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Review

Intratumoural immunotherapy: activation of nucleic acid sensing pattern recognition receptors

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ABSTRACT

Recently, it has become clear that the tumour microenvironment (TME) is important in cancer immunotherapy. While immune checkpoint inhibitors are effective for some patients, the heterogeneous nature and status of the TME ('cold' tumours) play a critical role in suppressing antitumour immunity in non-responding patients. Converting 'cold' to 'hot' tumours through modulation of the TME may enable expansion of the therapeutic efficacy of immunotherapy to a broader patient population. This paper describes advances in intratumoural immunotherapy, specifically activation of nucleic acid sensing pattern recognition receptors to modulate the TME.

Introduction

Over the last decade, immune checkpoint inhibitors (CPIs) have transformed cancer treatment. Antibodies against the key immunological checkpoints cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed death receptor-1 (PD-1)/programmed death receptor ligand-1 (PD-L1) have resulted in unprecedented durable antitumour responses with survival benefits across many cancer types [1–3]. However, only a minority (10–30%) of patients benefit from CPI treatment [4,5] and options for CPI relapsed or refractory patients are limited [6]. Could CPI efficacy be improved by rational combination with other agents that may potentiate T-cell activation? Intratumoural (IT) immunotherapy, where immune-activating agents are injected directly into the tumour to modulate the tumour microenvironment (TME) for antitumour T-cell priming, is a promising possibility.

The discovery of pattern recognition receptors (PRRs) and their role in activating innate and adaptive immune responses led to the development of novel immune-activating agents. These agents mimic pathogen-associated molecular patterns (PAMPs) to activate targeted receptor-mediated immune cascades [7–9]. This paper summarizes recent clinical data and ongoing trials of such agents.

Converting 'cold' to 'hot' tumours is beneficial for immunotherapy

Patients with a T-cell inflamed or 'hot' TME are likely to respond to CPI therapy, while those with a non-T-cell-inflamed or 'cold' TME require additional interventions to make the tumours susceptible. The TME contains cancerous cells as well as various immune cells, such as tumour-associated macrophages, T cells, B cells, dendritic cells and myeloid-derived suppressor cells. A complex interplay between antitumour immunity and immunosuppression regulates the balance between tumour growth and eradication. Growing evidence suggests that the TME can be manipulated by direct IT injection of immunostimulatory agents that increase T-cell infiltration. This should convert 'cold' tumours to 'hot' tumours that are responsive to treatment with CPIs. For example, IT immunotherapy with talimogene laherparepvec (TVEC) has been approved for treatment of unresectable melanoma [10,11], and trials are underway combining TVEC with pembrolizumab (anti-PD-1) in advanced melanoma [12].

Nucleic acid sensing PRRs

IT immunotherapy aims to create a suitable TME for optimal T-cell priming within the injected tumour, leading to systemic anenestic

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tumour response [13]. This could involve either the direct use of cytokines or the induction of immune responses via engaging PRRs expressed in immune cells present in the TME. Both T-cell- and B-cell-mediated antitumour responses can be generated.

The role of individual components of bacteria and viruses in activating specific PRRs resulting in innate and adaptive immune responses has become clear [7]. These PRRs are evolutionarily conserved and expressed primarily by immune cells, but also by other cells. Specific receptors for various bacterial or viral components (e.g. lipopolysaccharide, lipopeptide, flagellin and nucleic acids) have been identified [7,8,14]. Nucleic acid sensing receptors can detect both pathogen- and host-derived nucleic acids and are broadly classified according to their location in endosomes or cytoplasm (Figure 1). For example, Toll-like receptors (TLRs) can be found in endosomal membranes, while RIG-I-like receptors (RLRs) and cyclic GMP-AMP synthase–stimulator of IFN genes (c-GAS-STING) are located in the cytoplasm [7] (Figure 1).

Of the 13 TLRs identified in mammals, TLR3, TLR7, TLR8, TLR9 and TLR13 detect distinct molecular patterns of RNA and DNA [7,8]. TLR3 is the receptor for viral and synthetic double-stranded RNAs [15]. TLR7 and TLR8 recognize single-stranded RNAs [16,17] but also small molecules like imidazoquinolines and certain nucleosides [18,19], while TLR9 is activated by unmethylated CpG motifs typically present in bacterial and viral DNA [20,21]. Although a human analogue has not been identified, TLR13 senses bacterial and viral 23S rRNA in mice [22,23].

