Introduction

Toll-like receptors (TLRs) are one of the families of pattern recognition receptors (PRRs) operating in the innate immunity, which also encompass RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs). PRRs share the ability to recognize relatively conserved microbial components, which are generally referred to as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs), as well as endogenous danger signals commonly known as damage-associated molecular patterns (DAMPs). Common TLR-activating MAMPs include viral and bacterial nucleic acids (which can signal through TLR3 or TLR9), flagellin (a TLR5 agonist), as well as lipopolysaccharide (LPS), lipoteichoic acid, and mannan (which signal through TLR2 or TLR4). Endogenous nucleic acids and the nuclear non-histone protein high mobility group box 1 (HGMB1) are prototypic TLR-activating DAMPs.

Toll was initially identified and characterized for its anti-fungal activity in Drosophila melanogaster, and TLRs are evolutionarily conserved from Caenorhabditis elegans through mammals. Thus far, 13 TLRs have been identified in mammals (TLR1–TLR13), 10 of which are encoded in the human genome (TLR1–TLR10). Notably, human TLR11 is a pseudogene, and human cells lack Tlr12 and Tlr13. TLRs are type I integral membrane glycoproteins characterized by an extracellular domain with a leucine-rich-repeat (LRR) motif and a cytoplasmic signaling domain, which is homologous to the interleukin 1 receptor (IL1R) and is classified as the Toll/IL-1R homology (TIR) domain. TLRs either reside in the plasma membrane (TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10) or in endosomal membranes (TLR3, TLR7, TLR8, and TLR9). As a family, TLRs are expressed by multiple immune cells, including macrophages, dendritic cells (DCs), B cells, and natural killer (NK) cells, as well as by non-immune cells including epithelial cells, fibroblasts and malignant cells. Thus, besides controlling the activation, maturation and immunological functions of immune cells (notably cytokine secretion), TLR signaling can influence tumor metabolism, proliferation and dissemination.

Several TLR ligands demonstrated potential therapeutic efficacy against malignant disorders. Imiquimod, also called Aldara® (imiquimod 5% cream as commercialized by 3M Pharmaceuticals) or R-837, is a TLR7 agonist that is approved by the US Food and Drug Administration (FDA) for the treatment of actinic keratosis, external genital/perianal warts (condylomata acuminata), and superficial basal cell carcinoma. The structure of imiquimod is similar to an attenuated form of M. tuberculosis (BCG), which is immunologically related to M. tuberculosis. It was initially used as a vaccine against tuberculosis, but it is now FDA-approved for the treatment of non-invasive transitional cell carcinoma of the bladder. Although the mechanism of action of BCG...
is not entirely understood, TLR2, TLR4, and TLR9 have all been implicated in the host response to this TLR agonist.93 Finally, monophosphoryl lipid A (MPL) is a derivative of Salmonella minnesota LPS that is employed as an adjuvant in Cervarix84,85—a prophylactic vaccine against human papillomavirus (HPV) type 16 and 18, which are strongly associated with cervical carcinoma.86 In this setting, MPL, which mostly signals through TLR2 and TLR4, forms part of so-called “Adjuvant System 04” (AS04), together with aluminum salt.14,87

Here, we discuss recent preclinical and clinical progress on the development of TLR agonists for cancer therapy.

Update on the development of TLR agonists for cancer therapy

Completed clinical studies

Since the publication of the latest Trial Watch dealing with this topic (September 2015),88 various clinical trials investigating the safety and therapeutic profile of TLR agonists in cancer patients have been completed. Only three of these studies, however, reported results to the National Library of Medicine accessible at https://clinicaltrials.gov/ or https://www.ncbi.nlm.nih.gov/pubmed/. The remaining studies have been presented during national or international meetings in the form of oral or written abstracts.

Levy and colleagues (Stanford University, Stanford, CA, USA) in collaboration with the National Cancer Institute (NCI) investigated the side effects and the dose-limiting toxicity of ipilimumab, an anti-CTLA-4 monoclonal antibody, in combination with the TLR9 agonist SD-10189 and radiation therapy90 in patients with recurrent low-grade B-cell lymphoma (NCT02254772). These results were first presented as a poster at the American Society of Clinical Oncology (ASCO) in 201591 and have been featured in several peer-reviewed journal articles.92,93 In this Phase II study, nine participants received intratumoral injections of 10 mg ipilimumab on day 2 of week 1 and 1 mg/week of SD-101 for up to 5 weeks. On days 1 and 2 of the study, participants underwent local radiation therapy. The safety and tolerability of the treatment was assessed over the course of 10 weeks. Tumor response to the treatment and lesion growth were evaluated over the course of 2 years. Of the nine participants included in the study, one experienced at least one serious adverse event (AE). All participants experienced other Grade 3 AEs including fatigue, fever, gastrointestinal disorders or chills. Of the nine participants, seven completed the study. Six out of the seven patients (85.7%) had progressive disease (PD), developing new lesions or significant increases to existing lesion sites. Only one participant had stable disease (SD). The results from this study suggest that combining the intratumoral administration of ipilimumab with SD-101 and radiation at these dose levels does not constitute a promising therapeutic option.

