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TRIAL WATCH

Trial watch: Toll-like receptor ligands in cancer therapy

Julie Le Naour[a,b] and Guido Kroemer[a,c]

[a]Centre de Recherche Des Cordeliers, Equipe Labellisée Par la Ligue Contre le Cancer, Université de Paris Cité, Sorbonne Université, Inserm U1138, Institut Universitaire de France, Paris, France; [b]Metabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France; [c]Institut du Cancer Paris CARPEM, Department of Biology, Hôpital Européen Georges Pompidou, AP-HP, Paris, France

ABSTRACT

Accumulating evidence indicates that Toll-like receptor (TLR) agonists proficiently (re)instore cancer immunosurveillance as immunological adjuvants. So far, three TLR agonists have been approved by regulatory agencies for use in oncological applications. Additionally, these immunotherapeutics have been extensively investigated over the past few years. Multiple clinical trials are currently evaluating the combination of TLR agonists with chemotherapy, radiotherapy, or different immunotherapies. Moreover, antibodies targeting tumor-enriched surface proteins that have been conjugated to TLR agonists are being developed to stimulate anticancer immune responses specifically within the tumor microenvironment. Solid preclinical and translational results support the favorable immune-activating effects of TLR agonists. Here, we summarize recent preclinical and clinical advances in the development of TLR agonists for anticancer immunotherapy.

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Introduction

Toll-like receptors (TLRs) are highly conserved throughout evolution from plants to mammals.[1,2] Among the 13 TLRs that have been characterized in mammals, the human genome encodes 10. These gatekeepers of the innate immune system can be found either in endosomes (TLR3, TLR7, TLR8, and TLR9) or at the plasma-membrane (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10), correlating with their function.

Most TLRs bind to conserved motifs of microbes that are referred to as microbe-associated molecular patterns (MAMPs). In addition, TLRs interact with host-derived molecules, which are commonly referred to as danger-associated molecular patterns (DAMPs)[3,4] Both MAMPs and DAMPs ultimately trigger the induction of inflammatory cytokines essential for the initiation of an innate immune response.[5] In line with this notion, *Bacillus Calmette–Guérin*, an attenuated variant of *Mycobacterium bovis*, is currently approved by the United States Food and Drug Administration (FDA) and other government agencies as a standalone therapeutic intervention for the treatment of noninvasive transitional cell carcinomas of the bladder.[6,7]

Three more specific TLR agonists are approved in some countries for oncological indications: (1) imiquimod, an imidazoquinoline derivative is used topically for the treatment of superficial basal cell carcinoma, actinic keratosis, and external genital/perianal warts (Condylomata acuminata); (2) monophosphoryl lipid A, a derivative of *Salmonella minnesota* lipopolysaccharide is used as immunological adjuvant in a prophylactic peptide-based vaccine for human papillomavirus (HPV)-16$^+$ or HPV-18$^+$ cervical carcinoma;[8] and (3) picibanil, a lyophilized preparation of *Streptococcus pyogenes*, is used in Japan for the treatment of various carcinomas in combination with chemotherapy.

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

Recently initiated clinical trials

Since the publication of the last Trial Watches dealing with TLR agonists (September 2018),[9] TLR7/8 agonists (July 2020)[10] and intratumoral (*i.t.*) TLR-targeting therapies (October 2021),[11] 22 clinical trials (source http://clinicaltrials.gov/) involving the use of TLR agonists as cancer therapeutics have been initiated (Table 1).

TLR1/2 agonist

XS15

XS15 is a water-soluble synthetic tripalmitoyl-S-glycerylcysteine (Pam_3Cys)-derivative and a mimetic of bacterial lipopeptide, which is an agonist of TLR1/2.[12] The trial NCT04688385 (phase I) will evaluate the safety, toxicity, and efficacy of a personalized multi-peptide vaccine in combination with the TLR1/2 ligand XS15 in chronic lymphocytic leukemia (CLL) patients that achieved at least a partial remission with detectable minimal residual disease (MRD) after at least 6 and less than 9 months of an ibrutinib-based treatment regime.[13] Of note, MRD positivity is defined by flow cytometry as more than 10^4 CLL cells in bone marrow or peripheral blood. As patients frequently experience disease relapse due to the persistence of MRD, peptide vaccination will be administered following ibrutinib monotherapy to target residual CLL cells.[12–14]

CONTACT Julie Le Naour julie.lenaour@gmail.com Centre de Recherche Des Cordeliers, Equipe Labellisée Par la Ligue Contre le Cancer, Université de Paris Cité, Sorbonne Université, Inserm U1138, Institut Universitaire de France, Paris, France

Table 1. Clinical trials testing TLR agonists in oncological indications.