TLRs are transmembrane receptors containing extracellular leucine-rich repeats and a cytoplasmic Toll/IL-1R (TIR) domain [24,25]. Activation results in TLR dimerization through the TIR domain and recruitment of adapter proteins. For TLR3, these are TIR domain-containing

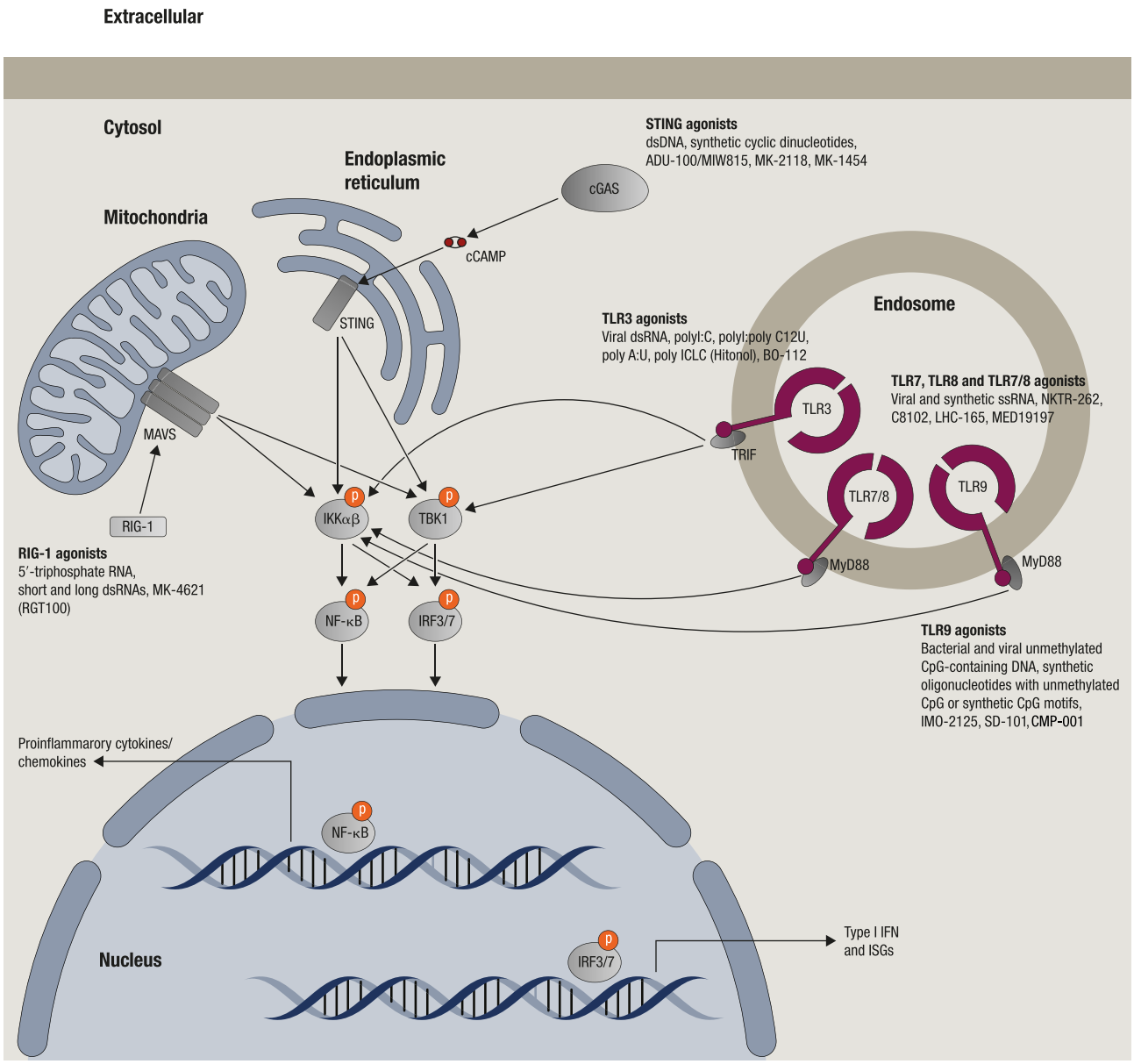

Figure 1. The pattern recognition receptors (PRRs) that sense nucleic acids and their derivatives and brief signalling pathways. The natural ligands and clinical candidates for each receptor are shown. The clinical status of PRR activators and the combination agents for immuno-oncology are shown in Table 1 cGAMP, cyclic guanosine monophosphate–adenosine monophosphate; cGAS, cyclic GMP-AMP synthase; IFN, interferon; IKKα/β, inhibitor of nuclear factor κ-B kinase subunit α or β; IRF3/7, interferon regulatory factor 3 or 7; ISGs, interferon stimulatory genes; MyD88, myeloid differentiation factor 88; MAVS, mitochondrial antiviral-signalling protein; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; RIG-I, retinoic acid inducible gene I; STING, stimulator of interferon genes; TLR3/7/8/9, Toll-like receptor 3, 7, 8 or 9; TBK1, TANK binding kinase 1.