Novartis Pharmaceuticals (Arlington Heights, Illinois, USA) tested the safety, tolerability and efficacy of the investigational drug, LFX453, against placebo in treating the pre-cancerous HPV-induced external genital warts (EGWs) in circumcised men in parallel with an additional open label arm using Aldara94,95 (NCT02482428). In this Phase II study, 88 participants were randomized and separated across five treatment interventions. Of the two experimental arms, one received LFX453 0.1% nanomedicinal cream (NMC) and the other LFX453 0.15% liquid crystal cream (LCC). The placebo comparator arms received vehicle. Across these groups, treatment was applied twice daily for up to 12 weeks. Aldara96 was applied 3 times per week for a maximum of 16 weeks to participants in the final active comparator arm. The treatment efficacy in clearing warts was assessed for up to 14 weeks and the safety and tolerability were assessed for up to 30 weeks. Participants were also evaluated as to whether they had a clearance rate of at least 75% reduction in counts of EGWs by the end of treatment (EOT) at weeks 12 or 16. Between the groups that received NMC (n = 24), LCC (n = 22), combined vehicle (n = 20) and Aldara97 (n = 22), only one individual (4.16%) from the NMC group had complete clearance of disease at week 14. By week 30, 3 participants from the NMC group (12.5%), 5 from the LCC group (22.7%), 3 from the vehicle to NMC group (30%), 2 from the vehicle to LCC group (20%) and the 10 participants given Aldara98 (45.5%) experienced AEs only. Two subjects from the NMC group (8.3%), 1 from the LCC group (4.5%), none from the combined vehicle group and 3 from the Aldara group (13.6%) had a partial clearance rate of at least 75% reduction in EGWs. These results support the potential efficacy LFX453 NMC after further testing and refinement.88

Griffiths et al. (Roswell Park Cancer Institute, Buffalo, NY, USA) investigated the safety and therapeutic efficacy of treating myelodysplastic syndrome or acute myeloid leukemia with a DEC-205/NY-ESO-1 fusion protein (CDX-1401)96,97 with an adjuvant, Hiltonol99,100,101 with standard decitabine-based chemotherapy99 (NCT01834248). In this Phase I study, organized into 4 cycles of chemotherapy and 5 vaccinations, 9 participants receiving 20 mg/m²/day decitabine i.v. per 5 days were treated with s.c. and i.d. injections of CDX-1401 along with Hiltonol99,100 s.c. on days 14 and 15 of cycle 1 and day 15 on cycles 2–4. Treatment was repeated every 28 days for a total of 4 cycles as long as there was no disease progression or unacceptable toxicity. Any incidence of toxicity over the course of a 30-day period following the last dose of the study treatment was assessed according to the NCI Common Terminology Criteria for Adverse Events. Immune and molecular responses were monitored for up to 16 weeks. Patients were followed at days 30, 60, 90 and 180 to evaluate their response to the treatment. The most frequent AEs, which were attributed to chemotherapy or the underlying hematological malignancy, included cytopenia (Grade 3/4), elevated liver enzymes (Grade 3), fatigue (Grade 2), edema (Grade 2/3) and diarrhea (Grade 1/2). Patients also developed localized skin reactions to the vaccine. Only 7 participants completed the study, 2 of whom experienced SAEs. In 6 out of 7 (85.7%) and in 4 out of 7 (57.1%) patients, NY-ESO-1-specific CD4+ and CD8+ T-lymphocytes, respectively, could be documented. NY-ESO-1-expressing myeloid cells isolated from patients at different time points during chemotherapy were able to activate a cytotoxic response from autologous NY-ESO-1-specific lymphocytes. These data indicate that Hiltonol99-adjuvanted vaccinations against NY-ESO-1 can drive an antigen-specific immune response, highlighting the therapeutic potential of
antigen-specific immunotherapies combined with potent TLR agonists.  

