The Challenges and Emerging Opportunities of Targeting Cytokines and Chemokine-Driven Inflammatory Signals in Metastatic Castrate-Resistant Prostate Cancer

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ABSTRACT: Inflammation is a key risk factor and functional driver in the initiation and progression of prostate cancer (PCa). De-regulated cytokine and chemokine signaling facilitates critical communication between tumor cells and multiple cell lineages within the tumor microenvironment (TME). Historical attempts at using targeted approaches to disrupt inflammation have been disappointing, with sub-optimal or negligible clinical benefit. Our increased awareness of the myeloid infiltrate in supporting the acquisition of castrate resistance and underpinning the abject response of advanced PCa to immunotherapy has re-focused attention on improved strategies to disrupt these complex cytokine and chemokine signaling networks within the TME. These ongoing and prospective strategies are principally focused on employing cytokine-/chemokine-directed therapies in informed combination with androgen signaling inhibitors or immunotherapeutic agents and, increasingly, with due consideration of the genetic context of the tumor. The availability of molecular-targeted therapeutic agents directed against the critical signal transduction nodes activated by cytokine and chemokine signaling in tumor cells provides opportunities to reduce the impacts of biological redundancy. Precision-based trials that deploy this latest generation of cytokine- and chemokine-directed therapeutics, directed to enriched patient cohorts in a biologically informed and biomarker-guided manner, have the potential to diversify the armamentarium of agents that is required in order to transform long-term outcomes for a currently incurable and genetically heterogenous disease.

KEY WORDS: prostate cancer, cytokines, chemokine, inflammation, microenvironment, therapeutics, tumor genetics, IL-8, TNF, MCP-1, IL-23

I. THE CONTEXT OF INFLAMMATION IN PCA

Prostate cancer (PCa) is a major healthcare burden. Over 375,000 men worldwide are reported to succumb to this disease annually.1 The majority of these deaths relate to the acquisition of a castrate-resistant state, arising from the development of multi-factorial resistance to androgen signaling inhibition, the principal mode of systemic therapy for advanced disease.2 Over the past decade, the number of agents approved for use or undergoing advanced clinical development in late-stage PCa has increased significantly, including new generation androgen signaling inhibitors (ASIs), taxane chemotherapeutics, radionuclides and poly(adenosine diphosphate)-ribose polymerase (PARP) inhibitors.3 Improvements in overall survival and progression-free survival of patients with metastatic disease have been realized by the earlier introduction of agents like abiraterone-acetate and docetaxel in hormone-sensitive patients upon first detection of metastatic disease.4–6 Biologically informed use of PARP inhibitors has demonstrated biomarker-associated responses for patients with tumors defined by DNA-damage repair deficiency, and preferentially BRCA2 mutations.7 This latter advance especially highlights the opportunity of employing biologically informed and biomarker-guided approaches as the future paradigm for extending survival and improving quality of life. However, the optimism of deploying new agents and employing them earlier in the disease spectrum must be qualified against the continuing observance that metastatic castration-resistant PCa (mCRPC) remains incurable. A further extension of the current
therapeutic armamentarium is essential to cover the significant genetic heterogeneity of mCRPC. This new arsenal of clinical agents must also be deployed in conjunction with relevant biomarkers to ensure novel but precise interventions are used to abrogate the “active and driving biology” associated with the lethal clones of mCRPC. Moreover, identifying new interventions that can be used in a strategic manner to expand the clinical use of immunotherapeutics, beyond that of a narrow genetically defined cohort of patients, will be especially beneficial to patients with high-risk PCa.

Inflammation is a well-established risk factor in the initiation and progression of PCa, that may arise from and be sustained by a multitude of contributing factors including infection from microbiota, and the effects of obesity and diet and which can promote an immune infiltrate of immune cells of both myeloid and lymphocytic origin. A milieu of inflammation-associated cytokines and chemokines are released from these infiltrating immune cells to co-ordinate the physiological response to injury and infection. However, in conditions of unresolved or sustained inflammation, the potentiating of these inflammatory stimuli can have deleterious and damaging impacts on the tumor epithelium. The accumulation of free radicals and nitrosylation by-products are considered to underpin early-onset genetic changes within the affected prostate epithelium resulting in morphological changes in the epithelial barrier, termed prostatic inflammatory atrophy and prostatic intra-epithelial neoplasia.

Cytokine-mediated signaling is also reported to modulate tumor-suppressor gene expression, resulting in the elimination of critical cell checkpoints and thus facilitating the expansion of precursor and cancerous cells. In addition, the evolving and cumulative genetic changes within the epithelial cells modulates the epithelial-derived cytokine secretome, supplementing and complementing the stromal-derived production of inflammatory mediators. Such alterations in the cytokine milieu within the tumor microenvironment (TME) can exert profound effects on the lineage profile of the immune population and the preferential differentiation status of immune cell populations, driving associated changes in phenotypic behavior. Moreover, after initial diagnosis, the initiation of conventional radiation therapy or androgen deprivation therapy can further modulate the overall inflammatory stimulus to which malignant PCa cells are subjected. This treatment-induced cytokine signaling can further orchestrate the microenvironment-mediated contribution to disease pathogenesis and treatment resistance. Consequently, inflammatory-signaling and the actions of specific cytokines and chemokines play active roles across the clinical spectrum of PCa, with evidence that several of these networks are highly selected within mCRPC.