adapter-inducing IFN-β protein [26,27] and TNF receptor-associated factor 6 (TRAF6) [28]. Of note, the widely used 'TLR3 agonist' polyinosinic-polycytidylic acid (poly I:C) also activates the cytosolic RNA helicases retinoic acid inducible protein I (RIG-I) and melanoma differentiation-associate gene 5 (MDA5) [29]. In general, TLR3 agonists induce the cytokines interferon (IFN)-β, tumour necrosis factor (TNF)-α, interleukin (IL)-6 and CXCL10.

In contrast to TLR3, TLR7, TLR8 and TLR9 utilize the myeloid differentiation factor 88 as an adapter molecule [30]. Subsequent assembly of a large helical oligomer called the 'myddosome' [31] leads to

Table 1
Clinical trials of intratumoural immunotherapy of compounds that activate nucleic acid sensing pattern recognition receptors (PRRs) alone or in combination with checkpoint inhibitors.

PRR activator	Combination agent	Patient population	Clinical stage	Clinical trial number/status
TLR9 agonist[f]				
IMO-2125	Ipilimumab	Anti-PD-1 refractory melanoma	Phase 3	NCT03445533[a]
	Ipilimumab or pembrolizumab	Metastatic melanoma	Phase 1/2	NCT02644967[b]
	Ipilimumab + nivolumab	Solid tumours	Phase 2	NCT03865082[c]
	Monotherapy	Melanoma, advanced solid tumours	Phase 1b	NCT03052205[b]
SD-101	Pembrolizumab	Metastatic melanoma, recurrent/metastatic SCCHN	Phase 1b/2	NCT02521870[b]
	Pembrolizumab + radiation	Hormone-naive OMPC	Phase 2	NCT03007732[a]
	BMS986178 (anti-OX40 antibody)	Advanced solid malignancies	Phase 1	NCT03831295[a]
	BMS986178 + radiation	Low-grade B-cell NHL	Phase 1	NCT03410901[a]
	Epacadostat + radiation	Advanced solid tumours and lymphoma	Phase 1/2	NCT03322384[a]
	Ipilimumab + radiation	MALT lymphoma, NMZL, MZL, SLL, grade 1/2 FL and SMZL	Phase 1/2	NCT02254772[d]
	Pembrolizumab	Breast cancer	Phase 2	NCT01042379[a]
CMP-001	Pembrolizumab	Melanoma	Phase 1	NCT02680184[a]
	Pembrolizumab	Advanced melanoma	Phase 1b	NCT03084640[a]
	Nivolumab	Melanoma with lymph node disease	Phase 2	NCT03618641[a]
	Radiosurgery + nivolumab + ipilimumab	Metastatic CRC	Phase 1	NCT03507699[a]
	Atezolizumab	NSCLC	Phase 1	NCT03438318[a]
AST-008[f]	Pembrolizumab	Advanced/metastatic melanoma, MCC, SCCHN, cSCC and solid tumours	Phase 1b/2	NCT03684785[a]
MGN1703[f]	Ipilimumab	Melanoma, advanced solid tumours	Phase 1	NCT02668770[a]
TLR3 agonist				
Poly-ICLC (Hiltonol)	Monotherapy	Prostate cancer	Phase 1	NCT03262103[a]
	Tremelimumab/durvalumab (MEDI4736)	SCCHN, MCC, CTCL, breast cancer, melanoma, renal cancer, bladder cancer, prostate cancer, testicular cancer and other solid tumours	Phase 1/2	NCT02643303[a]
	Monotherapy (*in situ* vaccination)	Melanoma, head and neck cancer, sarcoma and non-melanoma skin cancers	Phase 2	NCT02423863[a]
	Pembrolizumab	MRP colon cancer	Phase 1/2	NCT02834052[a]
BO-112[g]	Pembrolizumab, nivolumab	Aggressive solid tumours	Phase 1	NCT02828098[b]
TLR7/8 agonists				
NKTR-262[g] (TLR7/8)	Peg-CD-122 agonist (NKTR-214) + nivolumab	Melanoma, MCC, TNBC, RCC, CRC, ovarian cancer, urothelial carcinoma and sarcoma	Phase 1/2	NCT03435640[a]
CV8102[g,h] (TLR7/8/RIG-I)	Anti-PD-1 antibody	Advanced melanoma, SCC, SCCHN or ACC	Phase 1	NCT03291002[a]
LHC-165 (TLR7)	Spartalizumab	Solid tumours	Phase 1/1b	NCT03301896[a]
MEDI9197 (TLR7/8)	Durvalumab	CTCL and solid tumours	Phase 1	NCT02556463[e]
RIG-I agonist				
RGT100 (MK-4621)	Monotherapy	Advanced solid tumours	Phase 1/2	NCT03065023[e]
	Pembrolizumab	Advanced solid tumours	Phase 1/1b	NCT03739138[a]
STING agonist				
ADU-S100/MIW815	Spartalizumab	Advanced/metastatic solid tumours or lymphomas	Phase 1	NCT03172936[a]
	Pembrolizmab	Metastatic/recurrent SCCHN	Phase 2	NCT03937141[a]
	Monotherapy and with ipilimumab	Advanced/metastatic solid tumours and lymphomas	Phase 1	NCT02675439[a]
MK-2118	Monotherapy and with pembrolizumab	Advanced/metastatic solid tumours	Phase 1	NCT03249792[a]
MK-1454	Monotherapy and with pembrolizumab	Advanced/metastatic solid tumours or lymphomas	Phase 1	NCT03010176[a]
SB 11285[i]	Nivolumab	Melanoma, SCCHN and solid tumours	Phase 1	NCT04096638[c]
GSK3745417[i]	Monotherapy and with pembrolizumab	Solid tumours	Phase 1	NCT03843359[a]

