Immunology and Cell Biology (2017) 95, 325–332

www.nature.com/icb

REVIEW

Targeting cancer-related inflammation in the era of immunotherapy

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Recent advances in cancer immunotherapy, particularly immune checkpoint blockade therapy have dramatically changed the therapeutic strategy against advanced malignancies. Still, only a subset of patients shows a good response to any single therapy. Moreover, it remains largely unsolved how we can maintain durable clinical responses, or how we can successfully treat a broader range of cancers by immunotherapy. Growing evidence suggests that the major barrier to more successful cancer immunotherapy is the tumour microenvironment (TME), where chronic inflammation has a predominant role in tumour survival and proliferation, angiogenesis and immunosuppression. Over the past decades, our understanding of cancer-related inflammation has significantly evolved, and now we have various therapeutic options tailored to the TME. These therapeutic strategies include inhibiting inflammatory mediators or their downstream signalling molecules, blocking the recruitment of myeloid cells, modulating immunosuppressive functions in myeloid cells and re-educating the TME. In this review, we discuss the role of cancer-related inflammation as a potential target in the era of immunotherapy.
Immunology and Cell Biology (2017) **95,** 325–332; doi:10.1038/icb.2016.126

INTRODUCTION

In the nineteenth century, Rudolf Virchow observed 'lymphoreticular infiltrates' in neoplastic tissues, suggesting the causal link between cancer and inflammation.[1] Over the past decades, a considerable number of studies have provided evidence to support his hypothesis, and now it is recognized that inflammation is a hallmark of cancer.[2] Moreover, there is growing evidence that the composition of 'lymphoreticular infiltrates' in tumour tissues, namely tumour-infiltrating lymphocytes and myeloid cells, is a key determinant for the therapeutic efficacy of conventional chemotherapy and immunotherapy.[3] Cancer immunoediting is a process by which immune system control the quantity and quality of malignant transformed cells.[4] Under the immunoediting process, the intricate interplay among tumour cells, immune cells, and inflammation, gradually generates the pro-angiogenetic and immunosuppressive tumour microenvironment (TME), which eventually allows tumour cells to evade control by the immune system. Cancer-related inflammation has a prominent role in the acquisition of the immune-privileged character of the TME.

Recent advances in cancer immunotherapy, particularly immune checkpoint blockade therapy, have brought a turning point for the therapeutic strategies against melanoma and other advanced cancers. However, we have not yet found a way to sustain durable clinical responses by immunotherapy, or to bring the clinical benefits into a broader range of cancer types. An in-depth understanding of cancer-related inflammation will provide more solutions to overcome these challenges. Here, we will provide an overview of the role of inflammation during the process of cancer immunoediting, and the mechanisms of cancer-related inflammation. On the basis of the successful translation of anti-inflammatory agents into clinical therapy for inflammatory diseases, many attempts have been made to use anti-inflammatory against cancer. Furthermore, various novel drugs have been developed to target the immunosuppressive myeloid cells in the TME. We will also discuss the potential role of these therapies against cancer in the era of immunotherapy.

CANCER IMMUNOEDITING AND CANCER-RELATED INFLAMMATION

Chronic inflammation is an established risk factor for cancers. It was estimated that 16.1% of newly diagnosed cancers were attributable to infections in 2008.[5] In addition to microbial insult, long-term exposure to environmental stimuli also causes cancer, such as lung cancer (by cigarette smoke, silica and asbestos), mesothelioma (by asbestos) and oesophageal adenocarcinoma (by gastric acid).[6] Furthermore, chronic inflammatory diseases are associated with increased risk of cancer, as seen in inflammatory bowel diseases (risk of colon cancer), rheumatoid arthritis (lymphoma) and obesity (postmenopausal breast, colon and endometrial cancer).[7,8] More recently, Zhong *et al.* reported that patients with germline mutations in NLRP1, a member of the NOD-like receptor (NLR) superfamily, were predisposed to skin cancer due to dysregulated production of IL-1β,[9] providing the clear causative link between genetically driven

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Received 20 October 2016; revised 24 November 2016; accepted 26 November 2016; accepted article preview online 21 December 2016; advance online publication, 10 January 2017