The results of a large portion of the clinical studies completed between 2016 and 2018 have not been publically posted on https://clinicaltrials.gov/ and have not been published in the peer-reviewed literature. Most of these studies, however, have been presented at annual meetings, and only abstracts or preliminary results are publically available. Although momentarily this precludes obtaining robust insights into the progress and versatility of TLR agonists for cancer therapy, an introduction to the aims and designs of these studies enables understanding of the current and future directions of these immunotherapeutic agents.

GlaxoSmithKline (Berlin, Germany) investigated the safety, tolerability, pharmacokinetic and pharmacodynamic profile of GSK1795091, a TLR4 agonist, in 42 healthy subjects (NCT02798978). This three-armed randomized, double-blinded, placebo-controlled Phase I study was split into two parts. Part 1 assessed the safety of ascending single doses (starting at 7 ng) of GSK1795091 i.v., while Part 2 was a parallel evaluation of two cohorts that received the drug as in Part 1 but at different time points during the trial. In Part 1, participants were given either the drug or placebo on day 1 administered as an i.v. bolus for 2 to 5 min, followed by an i.v. bolus of 10 mL saline. In Part 2, cohort 1 received an i.v. injection of GSK1795091 on day 1 and a dose on day 8, one week later. Cohort 2 received an i.v. injection of the drug on day 1 and a second dose on day 15. The measures for determining the safety of the drug include the number of AEs, oral body temperature, blood pressure and respiratory rate assessed for up to 11 weeks. In assessing the pharmacokinetics and pharmacodynamics of GSK1795091, measures included the maximum observed drug concentration (C_{max}), the time occurrence of C_{max}, the terminal half-life, immune cell and plasma cytokine phenotype, the white blood cell count and the clearance of GSK1795091 in Parts 1 and 2 for up to 144 hours. These results will determine the future of clinical trials in which GSK1795091 will be administered together with immunomodulators in patients with cancer.

Fox et al. (LSU Stanley S. Scott Cancer Center, New Orleans, LA, USA) in collaboration with the NCI and Mayo Clinic hypothesized that a DRibble-based vaccine could induce an immune response against tumor-associated antigens (TAAAs) in patients afflicted with Stage III non-small cell lung carcinoma (NSCLC) and that cyclophosphamide plus a HPV vaccine combined with the Dribble-based vaccine alone, with the Dribble-based vaccine plus Aldara® or with the Dribble-based vaccine plus colony stimulating factor 2 (CSF2; best known as GM-CSF) could have therapeutic effects (NCT01909752). DRibbles are tumor cell-derived defective ribosomal products and short-lived proteins packed within autophagosomes. To each of the three arms of this randomized Phase II study, cyclophosphamide 300 mg/m² was administered as a single dose 3 days prior to vaccination along with a 0.5 mL i.m. injection of the HPV vaccine at the time of the first and third Dribble-based vaccinations. In the first arm of 12 participants, the Dribble-based vaccine was administered every three weeks for 43 weeks. The second arm received the same vaccine and, in addition, applied one 250 mg packet of Aldara (containing 12.5 mg of imiquimod) to a 4 × 5 cm outlined area of skin (including the vaccine site) daily starting at week 4 (with the second Dribble-based vaccine) and for four days following each vaccine cycle. The third arm received the Dribble-based vaccine and was administered GM-CSF 50 μg/day via a CADD-MSTM 3 Ambulatory infusion pump starting at week 4. The goals of the study were to determine which treatment combination elicited the greatest antibody response in a 95-day time frame, and to evaluate safety within a 43-week period, progression-free survival (PFS), and the correlation between PFS and immune responses within a 2-year period. Preliminary results showed that compared to vaccination alone or vaccination plus GM-CSF, vaccination plus imiquimod significantly increased the number of antibody responses. This study was presented at the 31st Annual Meeting and Associated Programs of the Society for Immunotherapy of Cancer (SITC) 2016.