ACC, adenoid cystic carcinoma; CRC, colorectal cancer; CTCL, cutaneous T-cell lymphoma; FL, follicular lymphoma; MALT lymphoma, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue; MCC, Merkel cell carcinoma; MRP, mismatch repair proficient; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma; NMZL, nodal marginal zone B-cell lymphoma; NSCLC, non-small cell lung cancer; OMPC, oligometastatic prostate cancer; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of head and neck; SLL, small lymphocytic lymphoma; SMZL, splenic marginal zone lymphoma; TNBC, triple negative breast cancer.

[a] Recruiting.
[b] Active, not recruiting.
[c] Not yet recruiting.
[d] Completed.
[e] Terminated.
[f] Additional TLR9 agonists in clinical trials include DV281 by inhalation, and IT AST-008 and MGN1703 are in early trials with no data reported yet.
[g] Multiple modes of action.
[h] Single-stranded RNA-based compound.
[i] By intravenous administration.

engagement of IL-1-associated kinase, TLR-specific additional adapter proteins and TRAF6. Depending on the nature of the PAMP encountered and TLR activated, the transcription factors AP-1, ELK1, NF-κB or interferon regulatory factors are activated [30]. In general, TLR7 and TLR9 induce genes encoding Th1-type cytokines, such as IL-6, IL-12, TNFα, chemokines and type I IFNs [7,9]. However, the exact cytokine profile depends on the compound. Activation of TLR8 leads to mostly IL12, TNF, IL-6 and minimal IFN.

The cytosolic RIG-I senses viral RNA [32,33] with a 5′-triphosphate (5′-ppp) moiety combined with short blunt-ended double-stranded RNA (dsRNA) stretches; critical patterns that discriminate non-self from self RNA [34,35]. Activation of RIG-I and MDA5 leads to type I IFN gene expression [36]. The cGAS–STING pathway detects the presence of cytosolic DNA leading to expression of inflammatory genes, including type 1 IFN [37–42].

This increased understanding of nucleic acid sensing PRRs has allowed the development of novel immunostimulatory agents that can trigger receptor-mediated immune cascades. Table 1 lists the status of all nucleic acid sensing PRR agonists currently in clinical trials following IT immunotherapy. However, this paper will only discuss compounds for which clinical data are available in more detail.

TLR9 agonists

TLR9 expression is limited to plasmacytoid dendritic cells (pDCs) and B cells in humans, but is more widespread in rodent immune cells [43]. As discussed above, bacterial DNA or synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG motifs are known agonists of TLR9. Although TLR9 was not discovered until 2000 [21], the first evidence of immune activation in humans by a CpG ODN was observed in the mid-1990s [44,45].

The cytokine profile resulting from TLR9 activation is dependent on the nucleotide sequence, chemical modifications of the DNA backbone, CpG flanking sequences and secondary structures of the agonist [9,46–50]. Various categories of TLR9 agonists have been synthesized and studied [9,47]. Compounds may preferentially activate pDCs, leading to the secretion of type I IFNs [51,52] or B cells, thus producing proinflammatory cytokines, IL-6, TNF and minimal type I IFNs [20,53]. Certain agonists activate both pDC and B cells, inducing secretion of IFN-α and other inflammatory cytokines [54,55].