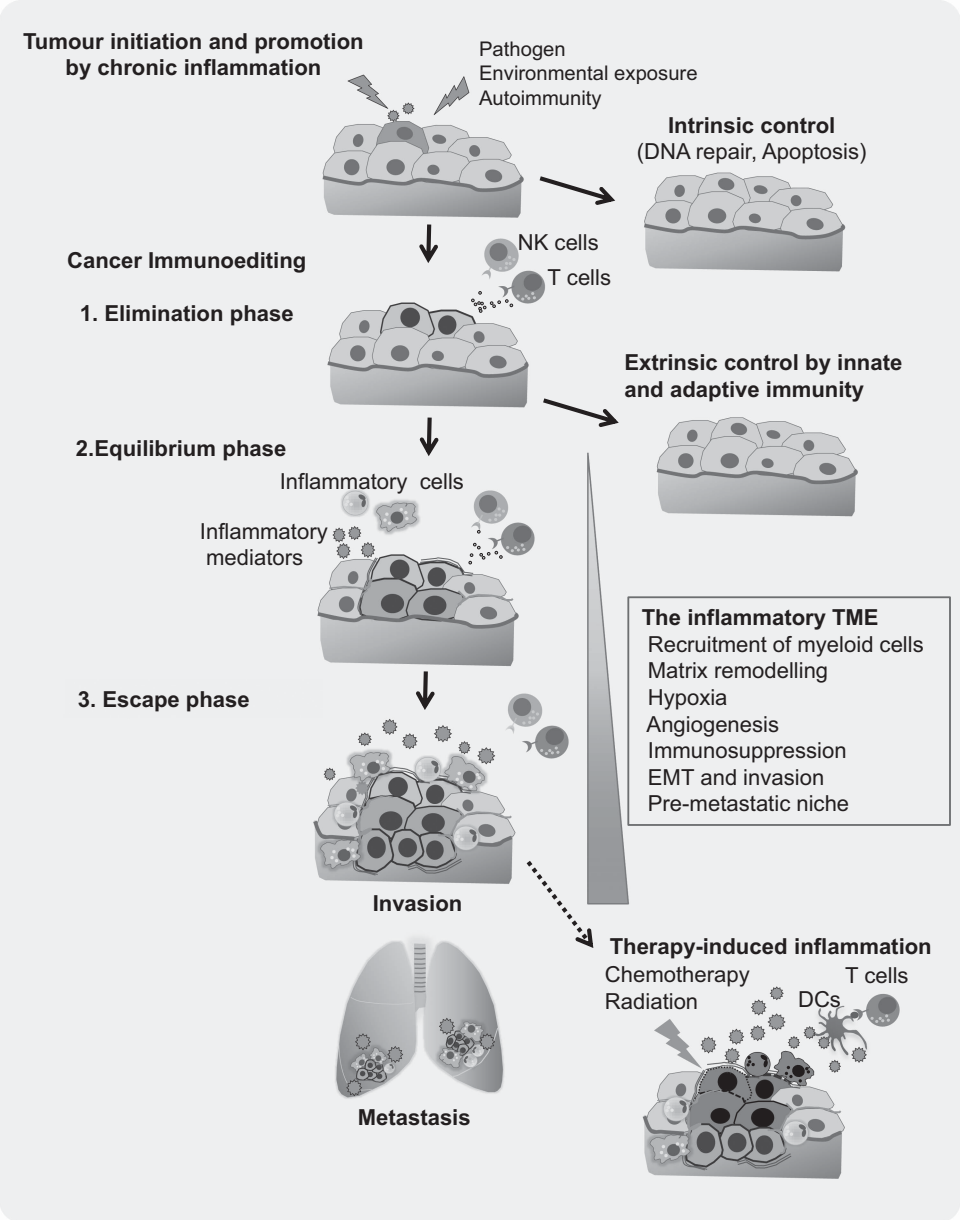

Figure 1 Cancer immunoediting and cancer-related inflammation. Chronic inflammation is involved in carcinogenesis. Malignantly transformed cells undergo intrinsic control (for example, DNA repair and apoptosis) and extrinsic control (innate and adaptive immune system). The process of immune-mediated control is now referred to as cancer immunoediting. In the elimination phase, innate and adaptive immune cells successfully recognize and kill tumour cells. In the next equilibrium phase, tumour cells are under functional dormancy by immune cells. During this phase, tumour cells are exposed to immunoselective pressure, which eventually leads to selection of immunoresistant variants. In addition, tumour-promoting inflammation confers the immunosuppressive activity to the tumour microenvironment (TME). As a result, tumour cells eventually evade the control by immune system. In the final escape phase, cancer-related inflammation contributes to the generation of the highly organized TME, characterized with marked immunosuppressive activity, angiogenesis, tissue remodelling and hypoxia. Cancer-related inflammation is also responsible for epithelial-to-mesenchymal transition (EMT), tumour invasion and generation of the pre-metastatic niche. Therapy-induced inflammation by some chemotherapy or radiotherapy can redirect the TME to fight against tumour cells through the activation of dendritic cells (DCs). Adapted from ref. 4. A full colour version of this figure is available online at the *Immunology and Cell Biology* website.

inflammation and carcinogenesis. Sustained inflammation can induce genetic instability and epigenetic modification, either directly through cytokine signalling or indirectly through generation of reactive oxygen and nitrogen species, leading to cancer initiation.[2,10]