Target	Molecule	Indication	Status	Co-therapy	Phase	NCT Number
TLR3	Ampligen	Ovarian cancer	Recruiting	Pembrolizumab, cisplatin	I/II	NCT03734692
TLR3	Ampligen	Breast cancer	Suspended	Celecoxib, cyclophosphamide, doxorubicin, doxorubicin hydrochloride, paclitaxel, rIFN-α-2b	I	NCT04081389
TLR3	BO-112	Melanoma	Active, not recruiting	Pembrolizumab	II	NCT04570332
TLR3	Poly-ICLC	Advanced solid tumor	Recruiting	Anti-PD-1	I	NCT04116320
TLR3	Poly-ICLC	Melanoma	Recruiting	6MHP, neoAg-mBRAF, CDX-1140	I/II	NCT04364230
TLR7	NJH395	Non-breast $HER2^+$ malignancies	Completed		I	NCT03696771
TLR7	SHR2150	Solid tumor	Unknown status	Anti-cancer agent	I/II	NCT04588324
TLR7/8	BDB001	Solid tumor	Active, not recruiting		II	NCT04819373
TLR7/8	BDB001	Solid tumor	Active, not recruiting	Atezolizumab	I	NCT04196530
TLR7/8	BDB018	Solid tumor	Recruiting	Pembrolizumab	I	NCT04840394
TLR8	SBT6050	$HER2^+$ solid tumor	Active, not recruiting	Pembrolizumab, cemiplimab	I	NCT04460456
TLR8	SBT6050	$HER2^+$ solid tumor	Terminated	Trastuzumab deruxtecan, tucatinib, trastuzumab, capecitabine	I/II	NCT05091528
TLR8	SBT6290	Advanced solid tumor	Withdrawn	Pembrolizumab	I/II	NCT05234606
TLR9	CAS3/SS3	Lymphoma	Recruiting	Radiation therapy	I	NCT04995536
TLR9	Imiquimod	Oral cancer	Recruiting		Early I	NCT04883645
TLR9	SD-101	Metastatic melanoma	Recruiting	Nivolumab, ilpilimumab	I	NCT04935229
TLR9	SD-101	Hepatocellular carcinoma and intrahepatic cholangiocarcinoma	Recruiting	Pembrolizumab, nivolumab, ipilimumab	I/II	NCT05220722
TLR9	SD-101	Advanced pancreatic adenocarcinoma	Recruiting	Pembrolizumab	I	NCT05607953
TLR9	TAC-001	Advanced solid tumor	Recruiting		I/II	NCT05399654
TLR9	Tilsotolimod	Malignant melanoma	Recruiting		II	NCT04126876
TLR9	Vidutolimod	Chronic lymphocytic leukemia	Recruiting	Multipeptide vaccine, XS15	I	NCT04688385
TLR9	Vidutolimod	Prostate cancer	Not yet recruiting	Nivolumab	II	NCT05445609

Abbreviations: CAS3/SS3, CpG-STAT3 siRNA CAS3/SS3; HER2, human epidermal growth factor receptor-2; HNSCC, head and neck squamous cell carcinoma; HR, hormone receptor; rIFN, recombinant interferon; mBRAF, mutated v-raf murine sarcoma viral oncogene homolog B; neoAg, neo-antigen; NSCLC, nonsmall cell lung cancer; poly-ICLC, polyinosinic-polycytidylic acid stabilized with poly-L-lysine carboxymethyl cellulose; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; TN, triple negative; 6MHP, 6 melanoma-associated peptides to stimulate helper T cells.

TLR3 agonists

TLR3 binds to double stranded ribonucleic acid (RNA) which can be synthetically designed to mimic viral infection and therefore elicit the secretion of type I interferon (IFN-I) and pro-inflammatory cytokines.[15] Over the past few years, numerous studies have demonstrated the efficacy of TLR3 agonists to reinforce tumor-specific immune responses in mice and patients, especially in combination with other therapeutic approaches.[16]