In collaboration with Oncovir, Britten and colleagues (Medical University of South Carolina, Charleston, SC, USA) tested the safety and tolerability of Hiltonol® in combination with a DC-based vaccine to patients with locally advanced unresectable pancreatic ductal adenocarcinoma (NCT01677962). Alongside, investigators aimed at elucidating whether combining DCs with Hiltonol® could serve as a therapeutic vaccine against the disease. In this Phase I study, 12 participants received i.t. injections on day 0 and day 14, followed by standard of care procedures for the remainder of the study. AEs were monitored until day 56 (the last day of treatment). Peereboom and collaborators (Cleveland Clinic, Cleveland, OH, USA) investigated the therapeutic profile of SL-701, a peptide-based vaccine, adjuvanted with imiquimod plus GM-CSF (Stage 1), or with Hiltonol® and bevacizumab, an FDA-approved monoclonal antibody specific for vascular endothelial growth factor A (VEGFA) in Stage 2, in patients with relapsed/refractory HLA-A2+ glioblastoma (GBM) (NCT02078648). Primary objectives were safety, tolerability, investigator assessed objective response rate (ORR), and 12-month OS rate. Alongside, SL-701-specific CDB++ T-cell frequency was monitored by flow cytometry. At reporting, 74 patients were treated, most frequent AEs related to treatment being fatigue (22%) and injection site reaction (18%). Amongst 46 patients enrolled in Stage 1, 1 partial response (PR) and 15 instances of SD were documented. Amongst 28 patients, enrolled in Stage 2, 2 CRs, 4 PRs and 19 instances of SDs were seen. OS at 12 months was 43% in Stage 2 and 37% in Stage 1. These data suggest that adjuvanted SL-701, alone or combined with bevacizumab is well tolerated and mediates clinically relevant antitumor activity.

Additional studies completed between 2016 and 2018 include NCT01957878, NCT02266147 and NCT02404389. To the best of our knowledge, the results of these studies have not yet been communicated to the public.

Preclinical and translational advances

A considerable body of preclinical and translational findings on the use of TLR agonists for cancer therapy has been disseminated since the publication of the latest Trial Watch dealing with this topic (source https://www.ncbi.nlm.nih.gov/pubmed/). Among
these studies, we found of particular interest the work of: (1) Molgora and colleagues (Humanitas Clinical and Research Center, Rozzano, Italy), who demonstrated that interleukin-1 receptor 8 (IL1R8, also known as TIR8 or SIGIRR), which is known to impair the signal transduction cascades elicited by several TLRs as well as by various interleukin receptors, negatively regulates the activity of NK cells as an immunosuppressive checkpoint; (2) Lou and co-authors (Institute for Molecular Bioscience. The University of Queensland, Brisbane, Australia), who showed that SLP adaptor and CSK interacting membrane protein (SCIMP), a transmembrane adaptor protein, facilitates TLR4 signaling upon direct binding to its TIR domain; (3) Acharya and collaborators (Benaroya Research Institute, Seattle, Washington, USA), who reported a new regulatory circuitry of B cell activation that involves the integrin-dependent recruitment of microtubule-associated proteins 1 light chain 3 (MAP1LC3, best known as LC3) to TLR-containing endosomes; (4) Combes et al. (Aix Marseille Université, Marseille, France), who reported that lysosomal associated membrane protein family member 5 (LAMP5, also known as BAD-LAMP) negatively regulates TLR9-driven type I interferon (IFN) production in the tumor microenvironment by plasmacytoid DCs (pDC); (5) New and colleagues (University of Oxford, Oxford, United Kingdom), who demonstrated that the sensitivity of hematologic cancers to histone deacetylase (HDAC) inhibitors is influenced by myeloid differentiation 88 (MYD88), a transducer of TLR signaling; and (6) Zhang and collaborators (The University of Tokyo, Tokyo, Japan), who harnessed crystallography to identify two different ligand-binding sites that regulate TLR7, (7) Okazaki et al. (USC Norris Comprehensive Cancer Center, Los Angeles, CA, USA), who suggested that the TLR7 rs3853839 G/G variant may constitute a positive prognostic factor as it is associated with a PFS benefit for patients with metastatic colorectal carcinoma (CRC) treated with cetuximab-based chemotherapy; (8) Takeda et al. (Hokkaido University Graduate School of Medicine, Sapporo, Japan), who demonstrated that the combination of a TLR3 agonist (ARNAX) and a PD-L1-specific immune checkpoint blocker and a TAA-derived vaccine may be used to overcome resistance to PD-1-targeting therapies, at least in mice; (9) Caronni and colleagues (International Centre for Genetic Engineering and Biotechnology, Trieste, Italy), who found that lactic acid blocks the ability of DCs to produce type I IFN in response to TLR3 and STING agonists in a mouse model of lung cancer, confirming previous reports on the major influence of local metabolism on the immune functions of the tumor microenvironment; (10) Dooduijn and collaborators (Leiden University Medical Center Leiden, The Netherlands), who showed that TLR7/TLR8 agonism, compared to TLR3 and TLR9 agonism, drives an NK cell-dependent immune response that can eradicate tumors that have escaped immunosurveillance following MHC Class I downregulation, which is in line with data from other groups demonstrating the ability of TLR7 ligands to trigger NK cell-dependent tumor control; (11) Klein and co-authors (University Hospital Essen, Essen, Germany), who showed that mice lacking TLR3, TLR7 and TLR9 are able to reject syngeneic wild-type malignant cells upon the activation of a tumor-targeting immune response involving both CD4+ and CD8+ T lymphocytes, suggesting that endosomal TLRs may operate as part of immunological checkpoints, at least in some settings; (12) Rashedi et al. (University of Toronto, Toronto, Canada), who demonstrated that mesenchymal stromal cells recruit T<sub>REG</sub> cells upon TLR3 or TLR4 activation, as a consequence of Notch signaling modulation and Delta-like 1 (DL1) upregulation; (13) Hotz et al. (University of Fribourg, Fribourg, Switzerland), who demonstrated that the sequential administration of polyribosinosinic polyribocytidylic acid (poly I:C, a TLR3 agonist) and R848 (a TLR7 agonist) 24 hours apart activates both MYD88-dependent and -independent pathways that culminate with a DC-driven, NK cell, and CTL-dependent antitumor immune response; (14) Le Noci and collaborators (Università degli Studi di Milano, Milan, Italy), who reported that combining a nebulized anti-MDSC antibody (RB6-8C5) with aerosolized CpG oligodeoxynucleotides (which operate as TLR9 agonists) and polyI:C results in the downregulation of multiple immunosuppressive molecules, including interleukin 10 (IL10); ultimately resulting in the activation of tumor-infiltrating NK cells that mediate robust therapeutic responses against lung metastases from syngeneic melanoma; (15) Müller and colleagues (University of Oslo, Oslo, Norway), who found that interferon gamma (IFNG, best known as IFN-γ) synergizes with various TLR agonists to trigger nitric oxide and pro-inflammatory cytokine production by M1 macrophages, coupled with robust anticancer activity; (16) Camargo and co-workers (University of Campinas, São Paulo, Brazil), who showed that BCG and imiquimod suppress chemical bladder tumorigenesis, while decreasing markers of proliferation (Ki67) and increasing markers of cell death (TUNEL), autophagy and as well as (17) Kim et al. (University of Minnesota, Minneapolis, MN, USA), who found enhanced tumor-targeting CTL activity following administration of nanoparticles containing mixed TLR7/TLR8 agonists.