Theoretically, malignant transformation may occur frequently in the body; however, according to the immune surveillance theory by Burnet[11] and Thomas,[12] our immune system continuously recognizes and eliminates malignant transformed cells. Now this hypothesis has been refined into a concept of immunoediting, which encompasses three phases: elimination, equilibrium and escape.[13] There is a complex crosstalk among immune cells, cancer cells and inflammation throughout these three phases (Figure 1). The elimination phase represents the original concept of immune surveillance theory. In response to oncogenic stress, malignant transformed cells express various tumour-specific antigens (neo-antigens) and self-encoded ligands, which enable innate and adaptive immune cells to recognize

and eliminate these cells by effector molecules (reviewed in ref. 4). Failure to complete elimination by the immune system leads to the progression into the equilibrium phase, where tumour cells undergo functional dormancy under immune system control. At least two factors are known to upset the equilibrium between the immune system and the tumour: reduction of tumour immunogenicity (by immunoediting) and the generation of an inflammatory TME that promotes tolerance. The importance of tumour immunogenicity is supported by the fact that carcinogen-induced sarcoma cells in the equilibrium phase are frequently rejected when they are transferred into syngeneic immunocompetent wild-type mice, whereas sarcoma cells in the escape phase can progressively develop tumour masses.[14] One of the TME factors is the balance between two pro-inflammatory cytokines, IL-12 and IL-23.[15] More recently, Wu *et al.*[16] reported that the ratio between myeloid-derived suppresser cells (MDSCs) and effector lymphocytes in sarcomas was associated with progression into the escape phase, providing further evidence that the inflammatory TME could be a driving force toward the escape phase.

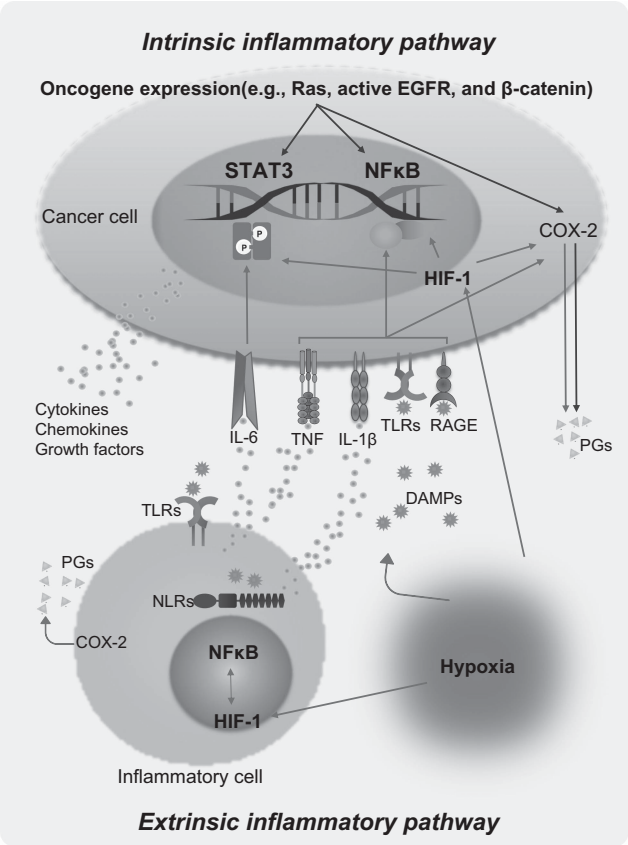

Figure 2 The mechanisms of cancer-related inflammation. Cancer-related inflammation is mediated by the crosstalk between intrinsic and extrinsic pathways. The intrinsic pathway is a cell-autonomous inflammatory response by oncogene-expressing cells. Many oncogenes (for example, K-ras) enable transformed cells to produce pro-inflammatory cytokines, chemokines and growth factors. Several oncogenes also induce the expression of cyclooxygenase-2 (COX-2) in tumour cells, which allows tumour cell to produce prostaglandins (PGs). These inflammatory mediators contribute to tumour proliferation and survival in an autocrine/paracrine manner. Moreover, these factors promote the recruitment of inflammatory cells, leading to generation of the tumour microenvironment (TME). The extrinsic pathway is mainly mediated by the recruited inflammatory cells (for example, myeloid cells) and environmental factors such as hypoxia. Disturbance of normal tissue structure in the TME results in the secretion of damage-associated molecular patterns (DAMPs). Innate immune cells recognize various DAMPs by Toll-like receptors (TLRs) or the inflammasome, leading to secretion of pro-inflammatory cytokines including TNF, IL-1β and IL-6. These cytokines support tumour survival and proliferation through the activation of NF-κB (by TNF and IL-1β) and STAT3 (by IL-6). Alternatively, some DAMPs can directly activate NF-κB in some tumour cells. Hypoxic environment stabilizes HIF-1 activity, which drives the expression of diverse pro-tumour genes, either independently or in cooperation with NF-κB and STAT3. Hypoxia-induced apoptosis and necrosis also provide a source of DAMPs. Thus, the extrinsic pathway amplifies cancer-related inflammation. A full colour version of this figure is available online at the *Immunology and Cell Biology* website.