Ampligen

NCT03734692 (phase I/II) will evaluate the efficacy of a chemoimmunotherapy regimen based on intensive locoregional intraperitoneal (*i.p.*) cisplatin with *i.p.* ampligen and intravenous (*i.v.*) pembrolizumab for patients with recurrent platinum-sensitive ovarian cancer. Patients will receive six treatment cycles administered every 3 weeks (Q3W), followed by cytoreduction of residual tumor (usually, laparoscopically) around 4 weeks after the 4th treatment cycle as well as the two additional courses of initial chemo-immunotherapy regimen post-surgery. Recently, Radolec et al.[17] described preliminary results for this trial in an interim analysis. Among 17 patients enrolled at the date of publication, the combination has been well tolerated, with mostly grade 1 and grade 2 toxicities. Several patients are in remission with prolonged progression-free survival. NCT04081389 (phase I) aims to assess the efficacy of cytokine modulation therapy and standard chemotherapy before surgery in early-stage triple negative breast cancer patients. Patients receive the nonsteroidal anti-inflammatory drug celecoxib *per os* (*p.o.*) twice daily, *i.v.* recombinant IFN-α-2b over 20 min, and *i.v.* ampligen on d 1–3 of weeks 1–3, as well as *i.v.* paclitaxel over 1 h once weekly starting on d1. One to 3 weeks after the last dose of paclitaxel, patients receive *i.v.* doxorubicin and cyclophosphamide over 10 and 30 min, respectively. Treatment repeats for a total of 12 weeks or every 2 weeks for 4 cycles in the absence of disease progression or unacceptable toxicity. Of note, this trial was suspended to analyze data from nine included patients, recently published by Gandhi et al.[18] The treatment was well tolerated with mostly grade 1 or 2 treatment-related adverse events (TRAEs) without dose-limiting toxicities (DLTs) or delayed or immune-related toxicities. Grade 3 TRAEs included anemia (11%) attributed to cyclophosphamide, neutropenia (33%) attributed to chemokine-modulating regimen (11%) or paclitaxel (33%) or pneumonia (11%). Additional pneumonia and skin squamous cell carcinoma (SCC) *in situ* were observed, unrelated to the study treatment. 5/9 (56%) patients reached pathologic complete response and one additional patient attained microinvasive residual disease. Of note, expression of CD8α was selectively increased after treatment in tumor biopsies (five patients at dose levels 3 and 4) but decreased in blood.

Poly-ICLC

NCT04116320 (phase I) evaluates the safety of focused ultrasound ablation treatments with and without PD-1 blockade for 3 weeks and/or *i.t.* poly-ICLC (polyinosinic-polycytidylic acid, poly-IC, mixed with the stabilizers polylysine and carboxymethylcellulose, also known as Hiltonolol) in advanced solid tumors.[19] NCT04364230 (phase I/II) evaluates the safety of a peptide vaccine composed of six melanoma helper vaccines comprised 6 class II major histocompatibility complex-restricted helper peptides (6MHP) and a mutated neoantigen peptide (BRAF585-614-V600E, referred to as NeoAg-mBRAF) combined with local adjuvants including a CD40 antibody (CDX-1140) and poly-ICLC in melanoma patients. 6MHP and NeoAg-mBRAF will be co-administered subcutaneously (*s.c.*) or intradermally on d 1, 22, 43, and 64 with poly-ICLC and CDX-1140.

BO-112

BO-112 is a formulation of poly-IC complexed with polyethylenimine.[20] NCT04570332 (phase 2) aims to confirm the safety of *i.t.* BO-112 in combination with *i.v.* pembrolizumab and assess the efficacy of this combination for the treatment of patients with advanced and/or metastatic melanoma that have progressed on PD-1- blockade therapy. Pembrolizumab will be administered *i.v.* 3 times per week (Q3W) before BO-112, which will be injected *i.t.* in up to 8 tumor lesions once weekly for the first 7 weeks and then Q3W at a total dose of 1–2 mg at each administration. If the lesion(s) remain(s) detectable by ultrasound or palpation, additional *i.t.* BO-112 doses may be delivered, after which only pembrolizumab will be administered. As of August 14th, 2021, recruitment for the trial was completed. All 42 patients had confirmed disease progression prior to inclusion and 41 patients had stage IV AJCC8 disease. Seventy-four percent of the patients (n = 31) had cutaneous melanoma, 19% (n = 8) had acral melanoma, and 7% (n = 3) had mucosal melanoma. As of October 14th, 2021, the median exposure to treatment was 12 weeks and 30 patients remained on treatment.[21] The combinatorial regiment elicited an objective response rate of 27% (n = 11) among which 2 patients achieved a pathologic complete response by week 8. Moreover, 37.8% of the patients (n = 14) exhibited stable disease at week 8. Regarding safety, 88.1% of patients experienced at least one TRAE of any grade, with 36% of individuals experiencing a grade 3–5 adverse effect.[20,21]

TLR7 agonists

TLR7 is an endosomal innate immune sensor which induces the production of IFN-I as well as other inflammatory cytokines in response to single-stranded RNA.[22]

Imiquimod

In this line, imiquimod (also known as Aldara) received FDA approval as a topical standalone agent to activate TLR7 for the treatment of superficial basal cell carcinoma. NCT04883645 (early phase I) will assess local and systemic safety, tolerability, and efficacy of self-administration of topical imiquimod in neoadjuvant settings for patients with early-stage oral SCC as determined by best response rate (complete and partial responses). Pre- and post-treatment tumor samples will be analyzed by quantitative multiplex immunofluorescence to assess the immunomodulatory activity of imiquimod.