Several excellent recent articles provide a comprehensive discussion of the circumstances underpinning the creation and amplification of “inflammatory cytokine storms” within the prostate TME and their contribution to the acquisition of aggressive hallmarks of cancer. Despite the significant burden of scientific evidence for inflammation as a driver of disease progression, there has been limited success in developing clinically effective strategies to target cytokine signaling in mCRPC, and so uncontrolled cytokine signaling remains a largely untapped therapeutic opportunity. This review focuses on the challenges, considerations, and future opportunities to perturb cytokine and chemokine signaling networks in mCRPC with the intent to disrupt microenvironment-mediated pathogenesis and, critically, to enhance the magnitude and duration of therapeutic response to current clinical treatment regimens. The importance of evaluating inflammatory cytokine/chemokine signaling as therapeutic targets in PCa is all the more acute given our current difficulty of treating a genetically heterogeneous disease with a narrow therapeutic arsenal, and the abject and sub-optimal responses observed with immunotherapy to date.

II. INDIRECT MANAGEMENT OF INFLAMMATORY CYTOKINES IN PCA

Glucocorticoids (GCs), predominantly prednisolone and dexamethasone (DEX) have been extensively employed in the clinical management of mCRPC, with intent to: (1) treat the pain, inflammation, and reduce the metastasis-associated edema, (2) alleviate disease and treatment-associated fatigue,
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and (3) as a pre-medication to support taxane chemotherapy.\textsuperscript{39} GCs provide clinical benefit and objective responses in mCRPC, measured by decreased prostate-specific antigen (PSA) levels,\textsuperscript{40,41} with DEX showing superior responses to prednisolone.\textsuperscript{41} Their long-term use in the clinic, predominantly in combination with androgen deprivation or antigen receptor (AR)–targeting agents, is justified by their inhibitory action on adrenocorticotropic hormone secretion, reducing systemic adrenal androgen levels, decreasing transcription factor activity, and attenuating cytokine gene expression and synthesis.\textsuperscript{39,42} Furthermore, GCs suppress AR expression\textsuperscript{43} and have been reported to reduce PCa cell-derived synthesis of pro-angiogenic factors,\textsuperscript{44} with further illustration of their impact in potentiating the antiangiogenic activity of the chemotherapeutic agent docetaxel.\textsuperscript{45}

GCs activate steroidal glucocorticoid receptors, one consequence of which is to regulate cytokine expression and function at the levels of transcription, mRNA stability, translation and/or post-translational processing.\textsuperscript{46} GC receptors are highly expressed in PCa cancer specimens, with reports of higher expression localised to stromal cells rather than malignant prostate cells.\textsuperscript{47} The high burden of tumor-associated macrophage (TAM) infiltration in mCRPC\textsuperscript{48} supports the continuing use of DEX to target inflammatory and cytokine-driven events. Future use of GCs and DEX in PCa therapy may be enabled through nanotechnology, employing lipid-encapsulated strategies to further potentiate the selective targeting of DEX therapy to these TAM populations.\textsuperscript{49}

Despite these aforementioned benefits and use of low dosage regimens, the ongoing use of GCs in clinical practice is a matter of debate. Sequencing analysis of advanced prostate tumors has characterized the existence of GC-responsive mutations in the AR, which may enable adverse contributions of low dose GCs to disease progression and castrate-resistance.\textsuperscript{50} Increased expression of glucocorticoid receptors within tumor cells has also been documented as a bypass mechanism of steroid hormone-mediated castrate resistance.\textsuperscript{51} Furthermore, GCs are potent immunosuppressants and inhibit the production of macrophage-derived tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β), which facilitate an immunosuppressive TME through the innate immune response.\textsuperscript{52} Indeed, immunosuppressive doses of DEX potentiate LPS-mediated metastasis of experimental PCa in vivo,\textsuperscript{53} consistent with the important roles of inflammation and macrophages in disease progression, and the importance of immune-surveillance and the innate immune system in reducing invasion and metastasis. The extensive and prolonged course of DEX and GC therapy may aid the establishment of an immunosuppressive TME, given the high myeloid infiltration reported in mCRPC.\textsuperscript{22,23,48} The widespread adoption and longitudinal use of GCs in treating patients with hormone-sensitive and mCRPC may therefore sustain an immunosuppressive TME. Together with the lower mutation burden of the disease, this GC-induced immunosuppression may contribute to the poor clinical response of mCRPC to immune checkpoint blockade therapeutics.\textsuperscript{10} Consequently, prospective uses of more targeted selective strategies to perturb specific cytokine signaling pathways may have greater future utility in abrogating key sustaining signals for the tumor, but importantly, not hindering the potential to maximise a therapeutically induced immune response within the TME of mCRPC.

