Emerging cytokine networks in colorectal cancer

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Abstract | Cytokine networks are crucial aspects of tumour immunology, particularly for colorectal cancer (CRC), in which inflammation and antitumour immunity are key determinants of disease progression. In this Review, we highlight new insights into the functions of well-known cytokines in CRC, describe recently discovered roles for a growing number of novel players, and emphasize the complexity and therapeutic implications of the cytokine milieu. We also discuss how cancer mutations and epigenetic adaptations influence the oncogenic potential of cytokines, a relatively unexplored area that could yield crucial insights into tumour immunology and facilitate the effective application of cytokine-modulatory therapies for CRC.

Carcinogenesis is the result of a complex interplay of cell-intrinsic and cell-extrinsic processes that promote sustained proliferation, resistance to apoptosis, reprogramming and reorganization of the stromal environment, and genomic instability. Inflammation, although necessary for damage repair, can influence these processes to promote neoplasia. As such, inflammation is now regarded as an enabling characteristic for the acquisition of the core hallmarks of cancer[1].

Chronic inflammation is an aberrant and prolonged response to a disruption of tissue homeostasis. Similarly, tumours often feature a dysregulated reparative response, thus earning the moniker "wounds that do not heal" (REF. 2). Chronic intestinal inflammation is a well-known risk factor for colorectal cancer (CRC) development[3], and several animal models of CRC depend on intestinal inflammation for tumour progression. Relative to colitis-associated cancer (CAC), sporadic CRCs that do not arise from colitic origins seem to develop along a distinct evolutionary track with differing molecular features (BOX 1). Nevertheless, sporadic CRCs also elicit an inflammatory response. Tumour-induced inflammation has a critical role in determining the fate of CRC, as antigen-driven cytolytic immune responses have the power to curtail cancer progression, whereas nonspecific inflammatory activity can potently augment it[4] (FIG. 1). Notably, numerous studies have supported a consensus that robust antitumour immunity is associated with favourable prognosis in patients with CRC[5,6], despite the association between chronic intestinal inflammation and CRC risk. These distinct sides of tumour immunity are not mutually exclusive and are critically dependent on cytokine networks that normally act to maintain gut homeostasis and manage the commensal microflora. Indeed, the gut contains the highest density of microorganisms in the human body and thus has a highly developed immune system with robust peripheral tolerance mechanisms. Aberrations of this system are critical lynchpins of both chronic colitis and CRC.

There is intense investigation aimed at identifying and characterizing the factors that drive chronic intestinal inflammation. Notably, diet and the gut microbiota are increasingly implicated in this process[7–12]. Reproducible changes in gut microbial diversity are observed during CRC progression and are associated with specific pathological features of tumours[13,14]. Although still a young field, it is already clear that certain genera of gut bacteria can have host-protective roles by promoting anti-inflammatory immune pathways, whereas others are pro-inflammatory or mutagenic[7–12]. Genetic susceptibility conferred by polymorphisms in key genes may also be an important factor in CRC development. For example, variants of several cytokine and cytokine receptor genes are strongly associated with inflammatory bowel disease risk, but less is known about their connection to CRC[15]. Finally, intestinal inflammation can also be driven through defective resolution of endoplasmic reticulum stress[15,16].

Cytokines such as tumour necrosis factor (TNF) and interleukin-6 (IL-6) are classically regarded as central players in CRC, driving activation of the key oncogenic transcription factors nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (STAT3), respectively, in intestinal epithelial cells to