SHR2150

NCT04588324 (phase I/II) aims to evaluate the safety and efficacy of SHR2150, developed by Jiangsu Hengrui Medicine Co Ltd., administered orally Q3W in combination with *i.v.* chemotherapy plus *i.v.* anti-PD-1 (for programmed cell death protein 1) or anti-CD47 antibody Q3W in patients with unresectable/metastatic solid tumors. This study is a first-in-man, dose escalation/expansion study, designed to assess the safety, tolerability, recommended phase 2 dose (RP2D), and clinical efficacy of this regimen.

BDB001

NCT04819373 (phase II) evaluates the safety and efficacy of *i.v.* BDB001, developed by Seven and Eight Biopharmaceuticals Inc., as monotherapy in anti-PD-1 or -PD-L1 (for programmed death-ligand 1) refractory-advanced solid tumor patients. In this dynamic, NCT04196530 (phase I) assesses *i.v.* BDB001 in combination with atezolizumab in subjects with advanced solid tumors.[23,24] Preliminary results demonstrated that this therapeutic regimen was well tolerated in 41 patients with 17 different tumor types enrolled across 4 dose levels.[23] No DLTs were observed and common TRAEs were transient grade 1 or 2 including fatigue (31.7%), fever (26.8%) and chills/rigor (26.8%). Only three individuals experienced grade 3 TRAEs of nausea and fatigue. Among the 19 individuals at dose level 4, durable and deep clinical responses were observed in 3 (16%) responders which remained on treatment, with a duration of response ranging from 7.1 to 34.1 weeks. Overall, marked and durable clinical responses were observed in both PD-1 refractory and untreated individuals, supported by robust systemic immune activation including marked increases in plasmatic IFN-γ and IFN inducible protein-10 at BDB001 dose level 4.[23] Similarly, NCT03915678 (phase II) evaluates *i.v.* BDB001 in combination with a 1-h infusion of atezolizumab Q3W and radiotherapy in solid tumor patients. Of note, radiotherapy will start at least 7 d after the first dose of atezolizumab and at the latest before the second administration. All patients will be treated by high-dose irradiation in stereotactic conditions on non-injected metastasis: 3–5 doses from 27 to 60 Gy. At last, BDB001 will be administered over 30 min before atezolizumab, on d 1, 8, and 15 of cycles 1–3. Then, from cycle 4, BDB001 will be administered on d 1 Q3W.

BDB018

BDB018 is an analog of BDB001 designed to further enhance immune activation against cancer, while maintaining a favorable safety profile.[23,24] NCT04840394 (phase I) assesses *i.v.* BDB018 in monotherapy and in combination with *i.v.* pembrolizumab in patients with unresectable or metastatic solid tumors that have relapsed or are refractory to standard treatment or for which there is no approved therapy. This study will include a dose escalation phase with BDB018 alone or in combination with pembrolizumab and a dose expansion phase of BDB018 in combination with pembrolizumab.

NJH395

NJH395 is composed of an anti-human epidermal growth factor receptor-2 (HER2) monoclonal antibody (mAb) conjugated to a TLR7 agonist. NCT03696771 (phase I) will assess the efficacy of a single (part I) and eventually multiple doses (part II) of NJH395 in non-breast HER2$^+$ advanced malignancies. Data from 18 patients enrolled in the single ascending dose escalation (part I) demonstrated that NJH395 could efficiently deliver the TLR7 agonist moiety in HER2$^+$ cancer cells.[25,26] Additionally, NJH395 induced IFN-I responses, which correlated with immune modulation in the tumor microenvironment.[25,26] Although cytokine release syndrome was common, it remained manageable. On the other hand, antidrug antibodies and neuroinflammation at high doses represent significant clinical challenges.[25,26]

BDC-1001

BDC-1001, developed by Bolt Biotherapeutics, is a novel immune-stimulating antibody conjugate consisting of a trastuzumab biosimilar chemically conjugated to a TLR7/8 agonist with a non-cleavable linker. NCT04278144 (phase I/II) is a dose escalation of BDC-1001 as single agent and in combination with nivolumab in advanced HER2$^+$ solid tumors. As of January 29th, 2021, 20 patients with a median age of 65 y old (46–85) have been enrolled in four dose groups (0.15 mg/kg to 5 mg/kg).[27] BDC-1001 appears to be well tolerated up to the dose tested to date.[27] After four cycles partial response was confirmed in one patient with microsatellite stable (MSS) colorectal cancer (CRC) with lung metastases and remains on study. Additionally, three patients had stable disease, including two individuals with metastatic MSS CRC and one with heavily pre-treated MSS endometrial cancer with lung metastases and remained on treatment for more than 17 weeks.[27] Of note, three of these patients were previously treated with anti-HER2 therapies.[27]

TLR8 agonists

TLR8 is highly expressed in human myeloid cell populations such as conventional dendritic cells (DCs) and macrophages.[28] Therefore, activation of TLR8$^+$ myeloid cells in the tumor bed has emerged as a promising approach in overcoming immunotherapy resistance. In this dynamic, Silverback Therapeutics developed two mAbs which aim to activate immune cells in close proximity to tumor cells: SBT6050 (also known as pertuzumab zuvotolimod) and SBT6290. These molecules, which carry an analog of motolimod, a TLR8 agonist, were designed to specifically activate tumor-infiltrating TLR8$^+$ myeloid cells, thus avoiding acute toxicities due to peripheral activation of the targeted cell population.