In preclinical models, systemic treatment with TLR9 agonists (either alone or in combination with chemotherapeutic agents), targeted therapies and monoclonal antibodies showed very encouraging antitumour activity [9,47,48,56–64]. However, in clinical trials, systemic treatment with TLR9 agonists alone or in combination yielded disappointing results [65–73], although local treatment with TLR9 agonists may exert better antitumour activity [74,75]. For example, TLR9 agonists administered locally in melanoma patients after excision of the primary tumour showed improvements in recurrence-free survival for over 10 years in two phase II trials [76]. Thus, the focus shifted to IT application of TLR9 agonists. This mode of administration resulted in strong antitumour activity in the injected tumour in preclinical models [74,77–79], and – more importantly – systemic immune responses and anenestic antitumour activity. Responses were more potent when TLR9 agonists were combined with CPIs [75,80–91].

Marabelle et al. showed that IT immunotherapy with CpG ODN and anti-CTLA4 and/or anti-OX-40 agonists not only reduced tumour burden at the injected site but also distant tumours in mouse models of lymphoma and breast [75].

The TLR9 agonist tilsotolimod (IMO-2125) was evaluated in preclinical models of colon, lung and pancreatic cancer, and melanoma [83–89]. IT immunotherapy with IMO-2125 led to dose-dependent growth inhibition of the injected lesion as well as anenestic tumours. Antitumour activity was associated with increased $CD3^+$ tumour infiltrating lymphocytes, $CD8^+$ T cells and CTL responses against tumour antigens [82,83]. Several immune checkpoint genes showed increased expression (IDO-1, PD-L1, TIM3, LAG3 and CTLA4) in both treated and distant tumours [82,85]. IMO-2125 in combination with anti-CTLA4 [82–84,89], anti-PD-1 [86], IDO-1 inhibitor [87] or both anti-PD1 and IDO-1 inhibitor [88] showed more potent antitumour activity than any agent alone.

Similarly, IT injection of another TLR9 agonist, SD-101, in the anti-PD-1 non-responder colon cancer model CT-26 resulted in complete durable responses in all injected tumours and a majority of distant tumours [81]. IT immunotherapy of a TLR9 agonist in combination with anti-OX40 antibody or anti-CTLA4 has also shown very potent antitumour activity [75,91].

Several TLR9 agonists are currently in clinical trials, either alone or in combination with CPIs (see Table 1). These are the ODNs SD-101, CMP-001 and IMO-2125, although each contains different features. SD-101 contains phosphorothioate backbone modifications, CpG motifs and palindromic sequences that allow formation of duplex and/or hairpin structures [92]. CMP-001 (previously known as 'CYT003/QbG10') is an ODN containing CpG motifs and regions of poly-dG nucleotides, formulated within a virus-like particle [93]. IMO-2125 contains synthetic immunostimulatory motifs and two 5′ ends for increased metabolic stability, and forms double-stranded structures [9,47,48].

There are limited clinical data using IT-delivered TLR9 agonists alone. In a clinical trial of IMO-2125 monotherapy, patients with histologically or cytologically confirmed metastatic refractory solid tumours were injected with doses of 8, 16, 23 or 32 mg into a single lesion. Treatment was well tolerated, and no dose-limiting toxicities or treatment-related adverse events were observed. Flow cytometry of tumour biopsies from two of the three analysed patients showed HLA-DR (major histocompatibility complex class II) upregulation at 24 h compared with pre-treatment. Robust activation and upregulation of the type I IFN pathway was demonstrated by increased expression of IFN and IFN-related genes. There were no clinical responses, although 13 of the 29 (45%) evaluable patients had stable disease according to RECIST v1.1 [94–96].

However, several studies using TLR9 agonists in combination with CPIs are in progress, including a phase 1/2 trial of IMO-2125 in combination with ipilimumab or pembrolizumab in patients with PD-1 refractory metastatic melanoma. Over 12 weeks, six doses of 4, 8, 16 or 32 mg IMO-2125 were injected intratumourally into single lesions while patients were treated with the standard dose of ipilimumab or pembrolizumab from the second week onwards. Patients showed a strong type 1 IFN gene signature, macrophage influx and robust dendritic cell maturation 24 h post injection of IMO-2125 alone, before standard CPI treatment was initiated. The combination therapy induced strong CD8+ T-cell proliferation and activation that was preferential to the tumour and not present in peripheral blood mononuclear cells. In responding patients, the main T-cell clones expanding on therapy were shared between local and distant lesions, indicating that priming/reactivation was to a shared antigen [97,98].