This large amount of preclinical and translation literature corroborates the notion that TLR agonism is a promising strategy for the development of combinatorial anticancer regimens based on the reactivation of immunosurveillance.

**Recently initiated clinical trials**

Since the submission of the latest Trial Watch dealing with this topic (September 2015), no less than 66 clinical studies involving the administration of TLR agonists to cancer patients have been initiated (source http://clinicaltrials.gov/). The majority of these trials involve the FDA-approved molecules imiquimod (17 studies) and BCG (11 studies), as well as the hitherto experimental TLR3 agonist Hiltonol®, a particular formulation of polyI:C that includes carboxymethylcellulose and poly-L-lysine as stabilizing agents, and the TLR9 agonist SD-101 (9 studies) (Table 1).

In particular, imiquimod is being tested (1) in combination with neoadjuvant sonidegib followed by surgery or imiquimod for the treatment of basal cell carcinoma (NCT03534947); (2) in combination with curative surgery as compared to surgery alone in patients with basal cell carcinoma (NCT02242929); (3) in combination with 5-fluorouracil for the treatment of squamous cell carcinoma (NCT03770406); (4) in combination with pembrolizumab, an FDA-approved immune checkpoint blocker specific for PD-1, in patients with melanoma (NCT03276832); (5) compared to 5-fluorouracil-based
chemotherapy or observation for the treatment of anal carcinoma (NCT02059499); (6) as a standalone immunotherapeutic agent or following large loop excision of the transformation zone (LEEP) in patients with cervical intraepithelial neoplasms (CINs) (NCT02917746); (7) in combination with a DNA-based vaccine, GX-188E (NCT03206138); (8) in combination with 5-fluorouracil for the treatment of patients with high-grade cervical intraepithelial neoplasia (NCT03196180); (9) in combination with conization of the uterine cervix based on loop electro surgical excision procedure (LEEP) as compared to LEEP alone for patients with CIN (NCT03233412); (10) as standalone therapy or in combination with a nonavalent HPV-specific vaccine for patients with CINs (NCT02864147); (11) as a single agent compared to LLETZ for patients with CINs (NCT02669459); (12) in combination with a DNA vaccine, VGX-3100,171–174 for patients with HPV-16 and/or HPV-18-related high grade squamous intraepithelial lesion (HSIL) of the vulva (NCT03180684); (13) in combination with doxycycline for the treatment of cutaneous T cell lymphoma (NCT03116659); (14) in combination with a peptide vaccine, iVAC-L-CLL01,175,176 and the immunomodulatory agent lenalidomide177–180 in patients with chronic lymphocytic lymphoma (NCT02802943); (15) combined with a DRibble-based vaccine181 and DC-activated cytokine-induced killer (DC/CIK) cells and GM-CSF in NSCLC patients (NCT03057340); (16) as adjuvant therapy for patients with anal HPV lesions (NCT03289260); and (17) in combination with DPV-001.