SBT6050

SBT6050 is composed of a HER2-directed mAb conjugated to a TLR8 agonist.[29] NCT05091528 (phase I/II) is an open-label dose-escalation and expansion study of 21-d cycles of *s.c.* SBT6050 in combination with other HER2-directed therapies (either *i.v.* trastuzumab deruxtecan or *p.o.* tucatinib plus *i.v.* trastuzumab ± *p.o.* capecitabine) for HER2$^+$ cancers in adult patients. This study has been terminated due to strategic re-alignment of the sponsor.[30] NCT04460456 (phase I) aims to assess the safety, tolerability, and activity of escalating doses of *s.c.* SBT6050 (every 2 weeks for up to 2 y) alone and in combination with *i.v.* PD-1 blockade (pembrolizumab and cemiplimab) in adult patients with locally advanced (unresectable) and/or metastatic HER2$^+$ solid tumors.[31] SBT6050 will be assessed as standalone therapy or in combination with pembrolizumab at escalating doses (parts 1 and 3), followed by expansion cohorts at the RP2D (parts 2 and 4). As of April 4, 2021, 18 patients across 10 tumor types were treated at four dose levels from 0.15 mg/kg to 1.2 mg/kg (part 1, n = 14; part 3, n = 4). Target saturation was reached at 0.6 mg/kg.[32] All doses were pharmacologically active as indicated by blood-based biomarkers associated with NK cells and myeloid cells or T lymphocyte activation, including IFN-γ.[32] In part 1, grade 3 DLTs were observed at 1.2 mg/kg Q2W and could be resolved with supportive care.[32] Preliminary data across all dose levels showed partial response (n = 1), stable disease (n = 3), and progressive disease (n = 10).[32]

SBT6290

SBT6290 is composed of a mAb directed against the tumor-associated antigen nectin-4 conjugated to a TLR8 agonist.[30,33] Nectin-4 is a cell surface adhesion molecule that is scarcely expressed in normal tissue but overexpressed in numerous cancer types including bladder, head and neck, nonsmall cell lung, squamous, and triple negative breast cancers. Additionally, Nectin-4$^+$ solid tumors display a marked myeloid cell infiltrate. NCT05234606 (phase I/II) investigates *s.c.* SBT6290 as 21-d cycles standalone therapy and in combination with *i.v.* pembrolizumab in Nectin-4$^+$ advanced solid tumors.[34]

TLR9 agonist

TLR9 translocates from intracellular vesicles within the endoplasmic reticulum to endosomes upon stimulation by unmethylated cytidine phosphate guanosine (CpG) oligodinucleotides (ODN). Stimulation of TLR9 leads to enhanced uptake and killing of cancer cells as well as the initiation of adaptive immune responses.[35] Mechanistically, TLR9 activation induces the production of pro-inflammatory cytokines such as IFN-I, IL-6, IL-12 and TNF-α which activates innate immune cells, including DC and NK cells. In turn, mature DCs activate T lymphocytes which eradicate cancer cells.[36]Therefore, unmethylated CpG ODNs have been developed to mimic the immunostimulatory activity of bacterial DNA on TLR9.[36]

Vidutolimod

Vidutolimod (formerly CMP-001) is a novel TLR9 agonist that consists of a synthetic immunostimulatory G10, encapsulated in a virus-like particle formed by purified recombinant Qβ, a bacteriophage capsid protein.[37] NCT05445609 (phase II) will assess the efficacy of vidutolimod with nivolumab in patients with castration resistant and metastatic prostate cancer. Patients receive *s.c.* vidutolimod on d 1 and 7 of cycle 1, *i.t.* on d 14 of cycle 1 and d 1 and 14 of cycle 2 along with nivolumab *i.v.* over 30 min, and then *s.c.* on d 1 of subsequent

cycles along with nivolumab. Cycles of nivolumab and vidutolimod repeat every 4 weeks for up to 2 y and up to 6 months, respectively, in the absence of disease progression or unacceptable toxicity.

SD101

NCT04935229 (phase I) and NCT05220722 (phase I/II) assess the use of pressure-enabled hepatic artery infusion of SD-101, a synthetic CpG ODN, Q3W alone or in combination with *i.v.* checkpoint blockade in adults with metastatic uveal melanoma (nivolumab, ipilimumab) or hepatocellular carcinoma and intrahepatic cholangiocarcinoma (pembrolizumab, nivolumab, ipilimumab), respectively.[38] Of note, patients will receive two cycles of SD-101 in NCT05220722. Similarly, NCT05607953 (phase I) evaluates the use of pressure-enabled intrapancreatic infusion of two doses of SD-101 over two cycles alone or in combination with *i.v.* pembrolizumab in adults with locally advanced pancreatic cancer.