III. THE IMPACT OF GENETICS ON CYTOKINE AND CHEMOKINE SYNTHESIS AND SIGNALING IN PCA

Multi-omic characterization of mCRPC has provided a detailed landscape of the genetic heterogeneity of the disease and a framework to rationalize the development and evaluation of novel molecular-targeted therapies at different stages of disease progression.\textsuperscript{36–38} Many of the principal genetic events detected in mCRPC can regulate and modulate cytokine and chemokine synthesis from malignant epithelium. As one of the prevalent re-arrangements in PCa, the TMPRSS2-ERG fusion regulates cytokine gene transcription, including IL-6 and TGF-β.\textsuperscript{54} PTEN deficiency/PI3K mutation–induced pathway activation is a further prevalent and early-onset genetic event, further enriched in mCRPC in PCa.\textsuperscript{18} PTEN loss-of-function potentiates intracellular
stimuli-induced Akt and MAPK signaling, promoting downstream NF-κB and AP-1 transcription, each of which serve as key regulators of transcription of multiple cytokine and chemokine genes. Transcriptional regulation of IL-8 (or CXCL8), its orthologs CXCL1 and CXCL2, its murine ortholog Cxcl15, and their receptors, CXCR1 and CXCR2 is strongly associated with PTEN loss of function in human cell-based models and genetically engineered mouse models (GEMMs) of PCa, and human PCa tissue, where definition of a PTENlow/CXCR1-CXCR2high signature is prognostic of adverse clinical outcomes. Prostate tumors defined by the elevation and promotion of Wnt signaling, with underpinning neuroendocrine characteristics, are also subject to elevated IL-8/CXCR2 expression and signaling, promoted through the activation of the TCF7L1 transcription factor. This highlights the further relevance of the pro-inflammatory IL-8 driven signal across a range of genetically defined disease stratifications. Further studies that define the hierarchy of specific cytokine and chemokine networks that are operational within genetically annotated tumor subgroups will be essential to defining optimal use of cytokine- and chemokine-directed therapeutic interventions.

Inflammatory cytokines are functional elements contributing to creation of a senescence-associated secretory phenotype (SASP) arising from stress-associated oncogene-signaling or tumor suppressor gene loss in epithelial cells at early stages of carcinogenesis. Many of these contributing cytokines serve to facilitate growth arrest and enable immune surveillance within the localized environment. Targeting cytokine function may therefore disturb the careful equilibrium of anti- and pro-tumorigenic forces. Use of prostate-restricted GEMMs has both demonstrated the acquisition of SASP and enabled greater understanding of the mechanisms regulating SASP function. For example, loss of PTEN induces the SASP, consistent with morphological characterization of PTEN-deficiency with pre-malignant prostatic intra-epithelial neoplasia in human prostate biopsy tissue. IL-6–induced STAT3 signaling promotes cellular senescence in these models. Subsequent transgenic models modeling the effects of concurrent PTEN and STAT3 deletion identified the importance of a STAT3-Arf-MDM2-mediated activation of p53-induced cell cycle arrest and apoptosis in maintaining the SASP phenotype. It is therefore intriguing to speculate on the importance of p53 functional status as a key regulator in transitioning cytokines from supporting a senescence phenotype towards enablement of a pro-tumorigenic phenotype when p53 is impaired. Such observations are consistent with results derived from earlier GEMMs where the progression to invasive adenocarcinoma was only observed in prostate-restricted PTEN-deficient models with co-operative loss of functional p53 loss or Myc gain-of-function. Importantly, PTEN-deficiency and p53 inactivation is heavily enriched in mCRPC with aberrations reported at 40.7% and 53.3%, respectively, and is correlated with adverse clinical outcome including reduced time-to-progression on ASI-based therapy. Contextually, this underlies the potential importance of adopting precision approaches based on the predominant genetic backgrounds to target the disruption of specific cytokine networks; for example, negating the pro-tumorigenic function of IL-6 signaling may demonstrate improved response under PTEN-deficient and p53-impaired contexts of aggressive disease.

IV. THE IMPACTS OF INHIBITING ANDROGEN SIGNALING ON CYTOKINE AND CHEMOKINE REGULATION

The persistent influence of androgen signaling across the spectrum of PCa and its amplified expression in mCRPC underpins its selection as the mainstay of targeted therapeutic intervention and the commercial focus on developing subsequent generations of ASIs. Many intrinsic mechanisms contributing to ASI drug resistance have been described, including amplification of the AR gene, the expression of AR gene variants, mutations within the androgen synthesis and metabolizing pathways, and more recently, the impact of adaptive or cellular plasticity. More recently, there has been an emerging awareness of the impacts of external microenvironment-associated factors, including the potential impacts of cytokine signaling on the acquisition of resistance.
Expression of the AR is not exclusive to the epithelial cells of the prostate gland, since it is expressed in other compartments of the prostate stroma, including cancer-associated fibroblasts (CAFs).64 Low AR expression in CAFs is prognostic of poor patient outcome, supported by functional studies indicating that activation of AR signaling in these cells inhibits cellular proliferation and supports the matrix attachment of PCa cells and CAFs, thus attenuating cellular invasion.65 Chromatin-immunoprecipitation analysis confirms that AR signaling represses the expression of motility promoting chemokines including CCL2 and CXCL8 in stromal fibroblasts.66 Consequently, in contexts of reduced stromal AR expression in CAFs, or ASI-attenuated AR function, the loss of stromal-derived AR-mediated gene repression may dynamically change how the TME orchestrates disease pathogenesis. Sfanos and colleagues have also demonstrated the ability of the AR to repress IL-8 gene transcription in PCa cell lines, such that under AR inhibition, the synthesis of IL-8 from tumor epithelium is also elevated in these experimental models.31