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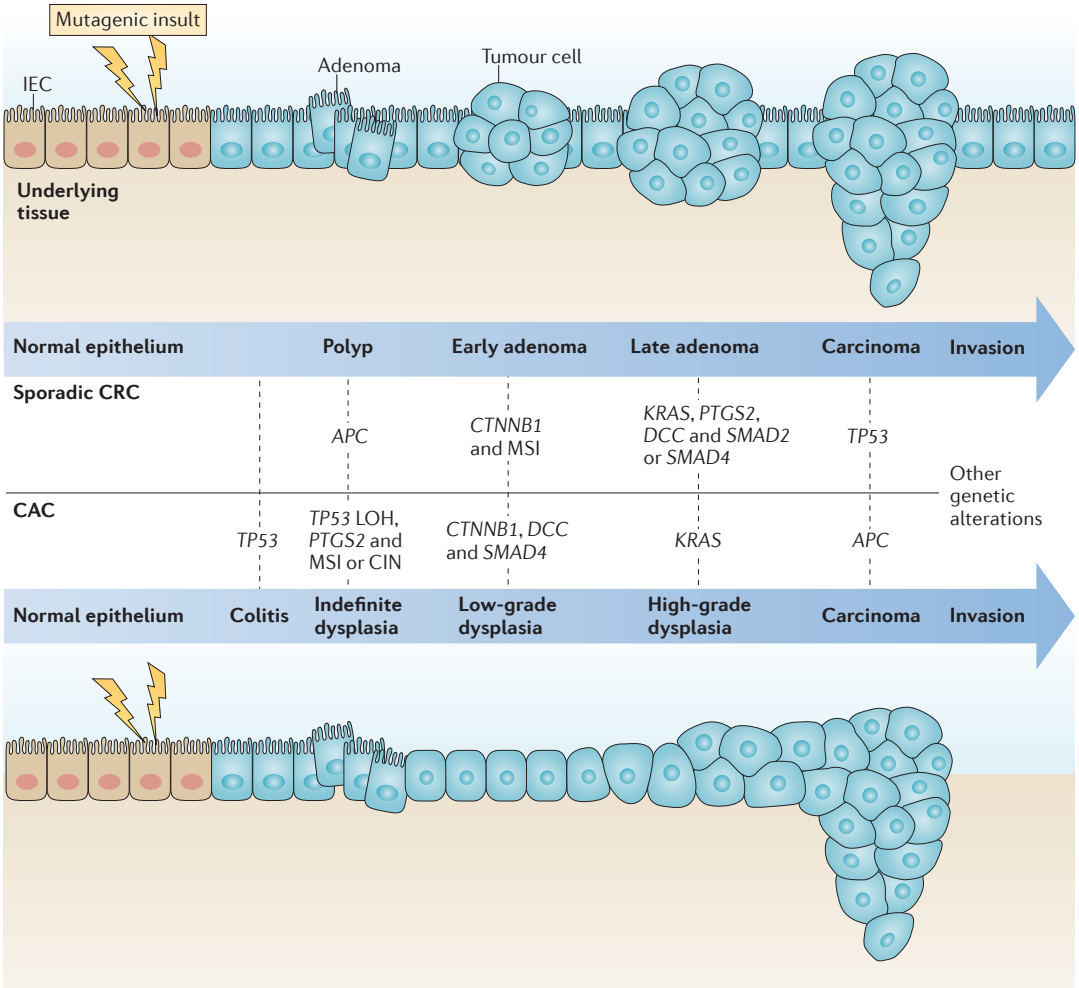
Checkpoint blockade immunotherapy
A therapeutic intervention (typically a monoclonal antibody) designed to block inhibitory 'checkpoint' signals that suppress or terminate immune activity, with the intention of enhancing or inducing antitumour immunity. Notable checkpoint targets include cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death protein 1 (PD1) and PD1 ligand 1 (PDL1).

promote proliferation and resistance to apoptosis[4]. More recently, cytokines with similar biochemical functions — including IL-11, IL-17A and IL-22 — have gained attention as facilitators of both human and mouse CRC (a summary of animal models discussed in this Review is provided in TABLE 1). By contrast, other cytokines — such as interferon-γ (IFNγ), IL-15 and IL-18 — promote protective host immunity mediated by cytotoxic cell types, particularly $CD8^+$ cytotoxic T lymphocytes (CTLs) (FIG. 2). Regulatory T (T_{Reg}) cells are essential for the control of intestinal inflammation, due in part to their production of the anti-inflammatory cytokines IL-10 and transforming growth factor-β (TGFβ). Although this suppressive role of T_{Reg} cells may be deleterious due to the impairment of protective immune responses, it may also be beneficial for the limitation of pro-tumorigenic inflammation[17]. Despite an impressive volume of supporting evidence from mouse disease models and detailed analyses of human tissue, modulation of the CRC cytokine milieu has rarely been attempted in the clinic.

The field of cancer immunology currently enjoys unprecedented attention, based in no small part on the exciting clinical successes of checkpoint blockade immunotherapy in several cancer types (reviewed extensively elsewhere[18,19]). Unfortunately, this modality has

Box 1 | **Adenoma to carcinoma sequence**

Canonical mechanisms of sporadic colorectal cancer (CRC) and colitis-associated cancer (CAC) development are shown in the top and bottom panels, respectively. CRC and CAC share similarities in their developmental pathways, including microsatellite instability (MSI), activation of the oncogene *KRAS*, activation of cyclooxygenase 2 (COX2; encoded by *PTGS2*), and mutation and eventual loss of heterozygosity (LOH) of *TP53*, adenomatous polyposis coli (*APC*), deleted in colon cancer (*DCC*) and *SMAD4*. However, the frequency and sequence of these events differs between the cancers. For example, mutation in *APC* is one of the first events in CRC, whereas it occurs at later stages in CAC. By contrast, *TP53* mutations usually occur early in CAC but at a later stage in the progression of CRC. Although CRC shows a clear progression of morphological changes, from polyp to carcinoma, CAC progression involves increasing histological grades of dysplasia that culminate in an invasive carcinoma.