The pleiotropic role of cancer-related inflammation has been perhaps most extensively studied in the escape phase. Pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α, either directly or indirectly, stimulate tumour survival, proliferation and angiogenesis.[1,6,10] In combination with these cytokines, stem-cell factor, GM-CSF and prostaglandins E_2 (PGE_2) from tumour sites induce aberrant myelopoiesis in bone marrow, leading to the expansion of MDSCs. Tumour-derived chemoattractants such as CCL2 and CSF-1 mobilize inflammatory monocytes and monocytic MDSCs into the TME, and both these cell types subsequently differentiate into tumour-associated macrophages (TAMs).[17] Now it is appreciated that both MDSCs and TAMs have central roles in immunosuppression and angiogenesis. It has long been speculated that inflammation promotes the invasion and metastasis of cancer. Recent studies have provided mechanistic explanations for the link between inflammation and invasion/metastasis. For example, TNF-α promotes epithelial-to-mesenchymal transition (EMT) through transcriptional and posttranscriptional upregulation of snail, a core regulatory factor for EMT.[18] Another group reported that IL-6 is also responsible for promotion and maintenance of EMT phenotype in colorectal cancer via STAT3-dependent suppression of miR-34a,[19] providing further evidence that pro-inflammatory cytokines can confer invasive capacity to tumour cells. According to the 'seed and soil' hypothesis proposed by Paget in 1889, tumour cells ('seeds') preferentially metastasize to specific sites ('soil') such as lung, liver and bone. Now this hypothesis is explained by the fact that inflammatory mediators derived from primary tumour sites can generate 'pre-metastatic niches' in distant organs, which allows engraftment of circulating tumour cells.[20]

More recently, the concept of therapy-induced inflammation has emerged as a powerful modulator of the TME. Several conventional classes of chemotherapeutic agents (for example, anthracyclines and oxaliplatin) elicit the immunogenic cell death (ICD) in tumour cells, and induce the secretion of damage-associated molecular patterns (DAMPs) from dying cells. The ICD-induced DAMPs activate dendritic cell (DC)-mediated antitumour T-cell responses. In fact, the host immune response is indispensable for the therapeutic efficacy of these drugs.[21] Similarly, radiation therapy dynamically alters the TME,[22] and increases the diversity of the T-cell receptor repertoire in tumour tissues.[23] Therefore, acute inflammation induced by some therapies can redirect the pro-tumour TME toward an antitumour immune milieu. However, it should be also noted that chronic death/injury-induced inflammation potentially promotes tumour progression,[24] implying that therapy-induced inflammation could be a double-edged sword for cancer.

MECHANISMS OF CANCER-RELATED INFLAMMATION

The mechanisms of cancer-related inflammation are undoubtedly complex. Cancer-related inflammation in the TME is differentially organized depending on cancer type, primary or metastatic site, clinical stage, past medical history or comorbidity in patients.

However, the molecular mechanisms underlying cancer-related inflammation can be summarized in the crosstalk between two pathways: intrinsic pathway (driven by oncogene expression) and extrinsic pathway (driven by noncancerous cell-derived inflammatory mediators and environmental factors). Transcription factors, NF-κB and STAT3, stand at the crossroads of these two pathways, regulating tumour survival, proliferation, angiogenesis and immunosuppression (Figure 2).

Intrinsic pathway

There is growing evidence that oncogene expression, by itself, confers pro-inflammatory capacity to malignantly transformed cells. For example, Sparmann and Bar-Sagi[25] showed that oncogenic Ras expression upregulates IL-8 (CXCL-8) in Hela cells, which leads to recruitment of myeloid cells and angiogenesis in a murine xenograft model. Other groups also showed that the expression of oncogenic Ras is sufficient to induce IL-1β, IL-6 and IL-8 production from normal ovarian epithelial cells,[26] or GM-CSF production from normal pancreatic ductal epithelial cells.[27] Similarly, active EGFR mutations can induce production of IL-6 from lung adenocarcinoma, leading to the activation of STAT3 in an autocrine manner.[28] Inducible cyclooxygenase, namely COX-2, is upregulated by various oncogenes such as K-ras, APC/β-catenin, EGFR mutations and HER2/neu.[28–30] Accordingly, the aberrant activation of the COX-2/PGE_2 pathway is frequently observed in colon, lung and breast cancer. These results indicate that malignantly transformed cells can actively initiate inflammatory responses in a cell-autonomous mechanism, which is presumably responsible for the initial development of the inflammatory TME. Subsequently, cancer-related inflammation will be amplified by recruited inflammatory cells and environment factors through the following extrinsic pathway.

Extrinsic pathway

The innate immune system acts as a first line of defence against infection. On recognition of pathogen-associated molecular patterns, pattern-recognition receptors initiate inflammatory responses to eliminate invading pathogens. It is now appreciated that pattern-recognition receptors are also responsible for sterile inflammation like that which occurs in cancer through recognition of endogenous ligands, namely DAMPs. Among the pattern-recognition receptors, toll-like receptors (TLRs) and NLRs are particularly implicated in the recognition of cancer-related DAMPs that are released in response to apoptosis/necrosis, cellular stress and matrix remodelling in the TME.[31] Although DAMPs–TLR interactions can produce IL-6 and TNF-α, the secretion of bioactive IL-1β is post-transcriptionally controlled by NLRs-containing protein complexes, called the inflammasome. As seen in antimicrobial inflammatory responses, TLRs and the inflammasome cooperatively augment inflammation in the TME.