AR is also expressed in the vascular endothelium of human prostate biopsy tissue.67 Administration of anti-androgens and clinically used AR antagonists causes a rapid vascular collapse and a sustained hypoxic TME in growing human PCa xenografts.68-70 The sustained hypoxia that develops within these prostate tumors promotes a dynamic angiogenic response to AR-targeted therapy, co-incident with a loss of tumor control, indicating a potential contributory role of the TME in therapeutic resistance. Hypoxia and acidosis are key regulators of IL-8 gene expression, while CXCR1 and CXCR2 expression in PCa cells is also induced by hypoxia-induced HIF-1 and NF-κB-driven transcription.71,72 Consequently, experimental evidence suggests that malignant, vascular and immune cells are all subject to a higher level of autocrine and paracrine IL-8/CXCR2 signaling as a consequence of ASI-mediated re-activation of IL-8 gene expression and the indirect actions of ASI-induced hypoxia on IL-8 and IL-8 receptor expression (Fig. 1). Therefore, ASI-treated prostate tumors, especially those with an underlying PTEN-deficient genotype, may be especially sensitive to inhibitors that abrogate the signal transduction of this chemokine across the TME.

High neutrophil-to-lymphocyte ratios (NLRs) are associated with adverse outcomes to ASIs and chemotherapy.10 The AR is universally expressed in numerous immune lineages, including neutrophil lineages at all stages of differentiation, ranging from proliferative to mature.73 Evidence from both clinical and experimental conditional knock-out models (ARKO) indicates that AR functionally regulates neutrophil expansion and differentiation, leading to increased susceptibility to bacterial infections in the knock-out mice.74 Conversely, AR signaling exerts inhibitory actions on T cell development, T cell activation and inhibits Th1 differentiation.73 As such, there is a mechanistic basis to correlate NLRs with outcome. The cell-specific knockdown of AR expression in granulocytes confirms a lower production of key cytokines including IL-1β, IL-6, and TNF-α from the innate immune compartment, which may reduce overall inflammatory burden within the TME.75 However, AR-inhibition can simultaneously promote increases in macrophage-derived pro-angiogenic and pro-tumorigenic chemokines including CXCL1, CXCL8, and CCL2.31,64,66 These latter cytokine/chemokine profiles may drive alternate phenotypic behaviors, including the acceleration of tumor motility, proliferation and the development of therapeutic resistance. As such, the use of NLRs to predict clinical outcome to current ASI and chemotherapeutic options in metastatic hormone-sensitive and mCRPC could be enhanced by more detailed understanding of the ratio of critical anti-tumor, pro-tumor, and immunosuppressive cytokines/chemokines.

Increased expression of IL-6, CXCL10, IFN-α, and IFN-γ have also been reported after AR inhibition in adipocytes, indicating a further contribution of peri-prostatic adipose tissue to stromal adaptation to ASI treatment.76 Other studies modelling the impacts of high-fat diet and obesity on PCa growth have identified adipose-derived IL-17 signaling and IL-6 signaling in promoting tumor growth, while production of CCL7 was associated with adipose-promoted localized invasion.77,78 Perturbation of these cytokines and chemokines may therefore be considered or indicated in patients with high body-mass index.
V. MYELOID-DERIVED CYTOKINE AND CHEMOKINES IN THE TME OF MCRPC

The prevalence of TAMs and high levels of MDSC infiltration establish the critical functions of these two lineages in orchestrating the functional contribution of the TME to tumor pathogenesis.\textsuperscript{22,48} Several cytokines have prominent roles in regulating macrophage differentiation and function, leading to translational opportunities to directly target CSF-1R and GM-CSF, in addition to alternative indirect strategies that can be used to deplete or reprogram macrophages.\textsuperscript{48} The complexity of IL-6 signaling within the TME of PCas has also recently been reviewed, and therapeutic opportunities discussed.\textsuperscript{34,79} We will focus on summarizing the contributions of IL-8, monocytic chemotactic protein 1 (MCP-1) and TNF signaling in pathogenesis and drug resistance, as their serum expression levels correlate with shorter time-to-progression of patients on ASI therapy in large clinical trial cohorts.\textsuperscript{80,81} Moreover, we will introduce the emerging role of MDSC-derived IL-23 as a novel cytokine with a recently discovered role in promoting castrate-resistant disease.\textsuperscript{32}