Tilsotolimod

NCT04126876 (phase II) will assess the administration of tilsotolimod intradermally at the primary melanoma excision site, 1 week prior to sentinel lymph node (SLN) biopsy in advanced melanoma patients. According to interim data, patients who received an injection of tilsotolimod following the excision of a primary tumor achieved a 70% lower SLN positivity rate as compared to placebo-treated patients (SLN 40–50%).[39]

CpG-STAT3 siRNA CAS3/SS3 (CAS3/SS3)

CAS3/SS3 combines a CpG oligonucleotide and a small interfering (si)RNA which simultaneously targets TLR9 and silences signal transducer and activator of transcription 3 (STAT3).[40] NCT04995536 evaluates CAS3/SS3 in combination with localized radiation therapy in patients with relapsed or refractory B-cell non-Hodgkin lymphoma. Patients undergo radiotherapy on d 1 and 2 on tumor-bearing lymph node, and receive *i.t.* CAS3/SS3 on d 2, 4, 16, and 18 as well as on d 9, 11, 23, and 25 for patients assigned to dose level 3.

TAC-001 – TLR9 agonist

TAC-001 received FDA clearance for administration as an investigational new drug for the systemic treatment of advanced solid tumors.[41,42] NCT05399654 (phase I/II, also referred to as INCLINE-101) designed to evaluate safety, pharmacokinetics (PK), and preliminary anti-tumor activity of *i.v.* TAC-001 in patients with histologically or cytologically documented advanced, metastatic, unresectable, or recurrent solid tumors. Eligible patients must have breast, nonsmall cell lung or ovarian cancer; cutaneous melanoma; gastro-esophageal adenocarcinoma; cervical or head and neck SCC; cholangiocarcinoma; endometrial, Merkel cell, hepatocellular, colorectal, renal cell, or urothelial carcinomas that have progressed on or are intolerant to standard therapy, including checkpoint blockade. Phase 1 study will explore escalating dose levels of *i.v.* TAC-001 Q2W and identify the maximum tolerated and administered doses as well as RP2D. Once RP2D is determined, phase 2 will assess RP2D in four specific tumor-type cohorts in a 2-stage design.

Preclinical and translational advances

In this section, we summarize the key preclinical advances on the ability of TLR agonists to boost anticancer immunosurveillance, which have been released since the publication of recent Trial Watches dealing with this topic.[9–11]

XS15 – TLR1/2 agonist

Rammensee et al.[12] reported that a single injection of XS15 mixed to uncoupled peptides in a water-in-oil emulsion (Montanide™ ISA51 VG) could induce strong $CD8^+$ and type 1 T-helper cell (T_H1) $CD4^+$ responses in a human volunteer. Moreover, a granuloma containing highly activated functional $CD4^+$ and $CD8^+$ effector memory T cells, formed at the injection site. These vaccine peptide-specific functional T cells were also detectable in peripheral blood for more than 1 y and could be strongly boosted by a second vaccination. Of note, XS15 is currently assessed in combination with a severe acute respiratory syndrome coronavirus 2-derived multi-peptide vaccine in adults with congenital or acquired B-cell/antibody deficiency in a phase I/II trial (NCT04954469).[43]

Poly-IC and poly-ICLC – TLR3 agonists

Ohkuri et al. recently reported that combining poly-ICLC with temozolomide, T-cell transfer, and Pmel-1 peptide vaccine significantly enhanced the survival of glioma-bearing mice.[44] Moreover, Anfray et al. evaluated the combination of *i.t.* poly-IC and resiquimod. In lung cancer and fibrosarcoma immunocompetent murine models, this combination significantly controlled tumor growth and metastasis in a T-dependent manner. Treated mice demonstrated a systemic immunosurveillance and resistance to tumor rechallenge mediated by macrophage and T cells.[45] Increased infiltration of macrophages and M1/M2 ratio, recruitment of $CD4^+$ and $CD8^+$ T cells, along with a reduction of immunosuppressive $CD206^+$ tumor-associated macrophages and $FOXP3^+CD4^+$ T cells were observed in regressing tumors[45] At last, Le Naour et al. demonstrated that systemic *i.p.* injections of poly-IC could restore the chemotherapeutic response of tumors implanted in mice with an immunodeficiency caused by the inactivation of *Fpr1*, the gene coding for formyl peptide receptor-1. In contrast, no anticancer effects of poly-IC were found in normal, fully immunocompetent mice. Indeed, poly-IC repairs a signaling defect of FPR1-deficient DCs, allowing them to recover their function as tumor antigen-presenting cells. Since close to one-third of the population is affected by a partial or complete defect in FPR1 signaling (due to the loss-of-function of single nucleotide polymorphism rs867228, which has an allelic frequency of 30%), it will be interesting to determine whether such FPR1 signaling-deficient individuals will benefit more from TLR3 stimulation than individuals with intact FPR1 function.[46–48]