High-mobility group box-1 (HMGB1) is a ubiquitous nuclear protein with DNA chaperone activity, but it acts as a DAMP once it is released extracellularly from necrotic cells or activated inflammatory cells. Subsequently, extracellular HMGB1 is recognized by multiple receptors including the receptor for advanced glycation end products (RAGE), TLR2 and TLR4.[32] Notably, HMGB1 can form complexes with other inflammatory mediators such as IL-1, DNA, RNA and LPS, and synergistically augment inflammatory responses. Taguchi *et al.*[33] reported that HMGB1 increases invasive capacity in tumour cells via RAGE, and that blockade of the RAGE–HMGB1 interaction inhibits tumour growth and metastasis. Moreover, Bald *et al.*[34] recently reported that HMGB1 released from ultraviolet-damaged keratinocytes induces neutrophilic inflammation in a TLR4-dependent manner, which contributes to angiogenesis and metastasis of melanoma cells. By contrast, in the context of therapy-induced inflammation, extracellular HMGB1 released by ICD-inducing drugs stimulates DCs via TLR4, leading to the activation of antitumour immune responses.[21]

Similar to HMGB1, S100 proteins, specifically S100A8 and S100A9, are recognized as DAMPs, by their ability to bind to RAGE and TLR4. Apparently, S100A8 and S100A9 are important regulators of MDSCs. In transgenic mice that overexpress S100A9 in haematopoietic cells, MDSCs are expanded due to accumulation of reactive oxygen species in myeloid progenitor cells.[35] Another group showed that the S100A8/A9 heterodimer complexes enhance the migration ability of MDSCs by RAGE-dependent activation of NF-κB.[36] Moreover, S100A8 protein derived from the primary tumour induces serum amyloid A3 in lung, which, in turn, generates a pre-metastatic niche through the recruitment of myeloid cells.[37]

Among inflammasome family members, the NLRP3 inflamamsome has an ability to recognize a broad range of DAMPs involved in various inflammatory diseases such as gout (by uric acid crystals), atherosclerosis (by cholesterol crystals) and Alzheimer's disease (by amyloid β).[38] Nevertheless, it remains poorly understood what kind of DAMPs actually activate the NLRP3 inflammasome in the TME, and how the NLRP3 inflammasome contributes to cancer-related inflammation. Adenosine-5′-triphosphate (ATP) is perhaps one the best-studied DAMPs in the context of cancer. By using *in vivo* bioluminescence imaging, Pellegatti *et al.*[39] showed that ATP is highly expressed in the TME, whereas it is not detectable in the normal tissues. Given that ATP is catabolized to immunosuppressive adenosine by the ectonucleases (CD39 and CD73) in the TME,[40] ATP-induced NLRP3 inflammasome activation and adenosine-mediated immunosuppression might coexist in the TME. By contrast, under therapy-induced inflammation, high levels of ATP released by ICD-inducing drugs can stimulate antitumour immune responses in a NLRP3-dependent manner,[21] indicating that ATP acts differentially depending on concentration and context.

Hypoxia is an indispensable environmental factor for cancer-related inflammation. Hypoxic conditions stabilize a protein called hypoxia-inducible transcription factor (HIF)-1, which allows tumour cells to adapt to a hypoxic environment through the upregulation of genes associated with angiogenesis (for example, VEGF-A), anaerobic metabolism (GLUT1) and invasiveness (MMPs), either independently or cooperatively with NF-κB or STAT3.[41] Hypoxia also promotes immunosuppression at least by two mechanisms: HIF-1-mediated functional maturation of MDSCs/TAMs and dysfunction of T cells due to metabolic stress.[42] Furthermore, hypoxia-induced apoptosis and necrosis can be a source of DAMPs in the TME. Hence, hypoxia facilitates the vicious cycle of cancer-related inflammation.

THERAPEUTIC TARGETS IN THE TUMOUR MICROENVIRONMENT

Targeting inflammatory mediators and transcriptional factors

Nonsteroidal anti-inflammatory drugs (NSAIDs). Autocrine and paracrine COX-derived PGE_2 signalling has a strong impact on all hallmarks of cancer (reviewed in ref. 43). In addition, the COX-2/PGE_2 pathway negatively regulates antitumour immune responses by at least three different mechanisms: direct suppression of cytotoxic activity in effector lymphocytes, induction of exhausted/tolerogenic DCs and enhancement of immunosuppressive activity in Treg cells and MDSCs.[44] In cancer therapy, perhaps the most striking effects of NSAIDs is chemopreventive activity against solid

malignancies, such as colon cancer.[45] Furthermore, in a recent a prospective, observational study of NSAIDs in stage III colon cancer, patients who used NSAIDs showed a significantly improved disease-free and overall survival,[46] indicating that targeting the COX-2/PGE_2 pathway is rational approach even in more advanced stages of disease. Currently, long-term effects of aspirin on recurrence and survival in various types of cancer are being tested in a phase 3 clinical trial called 'Add-asprin trial (NCT02804815)'. Recently, Zelenay *et al.*[47] reported that NSAIDs improves the efficacy of anti-PD-1 therapy through attenuation of the COX-2-driven cancer-promoting inflammation in preclinical models, although it remains unknown whether NSAIDs can actually improve the efficacy of anti-PD-1 therapy in patients.