A. IL-8 Signaling

Cancer cell-associated IL-8 expression and expression of its receptors CXCR1 and CXCR2 correlate with stage of PCa, and correlate with increased proliferation and microvessel density.\textsuperscript{13} Autocrine effects in PCa cells include the potentiation of cell proliferation and promotion of castrate-resistant transition, evasion of apoptosis,
therapeutic resistance and acceleration of cellular invasion and experimental metastasis. These are underpinned by activation of signal transduction pathways that regulate the synthesis of oncoproteins and potentiate the activation of numerous transcription factors, including AR, HIF, and NF-κB. IL-8 signaling can promote castrate-resistant transition through direct regulation of AR expression and activation in PTEN-deficient PCa cells. Administration of a first-generation CXCR2 inhibitor potentiated bicalutamide response in PCa cells. IL-8 signaling stimulates expression of critical anti-apoptotic proteins, including Bcl-2 and c-FLIP, the latter of which serves to diminish the effectiveness of AR therapeutics in experimental models. Moreover, increased expression of CXCR2 has been proposed as a marker of neuroendocrine PCa (NEPC), although the detailed mechanistic basis and functional contribution of this signaling as a driver of plasticity needs to be defined.

CXCR1 and CXCR2 receptors are expressed on multiple cell types present within the TME including vascular endothelial cells and innate immune cells, indicating the diverse range of cancer hallmarks influenced by the complex inter-cellular signaling mediated by IL-8, including angiogenesis, escape from immune surveillance, and localized invasion. Paracrine CXCR2 signaling especially has been associated with two contemporary adaptive mechanisms associated with therapeutic resistance of mCRPC. Activation of CXCR2 signaling underpins both myeloid-derived suppressor cell (MDSC) infiltration and M2-macrophage differentiation in murine PTEN-deficient prostate tumors, and PTEN-deficient foci of human PCa biopsy tissue. The importance of IL-8 signaling in sustaining an immunosuppressive microenvironment has also been supported by recent publications, which demonstrate the correlation of IL-8 signaling inhibition with enhanced T cell activation, and highlighting its role in potential resistance mechanisms to immune checkpoint inhibitors. This raises the possibility that contextual administration of CXCR2 inhibitors may not only enhance therapeutic response to ASIs but importantly, modulate the immune landscape to such a degree that it creates a permissive microenvironment that reveals an improved response to immune-oncology therapies in mCRPC.

B. TNF Signaling

TNF-α is a pro-inflammatory cytokine principally secreted by inflammatory cells (macrophages) and cancer cells but is also produced by stromal cells within the TME. Cellular signaling induced by TNF-α occurs through interaction with two related single-spanning type I transmembrane protein receptors, TNFR1 and TNFR2. Although TNFR1 is almost ubiquitous in expression, TNFR2 expression is selectively expressed on myeloid cells, Tregs, some tumour cells and cells of the TME. Interestingly, TNF-α signaling has been implicated in opposing roles in cancer progression. Generally, signaling via TNFR2 primarily results in proliferation, modulation of immune response and cell survival, whereas signaling via intracellular death domain containing TNFR1 is considered to activate the classical NF-κB pathway and caspases to induce apoptosis and cell death. However, it is increasingly appreciated that TNF-α signaling through TNFR1 exerts complex effects on tumor cells. For example, in direct contrast to the classical scenario of caspase activation and cell death, TNFα1 signaling can also facilitate cell survival, mediated through the recruitment and activation of inhibitor of KB (IKB) kinase (IKK), which triggers the ubiquitination and subsequent degradation of IκB and promotion of NF-κB-promoted transcription of anti-apoptotic genes such as BCL-XL and c-IAP-1/2. Expression of c-IAP-1/2 is the key determinant of this phenotypic switch arising from TNFR1 signaling. In the presence of c-IAP-1, TNF-α signaling enables cell survival through activation of the canonical NF-κB pathway. Conversely, in the absence of c-IAP-1, which can be affected by the administration of c-IAP-1 antagonists, TNF-α signaling is channeled towards a cell death promoting activity.

TNF signaling is associated with several critical functions in PCa. Histological analysis of PCa tissue documents strong epithelial TNF-α expression compared with normal prostatic tissue.
Functionally, elevated TNF signaling potentiates androgen-mediated cell proliferation of LNCaP cells,67 supporting a role in castrate-resistant transition, and supporting prior clinical correlations of serum TNF concentrations and time-to-progression.80,81 The wider impacts of TNF signaling upon the modulation of immune cells within the wider prostate TME as an aspect of accelerating disease progression remains to be fully characterized. However, recent studies in a murine melanoma model has demonstrated important impacts of TNF signaling upon the lymphocytic infiltrate; TNF-induced TNFR1-signaling promoted death of CD8+ tumor infiltrating lymphocytes, underpinning an immune-evasive phenotype. This was reversed by inhibition of the TNF-signaling pathway.98 Although our understanding of TNF signaling in the prostate TME is complex, nuanced and incomplete, there is accumulating evidence to support the importance of targeting TNF signaling in PCa to combat advanced disease.

