% or 0.05% cream under the trade name Picato®),[152-154] to develop squamous cell carcinoma (NCT01926496). In addition, BCG is currently being evaluated as a standalone therapeutic intervention or combined with a viral vector encoding mucin 1 (MUC1), carcinoembryonic antigen (CEA) and three immunostimulatory molecules, i.e., CD80 (also known as B7–1), CD58 (also known as LFA-3) and intercellular adhesion molecule 1 (ICAM1),[155-159] in adults with high-grade non-invasive bladder carcinoma who failed at least 1 course of BCG (NCT02015104).

The clinical profile of Hiltonol™ is currently being assessed (1) in subjects affected by solid tumors easily accessible by needle, receiving Hiltonol™ as a standalone therapeutic intervention i.t. (NCT01984892); (2) in melanoma patients, who receive Hiltonol™ to improve the immunogenicity of a personalized, neoantigen-targeting peptide-based vaccine (NCT01970358); (3) in individuals with glioblastoma, who are treated with Hiltonol™ as adjuvant to a multipeptide-based vaccine (IMA950)[160] given in combination with temozolomide-based radiochemotherapy[82,161] (NCT01920191); (4) in cutaneous T-cell lymphoma patients, receiving Hiltonol™ to boost the therapeutic potential of radiation therapy coupled to the histone deacetylase inhibitor romidepsin[162-165] (NCT02061449); and (5) in B-cell lymphoma patients, who are concurrently treated with recombinant human FLT3 ligand (FLT3L, also known as CDX-301)[166-168] i.t. plus low-dose radiation therapy in the attempt to recruit DCs to neoplastic lesions and thus promote clinically relevant anticancer immune responses (NCT01976585). MGN1703 is under investigation as a standalone agent for the maintenance of metastatic colorectal carcinoma patients experiencing objective responses in the course of induction chemotherapy (NCT02077868). Finally,

Table 1. Clinical trials recently launched to evaluate the safety and efficacy of TLR agonists in cancer patients.*

Agent	Indication(s)	Status	Phase	Route	Notes	Ref.
BCG	Bladder cancer	Recruiting	II	Intravesical	Combined with a CEA- and MUC1-targeting vaccine	NCT02015104
GLA	Merkel cell carcinoma	Recruiting	I/II	Intratumoral	As single agent	NCT02035657
Imiquimod	Actinic keratosis	Recruiting	IV	Topical	As single agent	NCT01926496
	Anal intraepithelial neoplasia	Not yet recruiting	III	Anal	As single agent	NCT02059499
	Glioblastoma	Not yet recruiting	I/II	Topical	Combined with GM-CSF and a synthetic peptide-based vaccine (SL-701)	NCT01957878
	Low-grade HPV^+ cervical lesions	Recruiting	II	Topical	Combined with a HPV-16- and HPV-18-targeting vaccine	NCT02078648
	NSCLC	Recruiting	II	Topical	Combined with GM-CSF, cyclophosphamide and an autophagosome-derived vaccine	NCT01909752
	Pediatric brain tumors	Recruiting	I	Topical	Combined with DCs loaded ex vivo with autologous cancer cell lysates	NCT01902771
Hiltonol™	B-cell lymphoma	Recruiting	I/II	Intratumoral	Combined with recombinant human FLT3L	NCT01976585
	Cutaneous T-cell lymphoma	Recruiting	I	Subcutaneous	Combined with radiation therapy and romidepsin	NCT02061449
	Glioblastoma	Recruiting	I/II	Intramuscular	Combined with multipeptide vaccine, radiation therapy and temozolomide	NCT01920191
	Melanoma	Recruiting	I	n.a.	Combined with a personalized peptide-based vaccine	NCT01970358
	Solid tumors	Recruiting	II	Intratumoral Intramuscular	As single agent	NCT01984892
MGN1703	Metastatic CRC	Not yet recruiting	III	n.a.	As single agent	NCT02077868

Abbreviations: BCG, bacillus Calmette-Guérin; CEA, carcinoembryonic antigen; CRC, colorectal carcinoma; DC, dendritic cell; GLA, glucopyranosyl lipid adjuvant; GM-CSF, granulocyte macrophage colony-stimulating factor; HPV, human papilloma virus; FLT3L, FLT3 ligand; n.a., not available; MUC1, mucin 1; NSCLC, non-small cell lung carcinoma. *Between 2013, May 1st and the date of submission.

GLA in stable emulsion is being tested as a standalone therapeutic intervention in biopsy-confirmed Merkel cell carcinoma patients who bear at least one injectable lesion (NCT02035657).

The following clinical studies discussed in our previous Trial Watches dealing with this topic[10,11,102] have changed status during the past 13 mo: NCT00003715 and NCT01808950, which are now listed as "Terminated"; NCT01264731, which nowadays appears as "Suspended"; NCT00694551 and NCT00899574, which are listed as "Active, not recruiting" but are associated with preliminary results; as well as NCT01410968, NCT00923910, and NCT01171469, which are reported as "Completed" (source http://www.clinicaltrials.gov).

The reasons for the termination of NCT00003715 and NCT01808950 are not specified, while the temporary suspension of NCT01264731 appears to relate to the study staff being on medical leave. NCT00694551 aimed at evaluating the clinical profile of a peptide-based vaccine adjuvanted with Hiltonol™ in prostate carcinoma patients. At present, safety data referring to n = 29 patients treated with three different doses of the vaccine have been released, demonstrating that this regimen is very well tolerated. Efficacy data on NCT00694551 have not been disclosed yet. NCT00899574 was intended to assess the efficacy of Aldara® in breast cancer patients with chest wall recurrence or skin metastases. No serious adverse effects were documented. Moreover, the authors report an ORR of 20% (95% CI: 3–56) and an amelioration of pain or pruritus in 9 out of 10 patients analyzed. To the best of our knowledge, the results of NCT01410968, NCT00923910, and NCT01171469 have not been released yet (source http://www.clinicaltrials.gov).

Concluding Remarks

In spite of their robust and well-characterized immunostimulatory potential, the attention attracted by currently available TLR agonists for use as therapeutic agents in oncological indications is stably decreasing. One year ago, in the latest Trial Watch dealing with TLR ligands,[102] we speculated that such a trend may reflect, at least in part, the limited availability of clinical-grade reagents. Several groups were indeed refocusing their efforts on alternative sources of TLR agonists, including commonly employed prophylactic

vaccines.[169,170] A few chemically defined TLR agonists have recently been shown to exert therapeutic effects in preclinical tumor models, including dendrophilin,[135] yet the clinical interest in these agents appears to remain low. It is therefore tempting to speculate that oncologists may now be prioritizing other, relatively more specific, immunostimulatory interventions, such as immune checkpoint blockers. Several molecules that block immunosuppressive receptors expressed on activated immune cells, including the cytotoxic T lymphocyte-assocaited protein 4 (CTLA4)-specific antibody ipilimumab[171-173] and the programmed cell death 1 (PDCD1)-targeting antibody nivolumab,[174] have been shown to significantly boost the immunostimulatory potential of TLR agonists.[175-177] In this scenario, it will be interesting to see not only whether TLR agonists and checkpoint-blocking antibodies can be combined in a safe and efficient manner in patients, but also whether the latter might one day replace the former as standalone adjuvants to active immunotherapeutic interventions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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