Based on these clinical findings, the 8-mg dose in combination with the standard dosing regimen for ipilimumab was selected for a phase 2 trial. The interim data from this trial show a 32.4% overall response rate (ORR) for the first 34 (of 52 to date) patients, including 9% ($N = 3$) who achieved a complete response (CR), 24% ($N = 8$) with a partial response (PR) and 76.5% ($N = 26$) who achieved disease control [97,98].

A phase 3 trial of IMO-2125 in combination with ipilimumab versus ipilimumab alone in PD-1 refractory metastatic melanoma with ORR and overall survival as primary endpoints is ongoing (Table 1). There is also a phase 2 trial combining IMO-2125 with both nivolumab and ipilimumab for the treatment of recurrent/metastatic squamous cell carcinoma of the head and neck and microsatellite stable colorectal cancer (Table 1).

SD-101 is being studied in multiple trials in combination with pembrolizumab. In advanced melanoma patients naïve to anti-PD-1/L1 therapy, 47 patients received ≤2 mg of SD-101 in one to four lesions and 40 patients received 8 mg in a single lesion along with standard pembrolizumab dosing. The results showed 70% and 48% ORR in patients who had received ≤2 mg in one to four lesions and a single 8-mg

dose of SD-101, respectively. The combination was well tolerated, with adverse events related to SD-101 being transient, mild-to-moderate flu-like symptoms [99]. In 23 PD-1/PD-L1-resistant melanoma patients who received 2 mg of SD-101 intratumourally per lesion in one to four lesions (weekly x 4 doses followed by q3w x 7) with pembrolizumab, the best overall response was 88% with PD, 8% PR/CR and 4% stable disease [92,99]. The results in the PD-1/PD-L1-resistant patients are somewhat disappointing considering a recent report on response rates in this patient population [6].

Interim data of IT-administered SD-101 in combination with pembrolizumab in patients with squamous cell carcinoma of the head and neck from an ongoing phase 1b/2 study showed a 40% ORR in evaluable patients ($N = 10$) (four PR, one stable disease, and five PD). Biomarker analyses showed induction of broad immune activity, including increases in CD8+ T cells and a Th1 response in the TME [100]. Additionally, IT SD-101 in combination with pembrolizumab is being studied in a phase 2 trial for breast cancer (Table 1).

Interim results of CMP-001 in patients with advanced melanoma resistant to anti-PD-1 checkpoint inhibition have been reported [101]. CMP-001 was administered at doses of 1, 3, 5, 7.5 or 10 mg in two dosing schedules (weekly for 7 weeks, followed by q3w; or weekly for 2 weeks, followed by q3w). The combination of CMP-001 and pembrolizumab was generally well tolerated. Results in 69 patients showed 22.0% ORR (15/69, 95% confidence interval 13–33%), including two CR and 13 PR. Serum levels of IP-10, a chemokine induced by IFNs, were increased, as were CD8+ T cells and PD-L1 expression in injected and non-injected post-treatment biopsies [101].

TLR3 agonists

TLR3 is expressed in the endosomes of human myeloid dendritic cells, monocytes and natural killer cells [102,103]. TLR3 recognizes dsRNA and synthetic nucleic acid polymers such as poly I:C [15]. Several analogues of poly I:C, including poly I:poly $C_{12}U$ [104] and poly A:U [15], as well as formulations of poly I:C with poly-L-lysine-carboxymethylcellulose (poly-ICLC) [105], polyarginine (BO-112) [106] or kanamycin-calcium ions [107], have been used as TLR3 agonists. Synthetic TLR3 agonists of defined length have also been reported [108]. These TLR3 agonists have mainly been studied as anti-infective and anticancer agents, and as vaccine adjuvants [109–116], but none have evaluated IT TLR3 agonists in preclinical cancer models.

Poly-ICLC (also known as 'Hiltonol') and BO-112 have advanced into the clinic for IT immunotherapy (Table 1). As with poly I:C, BO-112 is an agonist of MDA5 and RIG-I in addition to TLR3. IT immunotherapy with BO-112 alone or in combination with anti PD-1 in 28 anti-PD-1 refractory cancer patients showed disease control rate in seven of 12 (58%) at 9–10 weeks and an objective response in two of 12 (17%; one melanoma, one renal cancer) [117].

In a case study, a patient with facial embryonal rhabdomyosarcoma was treated with IT administration of poly-ICLC followed by intramuscular poly-ICLC as maintenance therapy. Antitumour efficacy was observed and was correlated with induction of immune markers [118].