C. MCP-1 Signaling

MCP-1, also known as CCL2, is a potent chemotactic recruiter of monocytes and macrophages into the TME. Interactions between MCP-1 and its principal receptor, CCR2, have been implicated in PCa progression, metastasis, and relapse.99 Cancer cell-derived MCP-1 recruits CCR2-expressing myeloid-derived suppressor cells (MDSCs) to the TME, creating an inflammatory and immunosuppressive TME.100 MCP-1 is produced by many cellular constituents of the TME, including macrophages, fibroblasts, endothelial cells, and cancer cells themselves.101 In vitro and in vivo studies have demonstrated that MCP-1 enhances the proliferation, migration, and survival of PCa cells through a PI3K/Akt-dependent pathway,102 and that these effects can be abrogated with CCL2 attenuating antibodies.100,103 In a murine xenograft study, neutralizing antibodies that targeted either stromal-derived MCP-1 or tumor-derived MCP-1 resulted in attenuated bone metastases, suggesting that both tumor and stromal-derived MCP-1 contribute to PCa progression.103 The secretion of MCP-1 by the prostate TME contributes to the development of an inflammatory TME, sustained by the potentiation of MCP-1 secretion by additional inflammatory cytokines IL-1β, IFN-γ and IL-2, all of which are also implicated in PCa progression.104

Increasing MCP-1 and CCR2 expression are associated with progression into advanced PCa. Immunohistochemistry analysis of MCP-1 receptor, CCR2, in localized PCa or benign prostate tissue samples revealed that increased CCR2 expression correlated with higher Gleason score and clinical stage.105 Similarly, Loberg et al. described an increase in PCa epithelial tissue CCR2 expression that positively correlated with proliferative inflammatory atrophy and Gleason score.103

Serum MCP-1 levels are significantly increased in PCa patients with confirmed bone metastases compared with patients with localized disease.106 Furthermore, increased MCP-1 expression was found in tumour cells, endothelial cells, and extracellular areas surrounding neoplastic glands in advanced state PCa with higher Gleason score, compared with low stage PCa.107 This increased MCP-1 expression coincided with a higher degree of CD68+ macrophage infiltration into neoplastic lesions, and with a decrease in 5-year PSA recurrence free survival, supporting a functional role of increased MCP-1/CCR2 expression in poorer PCa outcomes.

Blocking AR signaling can drive the inflammatory process in PCa and promote disease progression, and interactions between surviving PCa cells and TAMs after AR-targeted therapy may facilitate PCa progression. Izumi et al. reported that ablating AR expression in LNCaP and C4-2 PCa cells and M2 macrophage-like THP-1 cells increased expression of MCP-1, and that MCP-1 expression was synergistically potentiated when the cells were co-cultured.108 The elevated MCP-1 signaling promoted the adoption of an EMT phenotype, increased cancer cell migration and activation of STAT3 (pSTAT3) within the tumor cells. Treatment with anti-androgens, enzalutamide and bicalutamide, has also been reported to enhance pSTAT3/MCP-1 pathway activation, which promotes macrophage migration and PCa cell invasion.109 These results suggest that standard AR targeted therapies contribute to the development of more aggressive
diseases and that MCP-1 may play a key role in this pathway.

D. IL-23 Signaling

The high burden of MDSCs within the TME has been shown to directly contribute to disease progression and the acquisition of castrate-resistance. Expression profiling conducted in castrated GEMMs of PCa revealed the concurrent increase in IL-23 and a subunit of the IL-23 receptor as two of the most up-regulated genes under castrate-resistant conditions. Using conditioned media, MDSC-derived IL-23 was shown to activate the IL-23 receptor expressed on PCa cells. Higher levels of this cytokine were also detected in CRPC patients supporting the clinical relevance of these observations. IL-23-induced signal transduction in tumor cells activated JAK2/STAT3/ROR-gamma signaling, leading to an induction of AR gene transcription and subsequent regulation of AR-regulated genes. Inhibition of IL-23 signaling using neutralizing antibodies or genetic-knockout strategies attenuated the onset of castrate-resistance in mice and moreover was shown to potentiate the sensitivity of murine tumors to AR-targeted therapy. More recent studies have suggested that IL-23 signaling prevents cellular senescence of CRPC cells induced by AR-targeted agents, enzalutamide and darolutamide.

VI. TRANSLATIONAL PERSPECTIVES TO OPTIMISING CYTOKINE AND CHEMOKINE DISRUPTION IN ADVANCED PCA

A. Overcoming the Pleitropic Nature of Chemokines

There are a number of obstacles that have hindered the targeting of cytokine and chemokine function in patients. The first significant barrier concerns the significant biological redundancy associated with their signal transduction properties, which arises from: (1) many cytokines inducing activation of common signaling pathways, (2) the multiplicity of orthologous family members activating their cell-surface receptors, or (3) the promiscuity of individual cytokines to induce activation of multiple cell-surface receptors.