Table 1. Clinical trials recently started to investigate the therapeutic profile of TLR agonists in cancer patients.

| Molecule | Indication(s) | Phase | Status | Route | Notes |
|----------|---------------|-------|--------|-------|-------|
| Ampligen™ | Colorectal carcinoma | I | Recruiting | Intravenous | In combination with celecoxib and recombinant interferon-α2b for patients with metastatic disease to the liver | NCT03403634 |
| BCG | Bladder carcinoma | I | Recruiting | Intravesical | In combination with pembrolizumab | NCT02549833 |
| DUK-CPG-001 | Urological tumors | II | Recruiting | Intravesical | In combination with pembrolizumab | NCT02544880 |
| G100 | Melanoma | II | Recruiting | Intravesical In combination with cyclophosphamide, IL-2 and a melanoma-specific vaccine | NCT00477906 |
| Hiltonoll™ | Colorectal carcinoma | I | Recruiting | Intramuscular | In combination with pembrolizumab | NCT02834052 |
| Breast carcinoma | Breast cancer | I | Recruiting | Intramuscular | In combination with a peptide vaccine and pembrolizumab | NCT02826434 |
| Gynecological tumors | Gynecological tumors | I | Recruiting | Intramuscular | In combination with a CA 125 monoclonal antibody | NCT03162562 |
| Lung cancer | Lung cancer | I | Recruiting | Subcutaneous | In combination with pembrolizumab and chemotherapy | NCT03300817 |
| Solid tumors | Solid tumors | I | Recruiting | Subcutaneous In combination with pembrolizumab and chemotherapy | NCT02721043 |
| Multiple myeloma | Multiple myeloma | I | Recruiting | Subcutaneous In combination with pembrolizumab and chemotherapy | NCT02544880 |
| Acute myeloid leukemia | Acute myeloid leukemia | I | Recruiting | Subcutaneous In combination with pembrolizumab and chemotherapy | NCT03358719 |
| Glioma | Glioma | I | Recruiting | Intramuscular | Followed by radical prostatectomy | NCT03262103 |

(Continued)
another DRibble-based vaccine,\textsuperscript{107,182–184} in patients with advanced prostate carcinoma (NCT02234921).

BCG is being investigated in clinical settings: (1) in combination with rapamycin\textsuperscript{185} for bladder carcinoma (NCT02753309); (2) in combination with pembrolizumab\textsuperscript{186,187} for the treatment of bladder carcinoma (NCT02808143); (3) following re-resection as compared to BCG with no re-resection\textsuperscript{188} for the treatment of bladder carcinoma (NCT03266900); (4) in combination with durvalumab\textsuperscript{189–191} for the treatment of bladder carcinoma (NCT03317158); (5) in combination with atezolizumab\textsuperscript{192–194} for the treatment of bladder carcinoma (NCT02792192); (6) in combination with ALT-803, an IL-15 superagonist\textsuperscript{195} for the treatment of bladder carcinoma (NCT02138734); (7) in a multicenter trial of BCG in combination with ALT-803\textsuperscript{195,196} for treatment of BCG-unresponsive, high-risk, non-muscle invasive bladder carcinoma (NCT03022825); (8) as nivolumab or nivolumab/BMS-986205 (an investigational IDO1 inhibitor) alone or in combination with BCG\textsuperscript{197–199} for the treatment of BCG-unresponsive, high-risk, non-muscle invasive bladder carcinoma (NCT03519256); (9) different strains of BCG\textsuperscript{200,201} for the treatment of bladder carcinoma (NCT03091660); (10) in combination with pembrolizumab\textsuperscript{202,203} for the treatment of urological tumors (NCT03345134); and (11) in combination with cyclophosphamide, IL-2, and a melanoma-specific vaccine\textsuperscript{204} for the treatment of melanoma\textsuperscript{171} (NCT00477906).