IL-1β. As IL-1β is known as a master regulator of inflammation, anti-IL-1 therapy has been used in clinic for the treatment of rheumatoid arthritis and autoinflammatory syndromes. Owing to the potent stimulatory effect of IL-1β on IL-6 production, a phase 2 clinical trial of anakinra (a recombinant IL-1R antagonist) was performed in patients with smouldering or indolent multiple myeloma.[48] The result of this trial showed that anakinra had a favourable safety profile and improved disease stability in these patients,[48] providing the first evidence that anti-IL-1 therapy could be a potential therapeutic strategy against cancer. Anakinra is currently being tested for the treatment of various advanced cancer including pancreatic (NCT02021422), breast (NCT01802970) and colon cancer (NCT02090101). Of note, given that preclinical studies have shown that IL-1β is required for the ICD-mediated activation of antitumour immune responses,[21] optimal treatment schedule needs to be carefully designed for the combination with ICD-inducing drugs.

TNF-α. TNF-α was originally discovered as a cytokine with anti-tumour activity.[49] In fact, high doses recombinant TNF-α treatment can induce tumour necrosis *in vivo*; however, the early clinical trial of systemic TNF-α treatment showed intolerable side effects at therapeutic doses.[49] It is now also appreciated that physiological levels of TNF-α exhibit multiple pro-tumour functions in the TME. Because TNF-α blocking therapy has been established successfully for the treatment of inflammatory diseases such as rheumatoid arthritis, infliximab (an anti-TNF-α mAb) and etanercept (a recombinant TNFR2 fusion protein) have been tested in patients with advanced cancer. In a first phase 2 clinical trial of infliximab monotherapy for renal cancer, 61% of patients achieved disease stability with a median duration of 7.7 months.[50] However, in a subsequent phase 2 clinical trial, the combination of infliximab plus sorafenib failed to show any beneficial effect over sorafenib alone group in renal cancer patients.[51] Overall, anti-TNF therapy was well tolerated, but showed limited antitumour efficacy in patients with pancreatic cancer,[52] breast cancer[53] and ovarian cancer,[54] though it successfully attenuated the levels of pro-inflammatory cytokines such as IL-6 and CCL2.[53,54] In a mouse chronic inflammation model, etanercept reduced immunosuppressive activity of MDSCs and enhanced their maturation toward DCs and macrophages,[55] suggesting the possibility that anti-TNF therapy might enhance the efficacy of immune-based therapeutic approaches.

IL-6. The IL-6/Jak/STAT3 signalling cascade is a key driver for tumour progression. Silutuximab (an anti-IL-6 mAb) has been widely tested in various advanced or refractory cancers as a monotherapy. Overall, silutuximab was well tolerated in these patients, but it failed to show good clinical activity.[56,57] Among various types of cancer, multiple myeloma cells particularly require IL-6 for survival and proliferation.[58] However, in a recent phase 2 clinical trial, the addition of siltuximab to bortezomib-based chemotherapy failed to improve overall survival or complete response rate over the chemotherapy alone group.[59] Tocilizumab (an anti-IL-6R mAb) has been recently tested in combination with carboplatin/pegylated liposomal doxorubicin (PLD) and interferon (IFN)-α2b for ovarian cancer.[60] Although the carboplatin/PLD therapy can enhance antitumour T-cell responses in ovarian cancer, carboplatin is known to stimulate IL-6 production from cancer cells, which confers chemo-resistance. Therefore, this phase 1 study was intended to block the negative feedback loop by tocilizumab and to boost T-cell responses by IFN-α2b during carboplatin/PLD therapy. In fact, this combination strategy showed an acceptable safety profile, and improved immunological parameters,[60] providing a possible role of anti-IL-6 therapy in combination with chemotherapy and immunotherapy. Currently, tocilizumab is being tested in combination with albumin-bound paclitaxel plus gemcitabine for pancreatic cancer (NCT02767557).

NF-κB. Aberrant activation of NF-κB is frequently seen in many types of cancer, especially in lymphoid malignancies. Significant efforts have been made to develop inhibitors against NF-κB or IKKβ (a catalytic component required for the canonical NF-κB signalling); however, none of these inhibitors have been successfully approved due to their off-target effects.[61] The proteasome inhibitor bortezomib is a clinically approved drug against multiple myeloma and mantle-cell lymphoma given its ability to inhibit the degradation of IκBα (an inhibitory subunit of NF-κB), though it is now appreciated that bortezomib exhibits antitumour activity by multiple mechanisms of action. To overcome off-target effects by NF-κB inhibitors, Tornatore *et al.*[62] focused on the downstream targets of NF-κB that are specifically upregulated in myeloma cells, and identified the interaction between anti-apoptotic factor GADD45β and the JNK kinase MKK7 as a disease-specific therapeutic target. Furthermore, they developed an inhibitor for the GADD45β/MKK7 complex called DTP-3, which showed potent and selective activity against myeloma both *in vitro* and *in vivo*.[62] Such a disease-specific approach will be a potential strategy to target signalling molecules.