CTNNB1, gene encoding β-catenin; CIN, chromosomal instability; IEC, intestinal epithelial cell.

Mismatch repair
(MMR). A DNA repair pathway that recognizes and corrects mismatched base pairs (typically those that arise from errors of chromosomal DNA replication). There are two main types of MMR components: MutS homologues (MSH1–MSH6) and MutL homologues (PMS1, PMS2 and MLH1).

shown limited efficacy for CRC[20,21], although a recent study identified mismatch repair (MMR)-deficient CRC as a potentially sensitive clinical subset[22]. Although the therapeutic potential of checkpoint blockade is clearly profound, these agents only benefit a subset of patients and are not designed to target the oncogenic elements of tumour inflammation, particularly cytokines with pro-growth and/or pro-survival functions. Therefore, although manipulation of cytokine pathways may be clinically beneficial in its own right, combining this approach with checkpoint blockade may yield even greater advantages by simultaneously unleashing anti-tumour immunity and blocking the pro-tumorigenic elements of inflammation.

Focusing on literature from the past 5 years, in this Review we discuss newly described aspects of well-known CRC-modulatory cytokines such as IL-6, cytokines with more recently described roles in CRC, and how cancer adaptations influence the oncogenic potential of pro-inflammatory cytokines. Finally, we address the challenges posed by the daunting complexity of the CRC cytokine milieu and how this knowledge could guide the deployment of immunomodulatory therapies.

Classical cytokines in CRC: an update

TNF. TNF is a key inflammatory cytokine that is produced widely by both haematopoietic and non-haematopoietic cells. TNF binds as a homotrimer to two distinct receptors on the cell surface: TNF receptor 1 (TNFR1; also known as p55 receptor) or TNFR2 (also known as p75 receptor)[23]. Although TNF was first identified as a tumour-suppressive cytokine, based on its capacity to induce apoptosis in certain cell types, it is now also recognized as a tumour-promoting agent that links inflammation and cancer[24–26]. Intestinal epithelial cells are highly sensitive to TNF due to high levels of expression of TNFR1, which potently activates NF-κB-dependent oncogenic pathways.