Additionally, Hiltonol\textsuperscript{*} is being evaluated: (1) in combination with pembrolizumab in patients with CRC (NCT02834052); (2) in combination with PVX-410, a peptide vaccine,\textsuperscript{168,205,206} and pembrolizumab or durvalumab, a PD-L1-targeting immune checkpoint blocker,\textsuperscript{207} in patients with breast carcinoma (NCT02826434; NCT03362060); (3) in combination with oregovomab, a CA-125-targeting monoclonal antibody,\textsuperscript{208} for the treatment of recurrent, advanced ovarian carcinoma (NCT03162562); (4) in combination with CDX-1401, guadecitabine and atezolizumab, another PD-L1-targeting immune checkpoint blocker,\textsuperscript{209–211} in patients with recurrent ovarian, fallopian tube, or primary peritoneal cancer (NCT03206047); (5) combined with a peptide vaccine specific for mucin 1 (MUC1) for the prevention of lung cancer in current and former smokers who are at high risk (NCT03300817); (6) in combination with a neoantigen vaccine, NEO-PV-01,\textsuperscript{131,212} pembrolizumab and chemotherapy for patients with lung cancer (NCT03380871); (7) in combination with PGV001, a peptide vaccine,\textsuperscript{213} for the treatment of non-hematologic malignancies in the adjuvant setting (NCT02721043); (8) in

### Table 1. (Continued).

| Molecular weight | Indication(s) | Phase | Status | Route | Notes | Ref. |
|------------------|--------------|-------|--------|-------|-------|------|
| **Imiquimod**    | Basal cell carcinoma | II | Not yet recruiting | Topical | In combination with a neoantigen vaccine for surgery | NCT03345134 |
|                  | III | Active, not recruiting | Topical | Combined with surgery | NCT02721043 |
| **Squamous cell carcinoma** | II | Not yet recruiting | Topical | In combination with pembrolizumab | NCT02242929 |
| **Melanoma**     | II | Recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
|                  | III | Recruiting | Topical | Compared to pembrolizumab or observation | NCT02721043 |
| **Anal carcinoma** | III | Recruiting | Topical | In combination with a DNA-based vaccine | NCT02721043 |
|                  | III | Recruiting | Topical | In combination with sunitinib and observation | NCT02721043 |
| **Cervical intraepithelial lesions** | n.a | Recruiting | Topical | Alone or upon tumor resection | NCT02721043 |
|                  | I  | Not yet recruiting | Topical | In combination with Pembrolizumab | NCT02721043 |
| **Genital warts** | II | Recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
| **Cutaneous T cell lymphoma** | II | Recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
| **Chronic lymphocytic lymphoma** | II | Recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
|                  | III | Recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
| **NSCLC**        | III | Not yet recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
| **Prostate carcinoma** | I | Recruiting | Topical | Combined with pembrolizumab | NCT02721043 |
| **Motolimod**    | Solid tumors | I | Recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
|                  | II | Recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
|                  | III | Recruiting | Topical | In combination with pembrolizumab | NCT02721043 |
| **SD-101**       | Lymphoma | I | Not yet recruiting | Intratumoral | In combination with pembrolizumab | NCT02721043 |
|                  | I  | Terminated | Intratumoral | In combination with pembrolizumab | NCT02721043 |
|                  | I  | Terminated | Intratumoral | In combination with pembrolizumab | NCT02721043 |
| **Advanced malignancies** | I/Ib | Terminated | Intratumoral | In combination with pembrolizumab | NCT02721043 |
| **Solid tumors** | II/Ib | Recruiting | Intratumoral | In combination with pembrolizumab | NCT02721043 |
| **Follicular lymphoma** | II/Ib | Recruiting | Intratumoral | In combination with pembrolizumab | NCT02721043 |
| **Advanced solid tumors & lymphoma** | II/Ib | Recruiting | Intratumoral | In combination with pembrolizumab | NCT02721043 |
| **Prostate carcinoma** | II | Suspected | Intratumoral | In combination with pembrolizumab and RT | NCT02721043 |