STAT3. Approximately 70% of solid and haematological cancers express constitutively active STAT3. Various STAT3 inhibitors have been developed and some of them are being tested in clinical trials. However, none of them has been clinically approved due to lack of sufficient efficacy (reviewed in ref. 63). Hong *et al.*[64] recently reported that an antisense oligonucleotide against STAT3 (AZD9150) decreased STAT3 expression in a broad range of preclinical cancer models, and that AZD9150 showed antitumour efficacy in patients with highly treatment-refractory lymphoma and lung cancer in a phase 1 dose-escalation study. The alternative strategy is targeting the JAK kinase, the upstream activator of STAT3. The first FDA-approved Jak1/2 inhibitor, ruxolitinib improves disease-related symptoms and survival in myeloproliferative neoplasms patients with active JAK2 mutations.[65] In solid malignancies, a JAL1/2 inhibitor, AZD1480, has been tested in a phase 1 clinical trial; however, it failed to proceed to the next phase due to lack of efficacy and dose-limiting toxicities. Currently, another JAK1 inhibitor, NCT02646748 is being evaluated in combination with anti-PD-1 therapy (INCB039110).

HIF-1. Using drug screening, various targets have been identified as regulators for HIF-1 activity at differential levels, such as PI3K/mTOR signalling (HIF-1 synthesis), heat shock proteins (HIF-1 stability) or hypoxia responsive element (DNA binding).[66] Interestingly, several clinically approved chemotherapeutic drugs (such as anthracycline, camptothecin and bortezomib) have shown inhibitory effects on

HIF-1 activity.[66] Obviously, these inhibitors lack specificity and have various off-target effects. An antisense oligonucleotide inhibitor (EZN2968) was tested in patients with refractory solid cancer; however, the trial was closed prematurely because the sponsor suspended the development of this inhibitor.[67] Another antisense oligonucleotide inhibitor (RO7070179) is currently being tested in patients with primary liver cancer (NCT02564614).

Targeting the myeloid cells in the TME

There are three possible strategies to target the myeloid cells in the TME: blocking their recruitment into the TME, inhibiting their suppressive activity and re-educating them to exhibit antitumour activity.[3] To block the mobilization of MDSCs/TAMs, carlumab (an anti-CCL2 mAb) was tested in a phase 2 clinical trial of advanced prostate cancer. Unexpectedly, carlumab treatment resulted in rebound increase of serum CCL2 levels, and thereby failed to show any beneficial effects.[68] CSFR1 signalling is another important regulator for the accumulation of TAMs. In murine breast cancer models, blockade of CSFR1 signalling during chemotherapy dramatically decreased the mobilization of TAMs and improved the therapeutic efficacy in a CD8 T-cell-dependent manner.[69] Various inhibitors of CSFR1 signalling have been developed (IMC-CS4, FPA008, PLX3397, AMG820, RO5509554 and ARRY-382), and they are currently being tested as monotherapy or in combination with anti-PD-1 or anti-PDL-1 therapy.

Immunosuppressive activity of MDSCs can, to some extent, be attenuated by anti-inflammatory agents described above. In addition, phosphodiesterase-V inhibitor (for example, tadarafil) decreases the immunosuppressive activity of MDSCs by downregulation of arginase1 and nitric oxide synthase-2.[70] In fact, in a recent clinical trial for head and neck cancer, tadarafil significantly decreased the percentage of MDSCs in blood and tumour tissues, and increased tumour-specific CD8 T cells.[71] Tasquinimod (a quinoline-3-carboxamide derivative) is another drug that modulates MDSCs activity by inhibition of HIF-1 activity or by blockade of the interaction between S100A9 and TLR4 or RAGE.[72] In a recent phase 3 clinical trial of tasquinimod for metastatic prostate cancer, tasquinimod significantly improved radiographic progression-free survival, but not overall survival.[73] TAMs and MDSCs highly express the tryptophan catabolic enzyme, indoleamine-2,2-dioxygenase (IDO), which induces T-cell dysfunction by depletion of L-tryptophan or by accumulation of toxic catabolites. By using IDO-deficient mice, Holmgaard *et al.*[74] demonstrated that IDO is responsible for the therapeutic resistance to anti-CTLA-4 and anti-PD-1 therapy. An IDO inhibitor, indoximod is currently being tested in combination with anti-CTLA-4, anti-PD-1 or anti-PDL-1 therapy in advanced melanoma patients (NCT02073123). The combination of indoximod and anti-PD-1 has shown good clinical responses, and the objective response rate was 53%.[75] Another strategy is to inhibit 'don't eat me' signal by blocking the interaction between CD47 and SIRPα.[76] In preclinical models, anti-CD47 blockade robustly enhanced rituximab-mediated lymphoma elimination,[77] or chemotherapeutic efficacy against pancreatic ductal adenocarcinoma,[78] indicating that restoration of phagocytic activity can redirect TAMs to eliminate tumour cells. Anti-CD47 mAb is currently being investigated in phase 1 clinical trials (NCT02678338, NCT02367196 and NCT02663518). More recently, phosphatidylinositol 3-kinase γ (PI3Kγ) in TAMs has emerged as a promising target. PI3Kγ is highly expressed by myeloid cells, and is required for the recruitment of myeloid cells into tumour tissues in response to diverse inflammatory mediators and chemoattractants.[79] In addition, PI3Kγ promotes transcription of M2-like macrophage-associated genes (such as *Arg1, Tgfb and Il10*) through activation of C/EBPβ,[80,81] indicating that PI3Kγ has a key role in both mobilization and functional polarization of TAMs. Preclinical studies showed that a pharmacological inhibitor of PI3Kγ (TG100-115) suppressed the growth of pancreatic ductal adenocarcinoma tumours and lung tumours, either as a monotherapy or in combination with anti-PD-1 therapy.[80,81]