TLR7/8 agonists

In primates and humans, TLR7 is expressed in pDCs and B cells, and TLR8 is expressed in human myeloid dendritic cells and monocytes. In rodents, TLR8 is non-functional and TLR7 is expressed more widely in several types of immune cells. Known agonists of TLR7 and TLR8 are short synthetic single-stranded RNAs that mimic viral RNA segments [16,17], imidazoquinolines [Imiquimod, Aldara, 052; 852; S-34240; 3M-052 (injectable)/MEDI9197; 854A], and nucleoside analogues such as loxoribine, 7-thia-8-oxo-guanosine and 7-deazaguanosine [18,19]. Extensive structure-activity relationship studies have supported the design of potent agonists of TLR7, TLR8 or TLR7/8 [9,47,119–124].

Compounds that activate both TLR7 and TLR8 have shown potent antitumour activity in preclinical cancer models. For example, IT immunotherapy of MEDI9197 induces innate and adaptive immunity in the injected tumour and tumour-draining lymph nodes [125]. Importantly, in cancer models that respond poorly to agents targeting either PD-L1 or CTLA-4, combination of these agents with MEDI9197 significantly improved antitumour activity [125]. Cellular depletion studies revealed that $CD8^+$ T cells were required for therapeutic activity. NKTR-262, a TLR7/8 agonist resiquimod (R848) conjugate, in combination with NKTR-214 (CD122-biased cytokine agonist) has shown potent antitumour activity and associated changes in immune biomarkers [126].

MEDI9197 (3M-052), NKTR-262, LHC-165 (a benzonapthyridine-based compound adsorbed on aluminum hydroxide) and CV8102 (a single-stranded RNA complexed with a cationic peptide) are in early clinical studies (Table 1). In a dose escalation trial (0.005–0.055 mg; q4w) of MEDI9197 IT immunotherapy in patients with subcutaneous/cutaneous tumours, the maximum tolerated dose was 0.037 mg [127]. Immunohistochemistry in the 0.037-mg cohort showed an increase in CD8 (T cells), CD40 (myeloid and B cells), CD56 (natural killer cells) or PD-L1 (tumour and immune cells) 3 weeks after treatment initiation. RNAseq analysis of paired tumour biopsies also showed an increase in innate and adaptive immune-activation signatures [127]. LHC-165 is currently in a phase 1/1b trial in combination with PDR001 (spartalizumab), an anti PD-1 agent, while CV8102 is in a phase 1 trial in patients with advanced solid tumours either alone or in combination with systemic anti-PD antibody [128–130].

RIG-I agonists

RIG-I belongs to the RLR family of receptors and is broadly expressed in most cells, including haematopoietic and cancer cells. Other members of the RLR family are MDA5 and LGP2. RIG-I recognizes viral and synthetic blunt-ended dsRNA structures with a 5′-triphosphate group [131].

The RIG-I agonist RGT100, a synthetic oligoribonucleotide, induces cytokine expression including IFN-α and IFN-β. In preclinical models, IT immunotherapy with RGT100 led to antitumour activity in treated and untreated contralateral tumours, and induced a strong and durable type I IFN response. Depletion of natural killer cells blocked antitumour activity of RGT100 [132]. In a phase 1/2 study, 0.2, 0.4, 0.6 and 0.8 mg of MK-4621 (RGT100) were administered IT twice per week over a 4-week period in patients with advanced or recurrent tumours. There were no dose-limiting toxicities and the safety profile was favourable at all dose levels. Treatment with MK-4621 increased circulating chemokine levels in serum and expression of genes involved in IFN signalling in tumours. Interim data showed that four of 15 patients (26.7%) experienced best overall response of stable disease [133]. It is also under clinical evaluation in combination with pembrolizumab (Table 1), although no data have been announced.

cGAS-STING agonists

STING is expressed in the endoplasmic reticulum membranes of various epithelial and endothelial cells as well as in haematopoietic cells, T cells, dendritic cells and macrophages [134]. There are significant differences between mouse (mSTING) and human STING (hSTING). Thus, agonists of mSTING may not show any activity for hSTING, and compounds need to be optimized for hSTING activation [42,135,136]. STING is activated by endogenously generated or exogenously provided synthetic cyclic dinucleotides (CDNs), leading to the production of type 1 IFNs and other inflammatory cytokines [39]. Synthetic CDN analogues [41,137] and small molecules that act as agonists of STING are available [138].