BO-112 – TLR3 agonist

Co-administration of *i.t.* BO-112 and DMXAA in one of the lesions of mice bearing concomitant bilateral tumors (MC38 and B16 OVA-derived) enhanced local and distant antitumor efficacy.[49] Indeed, in this setting, in 8 out of 12 mice, third-party untreated tumor lesions were also completely regressed.

Synergistic effects in the tumor-bearing host were dependent on $CD8^+$ T cells, conventional type 1 DC, responsiveness to IFN-I and stimulator of interferon genes (STING) function. At last, PD-1 blockade demonstrated synergistic efficacy.[49] Similarly, *in vitro*, the treatment of TS/A breast cancer cells with a combination of hypofractionated focal irradiation (3 × 8 Gy) and BO-112 efficiently induced immunogenic cell death.[50] Interestingly, the efficacy of radiotherapy on the contralateral non-injected tumor sites was enhanced when combined with *i.t.* BO-112, leading to complete rejections in 10 out of 10 treated mice.[50] This effect was similar to that obtained by combined treatment of both lesions.[50]

SBT6050 – TLR8 agonist

Solid tumors are highly infiltrated by myeloid cells which can, upon activation, drive potent anti-tumor immunosurveillance. Additionally, while *i.t.* administration is limited by tumor accessibility, systemic administration of myeloid cell agonists induces acute toxicities due to peripheral activation of the targeted cell types. Therefore, Silverback Therapeutics developed SBT6050, designed to activate human myeloid cells specifically in the presence of $HER2^+$ tumor cells with moderate or high (immunohistochemistry 2+ or 3+) expression levels.[30] Studies with human immune cells demonstrated that SBT6050 could potently induce direct activation of myeloid cells and subsequently T- and NK-cell cytolytic activity in a HER2-dependent manner. SBT6050 demonstrated a significant anticancer efficacy in various murine tumor models (including a model deficient in T, B, and natural killer (NK) cells) and good tolerance in nonhuman primates of SBT6050 as single agent.[31] Preclinical studies also support combinations with trastuzumab and checkpoint inhibitors to synergize with SBT6050.[31]

SBT6290 – TLR8 agonist

Systemic administration of SBT6290 has been shown to trigger the activation of nuclear factor NF-κB, inflammasome, DCs, neutrophils, NK cells, and T_H1; production of pro-inflammatory cytokines and chemokines; reversion of the suppression of senescent naïve and tumor-specific T cells; and enhanced cytotoxic T lymphocyte (CTL) immune response culminating in increased overall survival of $Nectin4^+$ tumor-bearing mice.[30,33] These broad-spectrum effects can be either direct through Nectin4-specific mechanisms or indirect via the engagement of Fcγ receptors on the surface of myeloid cells.[34] Interestingly, Nectin4 was recently described as a novel ligand of T cell immunoreceptor with Ig and ITIM domains (TIGIT), which is an immune checkpoint protein.[51] SBT6290 apparently prevents the interaction between Nectin 4 and TIGIT, potentially contributing to T and NK cell activation.

SD101 – TLR9 agonist

SD-101, synthetic class-C CpG ODN TLR9 agonist, stimulates human plasmacytoid DCs to release IFN-α and mature into efficient antigen-presenting cells. Preclinical studies have demonstrated that *i.t.* SD-101, combined with systemic PD-1 blockade, can lead to complete and durable tumor growth control locally and abscopally in multiple murine tumor models.[52–54] Similarly, Ghosh et al. recently described that SD101 inhibited the expansion of murine and human myeloid-derived suppressor cells (MDSCs) and enhanced their polarization toward tumor-suppressive macrophages (so-called M1), suggesting that SD-101 could synergize with other immunotherapies within poorly infiltrated liver tumors.[38] Clinically, *i.t.* SD-101 stimulates antitumor immunosurveillance when combined with local radiation in individuals with non–Hodgkin lymphoma (NCT02266147)[55] and with anti-PD-1 therapy in patients with advanced melanoma (NCT02521870).[54,56]