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\textsuperscript{107,182–184} in patients with advanced prostate carcinoma (NCT02234921).
combination with bevacizumab and a peptide vaccine for patients with recurrent glioblastoma (NCT02754362); (9) in combination with NEO-PV-01 and nivolumab, another PD-1-specific immune checkpoint blocker, in patients with melanoma, lung cancer, and bladder cancer (NCT02897765); (10) in combination with durvalumab and tremelimumab, a CTLA-4-targeting immune checkpoint blocker, for the treatment of advanced, measurable, biopsy-accessible cancers (NCT02643303); (11) in conjunction with a combination of the following agents- NEO-PV-01, APX005M, ipilimumab, and nivolumab for the treatment of advanced melanoma (NCT03597282); (12) in combination with MUC1- and influenza-specific vaccines along with taladafil, an inhibitor of phosphodiesterase 5 (PDE5) in patients with head and neck squamous cell carcinoma (NCT02544480); (13) in combination with CDX-1401 and pembrolizumab in patients with previously treated, advanced solid tumor (NCT02661100); (14) in combination with a peptide vaccine, GL-0817 and cyclophosphamide to prevent the recurrence of squamous cell carcinoma of the oral cavity (NCT02873819); (15) in combination with PVX-41 and durvalumab with or without lenalidomide in patients with multiple myeloma (NCT02886065); (16) in combination with DEC-205/NEW-ESO-1 fusion protein CDX-1401, decitabine, and nivolumab for the treatment of myelodysplastic syndrome or acute myeloid leukemia (NCT03358719); (17) in combination with GBM6-AD, a cancer cell-based vaccine, in patients with Grade II glioma (NCT02549833); (18) in combination with varilumab and a peptide vaccine, IMAl950 in patients with low-grade glioma (NCT02924038); (19) in combination with H3.3K27M, a peptide vaccine, in children with newly diagnosed diffuse intrinsic pontine glioma and other newly diagnosed HLA-A2+ H3.3K27M positive gliomas (NCT02960230); and (20) as standalone therapy for prostate carcinoma in the neoadjuvant setting (NCT03262103).

Furthermore, SD-101 is being studied in clinical trials: (1) in combination with an anti-OX40 antibody, BMS-986178, and RT for the treatment of lymphoma (NCT03410901); (2) in combination with RT after allogeneic hematopoietic cell transplant for the treatment of lymphoma (NCT01745354); (3) in combination with an anti-IL-10 agent for the treatment of advanced malignancies (NCT02731742); (4) in combination with pembrolizumab for the treatment of solid tumors (NCT02521870); (5) in combination with ibrutinib and RT for the treatment of follicular lymphoma (NCT02927964); (6) in combination with epacadostat, an inhibitor of indoleamine 2,3-dioxygenase-1, and RT for the treatment of advanced solid tumors and lymphoma (NCT03232384); (7) in combination with ipilimumab and RT for the treatment of lymphoma (NCT02254772); (8) in combination with RT and pembrolizumab for the treatment of prostate carcinoma (NCT03007732).

The status of the following clinical trials discussed in our previous Trial Watches dealing with TLR agonists, has changed during the last 35 months: NCT02501473, NCT02320305, NCT02180698, NCT02134925, NCT02149225, NCT02281682, NCT02454634, NCT02242929, NCT02293707, NCT02015104, NCT02035657, NCT01926496, NCT02061449, NCT01970358, and NCT02077868, which are now listed as “Active, not recruiting”; NCT02432378, NCT02427581, NCT02334735, NCT02394132, NCT02431559, NCT02521870, NCT02452697 and NCT02059499, which are currently listed as “Recruiting participants”; NCT02385188, which is listed as “Enrolling by invitation”; NCT02326168, NCT02333474, NCT02404389, NCT02482428, NCT02254772, NCT02266147, NCT01957878, NCT02078648, NCT01909752, and NCT01 920191, which are listed as “Completed”; NCT02202044, NCT02510950, NCT02413827, NCT02332889, NCT02329171, NCT01907271, and NCT01984892, which have been “Terminated”; as well as NCT02495636, which has been “Withdrawn” (source http://clinicaltrials.gov).

Concluding remarks

During the last 35 months (September 2015 – August 2018), more than 60 clinical trials have been initiated to investigate the potential therapeutic efficacy of TLR agonists in patients with cancer (Table 1). These metrics are notable for a decrease in the rate of initiation of clinical trials testing TLR agonists as immunotherapeutics for cancer as compared to the previous 15 months (May 2014 – August 2015), during which approximately 50 clinical trials were initiated. Although it is difficult to attribute such a decrease to one or more specific factors, we suspect that the extraordinary clinical achievement of other immunotherapeutics including immune checkpoint blockers and CAR-expressing T cells may have caused some refocusing in the attention of oncologists and pharmaceutical companies to investigate the ongoing role of TLR agonists. Currently available preclinical and clinical data strongly suggest that successful anticancer immunotherapy in a large fraction of patients requires combinatorial approaches. In this setting, TLR agonists present an opportunity to boost the immune response in patients in an effort to contribute to better clinical outcomes.

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Abbreviations

AE adverse event
AS04 Adjuvant System 04
ASCO American Society of Clinical Oncology
BCG bacillus Calmette-Guérin
CIN cervical intraepithelial neoplasm
cRC colorectal carcinoma
CTL cytotoxic T-lymphocyte
DAMP damage-associated molecular pattern
DC dendritic cell
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