In preclinical models, IT immunotherapy with the STING agonists ADU-S100 and MK-1454 induced regression of established tumours in mice, and generated systemic immune responses capable of rejecting distant metastases and providing long-lived immunologic memory [41,136,139]. Agonists of STING have also shown antitumour activity

[140–142] in combination with radiation [139], vaccines [143] and CPIs [41,42,144].

Clinical trials of the STING agonists ADU-S100 (also referred to as 'MIW815'), MK-2118 and MK-1454 via IT administration (Table 1), and GSK3745417 and SB 11285 via systemic administration are ongoing. A trial of ADU-S100 at doses of 50–3200 μg on days 1, 8 and 15 of a 28-day cycle enrolled 41 patients with more than 20 different types of cancer. No dose-limiting toxicities were reported [145], and ADU-S100 IT immunotherapy increased key systemic cytokines, including IL-6, MCP-1 and IFN-β, indicating activation of the STING pathway. Two of the 40 patients (5%) had a PR: one patient with Merkel cell carcinoma and one patient with parotid gland cancer with prior anti-PD-1 therapy [145].

Interim data from a dose escalation trial of MK-1454 in patients with advanced solid tumour or lymphoma data were presented recently. Doses were 10–3000 μg for monotherapy and 90–2000 μg in combination with intravenous injection of 200 mg pembrolizumab every 3 weeks [146]. There were no responses in the monotherapy arm, but 24% of patients ($N = 6/25$) in the combination arm had PRs with median reductions of 83% in the size of both injected and non-injected tumours. Twenty percent and 48% of patients in the monotherapy and combination arms achieved disease control, respectively, while treatment-related adverse events occurred in 82.6% ($N = 19/23$) and 82.1% ($N = 23/28$) of patients, respectively [146].

MK-2118 is being evaluated in a study as IT injection as monotherapy, and in combination with pembrolizumab or by subcutaneous injection in combination with pembrolizumab for the treatment of adults with advanced/metastatic solid tumours or lymphomas (Table 1).

Conclusion and future prospects

Despite many notable successes in the treatment of cancer, many patients treated with CPIs have either primary resistance or recurrence. Low tumour mutational burden or tumours ignored by the immune system may be the cause, particularly in patients who are refractory to CPI therapy [147]. IT immunotherapy using immune-activating agents that modulate the TME (acting as *in situ* vaccination) and generate or expand pre-existing antitumour T cells could overcome this resistance and potentiate the response to CPIs.

Compounds designed to activate nucleic acid sensing PRRs and thus receptor-mediated immune responses are a novel class of immune-activating agents. Based on the available data, the type of immune cascade induced depends on the nature of the compound, targeted PRR, immune cells in which the PRR is expressed, and the presence of these cells in the TME. Most compounds induce a milieu of cytokines, including type I IFNs and cell surface markers. IFNs play a critical role in modulating the TME; however, the function of IFNs may be impacted by the levels of other cytokines (e.g. TNF, IL-6, IL-10). While IT immunotherapy with these agents alone modulates the TME and causes some responses in the injected tumour lesion, monotherapy has not resulted in systemic responses. However, IT immunotherapy with nucleic acid sensing PRRs in combination with anti-PD1 or anti- CTLA-4 has resulted in both local and systemic immune responses as well as significant clinical responses. Translational data show the similarities of the IT-treated TME in the injected and distant tumour sites, clearly demonstrating systemic immune responses. Draining lymph nodes are likely to play a very important role; however, no data are available to date for lymph node biopsies from any of these studies.

Although IT immunotherapy was initially administered to peripheral or surface lesions alone, image-guided IT immunotherapy now allows injection of deep visceral lesions [148] for broader applicability.

Gaining an understanding of the dose and dosing regimen requirements of IT immunotherapy is important; the clinical data indicate that lower doses may be more effective than higher doses [92,94–98,101]. This could be due to negative feedback between regulatory factors controlling inflammation. If so, it might be helpful to target such feedback regulatory factors to achieve maximal benefit of the agent. As discussed above, in the case of TLR9 agonists, inhibition of IDO-1 has been shown to potentiate efficacy [87,88]. Remaining questions also include frequency and duration of treatment, injection volume, site of injection, etc.

In conclusion, the discovery of PRRs has allowed the development of a novel class of immune-activating agents for IT immunotherapy of cancer. The first phase 3 trial of a TLR9 agonist, IMO-2125, in combination with ipilimumab, is in progress in patients with anti-PD1 refractory melanoma (Table 1). While the available data are encouraging, ongoing trials will further guide development to realize the full potential of these approaches. Combinations of IT innate immune modulator therapies with CPIs have the potential to revolutionize cancer treatments.

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Declaration of interests

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