Early attempts to reduce extracellular cytokine or chemokine stimuli employed neutralizing antibodies to the ligands (Fig. 2). Critical difficulties associated with using neutralizing antibodies to target chemokine signaling have been illustrated by early experiences with Carlumab (CNTO888), a human anti-CCL2 mAb which exhibited strong pre-clinical anti-tumor efficacy in experimental models of PCa but failed to achieve sufficient and sustainable reductions in serum CCL2 expression in patients. A monoclonal antibody (CNTO328; Silatuximab) has also been employed to attenuate IL-6 signaling in PCa patients. Although pharmacodynamic analysis of patient samples determined that the antibody was effective in repressing known IL-6 signaling pathways, including JAK/STAT3 and p44/p42 MAPK, and in reducing expression of IL-6-regulated genes, trials evaluating monotherapy with this antibody revealed no patient benefit in CRPC.

More recent trials have shown effective repression of serum IL-8 levels in patients with solid tumors after administration of the monoclonal anti-IL-8 antibody BMS-986253. Further evaluation of this antibody is ongoing in a number of solid tumors, informed by pre-clinical observations that repression of IL-8 signaling reduces MDSC infiltration and therefore modulates T-cell activity. Of major significance, the MAGIC-8 protocol is specifically evaluating the ability of BMS-986253 to improve the efficacy of immunotherapy (Nivolumab; anti-PD-1) when combined with androgen deprivation therapy in men with hormone-sensitive PCa. This design illustrates a major conceptual advance in trial design, employing the neutralizing antibody as part of a combination strategy, and secondly, informed by key observations in pre-clinical studies.

Defining the optimal strategy for attenuating chemokine signaling is a matter of debate. The use of a monoclonal antibody to suppress IL-8 signaling will not eliminate the pro-tumorigenic actions of the orthologous CXC-chemokines CXCL1, CXCL2, and CXCL5 that have been detected and reported to impact on PCa pathogenesis. Critical reductions in IL-8 expression alone may
prove to be sufficient to switch the TAM and MD-SC-enriched immunosuppressive TME of mCRPC to that of a more permissive setting, enabling a Nivolumab-promoted induction of a T-cell-mediated immune response in MAGIC-8. Translational studies measuring CXC-chemokine ligand expression together with detailed evaluation of changes in the immune infiltrate will be an important consideration in evaluating the results of the MAGIC-8 trial.

Alternative approaches have sought to target the impacts of CXC-chemokines using small molecule inhibitors of the cell-surface G-protein coupled receptors that transduce their biological activity. ACE, a phase I/II clinical trial in mCRPC is currently evaluating whether addition of the CXCR2-selective inhibitor AZD5069 increases the capacity to prolong and restore enzalutamide response in men with rising PSA. Translational studies will determine whether blockade of the
CXCR2 receptor is sufficient to reverse NLRs in patients and inhibit MDSC infiltration and activity within the metastatic microenvironment. However, the pharmacological focus on inhibiting ligand-induced CXCR2 receptor activation does not account for the potential redundancy of residual IL-8 to activate CXCR1 receptors also expressed on malignant, stromal and immune cell lineages within the mCRPC microenvironment (Fig. 2). The development of SX-682, SX-682 an orally bioavailable, potent allosteric inhibitor of CXCR1 and CXCR2 provides an alternate opportunity to explore this aspect of redundancy. Current trial designs are investigating the potential of SX-682 to improve the clinical response of Pembrolizumab in metastatic melanoma, which may inform future strategies to employ this agent in mCRPC.

B. Use of Chemokine or Cytokine Agents in Combination Therapeutic Approaches

Earlier trials of the monoclonal anti-CCL2 and anti-IL-6 antibodies highlight the concerns of using singular cytokine-directed therapies, and especially within the context of genetically heterogenous disease. More recent studies have concentrated on combining CXCR2 inhibitors with immunotherapy agents, indicating that the true value of disrupting cytokine and chemokine pathways may be revealed from combinatorial hits on the TME. Advocacy for greater use of cytokine/chemokine inhibitors in combination is also supported by earlier pre-clinical research where IL-6 and IL-8 have been identified as key drivers of androgen-independent AR signaling. CXCR2 pathway inhibition attenuated intracellular AR expression and gene transcription and potentiated bicalutamide sensitivity in LNCaP cell lines in vitro, whereas AZD5069 attenuated MDSC-mediated castrate-resistance and extended the anti-tumor effects of enzalutamide in vivo. Such results highlighting the multi-factorial benefits of employing CXCR2 inhibitors in combination with AR therapy have underpinned the design and conduct of the currently recruiting phase I/II ACE Trial. Other pre-clinical studies indicate the potential of CXCR2 inhibition to potentiate the efficacy of platinum-based chemotherapy, anti-metabolites, and TRAIL agonists in vitro.