Manipulating inflammation to harness antitumour immune responses

As described earlier, therapy-induced inflammation by ICD inducers (for example, anthracyclines) and radiotherapy potentially re-educate the immunosuppressive TME toward an antitumour immune milieu. In fact, preclinical studies have shown that ICD inducers and radiotherapy can sensitize tumour cells to immune checkpoint blockade therapy.[23,82] Growing evidence suggests that a type 1 IFN response is predominantly required for the re-education of the TME by therapy-induced inflammation, and accordingly, immunostimulatory effects can be expected from recombinant IFN and various adjuvants, such as poly I:C (an agonist for TLR3), imiquimod (for TLR7/8), CpG oligodeoxynucleotide (for TLR9) and 3′3′-cGAMP (for STING; reviewed in ref. 83). Multiple mechanisms contribute to type 1 IFN-mediated antitumour responses, including activation of innate and adaptive immune cells, regulation of suppressive functions of MDSCs and Treg, and upregulation of MHC-I expression in tumour cells (reviewed in ref. 84). Importantly, because IFN signalling upregulates both PD-1 and PDL-1 expression as a negative feedback regulation,[85,86] the combination of recombinant IFN (or IFN inducers) and anti-PD-1/PDL-1 therapy has shown synergistic antitumour efficacy against poorly immunogenic mouse melanoma models.[86,87] However, it should be noted that loss-of-function mutations in JAK1 or JAK2, and a truncating mutation in β-2-microglobulin gene have been recently identified in melanoma patients with acquired resistance to anti-PD-1 therapy,[88] raising a possibility that the IFN-based combination approach might have limited efficacy in melanoma patients with these mutations. Alternatively, CD40 agonists have shown promising results in preclinical models and clinical studies by their ability to activate DC-mediated antitumour immune responses.[89] Specifically, the pro-inflammatory cytokine IL-12 induced by CD40 agonists can attenuate T-cell exhaustion, and thereby the combination of an agonistic CD40 antibody and anti-PD-1 therapy shows antitumour efficacy against anti-PD-1 refractory tumour models.[90] However, immune-related adverse events such as cytokine release syndrome and liver toxicity are not negligible.[91]

FUTURE PERSPECTIVE

Over the past decades, significant progress has been made in understanding 'pieces of the puzzle' of complex cancer-related inflammation. Yet, we have not obtained the complete picture of the dynamic crosstalk among tumour cells, immune cells and inflammation. Recent new technologies such as *in vivo* imaging, multiplex IHC, three-dimensional cell culture systems and single-cell RNA sequencing will provide novel insights into the spatiotemporal dynamics of the TME.

As described above, most of the anti-inflammatory drugs have shown limited clinical efficacy as a single agent or in combination with chemotherapy. Presumably, these results have three implications for future cancer therapy. First, the lack of efficacy could be explained by the functional redundancy among cytokines or compensatory effects by other cytokines. In fact, even in the cytokine-driven disease such as

rheumatoid arthritis, less than half of the patients can achieve disease remission by anti-cytokine therapy.[92] Thus, multi-kinase inhibitors or dual cytokine blockade might bring more profound clinical efficacy, although we cannot ignore potential risks. Second, it will be a rational strategy to test anti-inflammatory drugs in combination with immune-based therapies, given that anti-inflammatory drugs can modulate the mobilization and functions of immunosuppressive myeloid cells. Alternatively, as supported by preclinical studies, exploiting the therapy-induced inflammation will be a promising approach to sensitize tumour cells to immunotherapy. In either approach, we need to carefully design the optimal combination, dose and schedule to maximize the therapeutic effects and to minimize the adverse effects. Lastly, because of diversity of the TME among patients, a somewhat personalized approach will bring a better outcome. Developing high-throughput biomarkers will be warranted to evaluate therapeutic effects of each drug on the TME. It will also help to predict the critical target in each patient from biopsy samples. Overall, targeting the cancer-related inflammation will potentially broaden therapeutic opportunities for patients with advanced malignancies.

CONFLICT OF INTEREST

MJS has a research agreement with Bristol Myers Squibb and Corvus Pharmaceuticals. The remaining author declares no conflict of interest.

ACKNOWLEDGEMENTS

KN is supported by the Naito Foundation and a QIMR Berghofer Seed Grant. MJS is supported by a National Health and Medical Research Council of Australia (NH&MRC) Senior Principal Research Fellowship (1078671) and a NH&MRC Development Grant (1093566).

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