TAC-001 – TLR9 agonist

Enrichment of tertiary lymphoid structure, memory B cells, and plasma cells is a positive prognostic factor in for various solid tumors.[57,58] Additionally, activation of TLR9 in B cells leads to the expression of co-stimulatory molecules with enhanced cross-presentation allowing for activation and proliferation of T cells, chemokine, cytokine and immunoglobulin secretion.[59] TAC-001 is an antibody–ODN conjugate, comprised of a differentiated TLR9 agonist (T-CpG) conjugated to an antibody against CD22, a receptor restricted to B cells, designed to deliver a potent and targeted immune activation via systemic administration. In-vitro stimulation of B cells with TAC-001 leads to increased expression of co-stimulatory molecules, immunoglobulin secretion, and cross-presentation leading to T cell proliferation.[42] Moreover, TAC-001 demonstrated efficient and durable single agent anti-tumor activity in checkpoint inhibitor resistant and refractory murine tumor models.[42] Systemic administration of TAC-001 in mice led to increased B cell infiltration, T cell effector functions, modulation of MDSCs, and a significant decrease in $IL\text{-}10^+$ regulatory B cells within the tumor microenvironment.[42] At last, *i.v.* administration of TAC-001 in monkeys showed favorable tolerability, PK, and pharmacodynamic profiles.[42]

Vidutolimod – TLR9 agonist

Cheng et al. showed that *in situ* vaccination of CMP-001 in combination with PD-1 blockade induced durable local and abscopal antitumor immune responses and significantly prolonged mouse survival as compared to anti-PD-1 alone.[60] Treatment with CMP-001 and anti-PD-1 led to increased IFN-γ producing $CD4^+$ and $CD8^+$ T cells as compared to control mice. Of note, these effects were completely abrogated by the depletion of $CD8^+$ T cells and relied on the presence of anti-Qβ.[60] Similarly, Lemke-Miltner et al. reported that the treatment of human peripheral blood mononuclear cell with CMP-001 induced cytokine production, including IFN-α from plasmacytoid DCs, but only in the presence of anti-Qβ antibody.[61] *In vivo, i.t.* CMP-001 treatment of lymphoma-bearing mice reduced growth of both treated and distant untreated tumors and enhanced survival. These effects were anti-Qβ antibody-dependent and abolished by the depletion of $CD4^+$ and/or $CD8^+$ T cells.[61] At last, *i.t.* CMP-001 combined with systemic PD-1 blockade enhanced systemic anti-tumor responses.[61]

Concluding remarks

Mounting preclinical and clinical evidence suggest that human TLR agonists are promising targets for the (re)elicitation of

anticancer immunosurveillance. Systemic immunotherapies have demonstrated their efficacy to induce a long-lasting antitumoral immunosurveillance and to increase overall survival in several solid tumors.[11] Nonetheless, numerous immunosuppressive circuitries as well as therapy resistance enable cancer progression and considerably limit the efficacy of conventional regimens. Additionally, preclinical studies have demonstrated that immunostimulatory products, such as PRR agonists, could overcome the resistance to systemic immune checkpoint blockers (ICBs).[62,63] Therefore, numerous combinatorial regimens consisting in the administration of TLR agonists together with chemotherapy, radiotherapy, or other immunotherapeutic agents (such as ICBs) are currently under clinical evaluation.

Additionally, the past few years have witnessed an increase in the number of trials evaluating intratumoral immunotherapy.[64,65] Among the 22 trials reported in this trial watch, 4 investigate a TLR agonist in combination with ICBs (NCT04116320, NCT04570332, and NCT05445609) or radiation therapy (NCT04995536). This dynamic has been supported by the FDA approval of i.t. talimogene laherparepvec (T-VEC) for oncolytic virotherapy of melanoma patients in 2015.[66] Intratumoral immunotherapy locally reshapes the tumor microenvironment, allowing to reinvigorate immunosurveillance. Additionally, this delivery route can reduce systemic leakage of the agonist and thus minimize off-target toxicity. Furthermore, this strategy requires a lower dose to impede tumor progression. However, intratumoral injection suffers from technical challenges including size, density, and accessibility criteria. Additionally, it is still unknown whether administration in one lesion rather than another could lead to systemic protection. Thus, the local immunomodulation induced by local anesthetics, which is required prior to i.t. injection, have not been much investigated.[67] Ongoing and future preclinical and clinical investigations are urgently awaited to define the recommendations for i.t. immunotherapy over systemic treatment for local monotherapies or combinatorial regimens.

Abbreviations

CpG, cytosine-phosphate-guanosine dinucleotide; DC, dendritic cell; HER2, human epidermal growth factor receptor-2; IFN, interferon; *i.p.*, intraperitoneally; *i.t.*, intratumorally; *i.v.*, intravenously; mAb, monoclonal antibody; MDSCs, myeloid-derived suppressor cells; MSS, microsatellite stable; NK, natural killer; ODN, oligodinucleotides; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; *p.o., per os; s.c.*, subcutaneously; SCC, squamous cell carcinoma; RNA, ribonucleic acid; TLR, Toll-like receptor; TRAEs, treatment-related adverse events; Q3W, administered every 3 weeks.

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ORCID

Julie Le Naour http://orcid.org/0000-0002-3749-2171

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