C. Targeting the Critical Intracellular Signaling Nodes of Multiple Cytokines

Many extracellular cytokines and chemokines converge to activate a bespoke number of intracellular signaling pathways. Identifying these critical signaling nodes provides therapeutic opportunities to circumvent aspects of biological redundancy arising from the synthesis of multiple cytokines and receptor promiscuity (Fig. 2). For example, activation of the IL-6R and IL-23R both result in the autophosphorylation of Janus-activated kinases (JAK)-1/2, which then promote activation of STAT3 signaling. Pharmacological strategies directed at targeting either of these receptors may therefore be compromised by the capacity of the alternate and untargeted receptor to maintain activation of the intracellular signaling pathway. STAT3 has also been identified as a key signaling intermediate of IL-8, thus providing a concurrent approach to attenuate the disease-promoting function of this chemokine. Blockade of JAK2 and STAT3 signaling has been shown to potentiate AR-targeted therapy in PCa models, while inhibition of STAT3 has been shown to induce both innate and adaptive immune pathways resulting in the resolution of primary and bone metastatic prostate lesions in vivo. No clinical data are yet available from targeting of this pathway. However, the plethora of pre-clinical studies indicates that use of the available small-molecule and antisense oligonucleotide-based inhibitors of JAK2 and STAT3 signaling, respectively, may provide desirable clinical outcomes if employed in combination with AR therapies or immune-oncology agents.

Akt activation is a common signaling effector of many pro-inflammatory chemokines. Moreover, Akt is a key intermediary that modulates the transcription of numerous CXC- and CC-chemokine genes. PTEN-deficient or hyperactivated PI3K-CA-mutated prostate tumors exhibit heightened levels of Akt activation, consistent with demonstration of elevated chemokine expression and sensitivity of these tumor cells to chemokine action. The Sawyers laboratory identified existence of a reciprocal
feedback pathway between the activation of the PI3K/Akt/mTOR and AR signaling pathways, such that under conditions of effective AR inhibition, PCa cells became dependent upon increased flux through the PI3K/Akt/mTOR signaling cascade to promote cell survival and proliferation. Akt inhibition using AZD5363 (capivasertib) was shown to extend the durability of enzalutamide response in Pten-deficient experimental models in vivo. The Re-Akt clinical trial has also confirmed this benefit in patients; administration of capivasertib demonstrated anti-tumour activity in a phase I study in patients, with clinical response correlating with tumors with confirmed genetic aberrations within the PI3K/AKT/mTOR pathway. Similarly, administration of the pan-Akt inhibitor ipatasertib has extended the radiographic progression-free survival (rPFS) observed in patients being treated with abiraterone-acetate, in independent phase II and phase III trials. Importantly, the positive effect on rPFS was greater in those patients with confirmed PTEN-loss. Further translational analysis of tumor samples will be essential to define the relationship of Akt inhibition to reduced chemokine function, changes in specific compartments of the immune infiltrate and the underpinning correlation to PTEN-deficient and PI3KCA-mutational status of the tumor. However, these early trial results give encouragement for precision-guided use of Akt inhibitors to attenuate chemokine and cytokine-drive tumor progression.

VII. CONCLUSIONS

Pre-clinical research continues to document the important contributions of de-regulated pro-inflammatory cytokine and chemokine signaling in the pathogenesis and therapeutic resistance characteristic of mCRPC. We now have an exciting opportunity to consider new approaches that go beyond the use of GCs to target inflammation-driven progression of disease.

Our more recent discoveries have been progressed using increasingly complex, genetically annotated experimental models of PCa, with an increasing emphasis on defining the essential function of cytokines and chemokines in driving communication between cancer cells, the immune infiltrate and the TME. Importantly, the discovery science strongly advocates that cytokine and chemokine inhibitors are best deployed in combination therapeutic approaches, including intent to extend the duration-of-benefit of ASI or to radically transform the role for immunotherapy in mCRPC. A new generation of cytokine/chemokine-directed therapeutics are now available for or are already undergoing clinical evaluation, including neutralizing monoclonal antibodies directed to specific cytokines or chemokines, small-molecule inhibitors or blocking antibodies to cell-surface receptors or agents directed at key intracellular mediators of cytokine/chemokine-promoted signaling. Intensive translational efforts must focus on generating an informed basis to select the optimal therapeutic approach, either through establishing the hierarchical importance of individual cytokines/chemokines within genetically annotated mCRPC disease subtypes, or in the context of emerging drug-resistance and tumor plasticity, or alternatively, in the presence of distinct immune infiltrates. Although high NLR values have been used as a surrogate for inflammation-driven disease and to predict adverse outcomes to therapy, prospective research will need to concentrate on the discovery of new companion diagnostics that offer more specific patient-enrichment strategies, including next generation sequencing-based diagnostics, plasma-based cytokine/chemokine profiling and in situ digitally enabled quantitation of immune infiltrates and molecular markers. The foundation to advance future precision-guided cytokine/chemokine-directed therapies has been laid by emerging successes with Akt inhibitors in PTEN-deficient/PI3KCA-mutant tumors. Further progress and translation from laboratory to clinic has the potential to add further cytokine/chemokine-directed therapeutics as part of an expanded therapeutic arsenal to treat mCRPC.

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