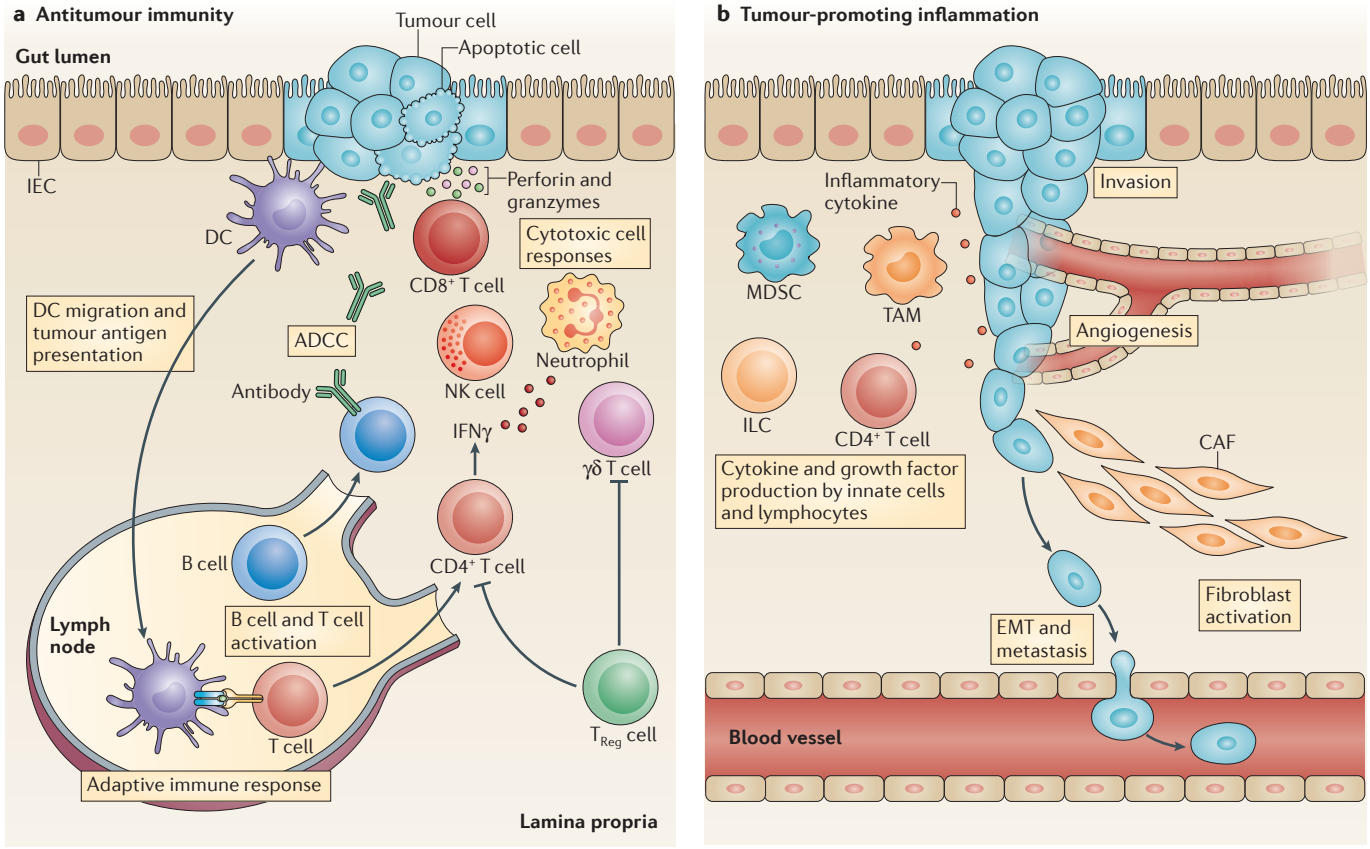


Figure 1 | **The double-edged sword of inflammation in colorectal cancer.** The immune system can have profound but disparate roles in the pathogenesis of colorectal cancer (CRC). Many new therapeutic approaches in cancer immunology aim to potentiate antitumour immunity (part **a**). Mechanistically, dendritic cells (DCs) sample tumour antigens and activate tumour-specific B cells and T cells in secondary lymphoid organs to initiate cytolytic and/or humoral responses. Conventionally, effective antitumour immunity is thought to be primarily driven by interferon-γ (IFNγ)-producing $CD4^+$ T cells, and cytotoxic $CD8^+$ T cells and natural killer (NK) cells. An increasing amount of data also supports a role for γδ T cells in antitumour immunity. This process is restrained by regulatory T (T_{Reg}) cells, which can impair antitumour immunity but may also help to restrain inflammation that could otherwise induce tissue disruption and tumour progression. Conversely, nonspecific inflammation (part **b**), potentially elicited by microbial stimuli in the gut (not shown), induces the production of inflammatory cytokines by several cell types including $CD4^+$ T cells, innate lymphoid cells (ILCs) and tumour-associated macrophages (TAMs). Many of the inflammatory cytokines can act directly on transformed intestinal epithelial cells (IECs) to promote proliferation, inhibition of apoptosis, invasion, angiogenesis, epithelial to mesenchymal transition (EMT) and metastasis. More recently, it has been appreciated that these cytokines can activate cancer-associated fibroblasts (CAFs) to produce cytokines and growth factors that modulate both neoplastic cells and the tumour microenvironment. ADCC, antibody-dependent cell-mediated cytotoxicity; MDSC, myeloid-derived suppressor cell.

Table 1 | **Mouse models of colorectal cancer discussed in this Review**

Model	Primary location	Invasiveness	Driver	Sporadic CRC or CAC
Apc$^{Min/+}$	Small intestine	Adenomas (non-invasive)	• Mice are heterozygous for an *Apc* loss-of-function mutation • Increased WNT–β-catenin signalling occurs following allelic loss of wild-type *Apc*	Sporadic CRC
Apc$^{\Delta 468}$	Small intestine	Adenomas (non-invasive)	Similar concept to the *Apc*$^{Min/+}$ system, but loss of function in *Apc* is achieved by a distinct truncating mutation	Sporadic CRC
CPC-APC	Colon and distal small intestine	Adenomas and a low frequency of carcinomas	• Colonic Cre-mediated deletion of a single *Apc* allele, causing increased WNT–β-catenin signalling upon allelic loss of wild-type *Apc* • Cre expression is driven by *Cdx2*, which is expressed preferentially by colonic epithelial cells	Sporadic CRC
Apc$^{Min/+}$ and ETBF	Colon (distal)	Adenomas and a low frequency of carcinomas	Increased WNT–β-catenin signalling and bacteria-driven T_H17 cell responses	Sporadic CRC and/or CAC
Vil1-Cre, *Trp53*$^{fl/fl}$ and AOM	Colon	Adenocarcinomas and invasion of local lymph nodes	Specific loss of p53 expression in the intestinal epithelium, combined with random mutagenesis induced by the carcinogen AOM	Sporadic CRC
DSS–AOM	Colon	Aberrant crypt foci and adenomas	Intestinal barrier dysfunction and inflammation driven by the chemical irritant DSS, combined with AOM-induced mutagenesis	CAC
Rag2$^{-/-}$, *Helicobacter hepaticus* and AOM	Colon and caecum	Dysplasia and adenocarcinoma	Innate inflammation following oral infection with the commensal pathobiont *Helicobacter hepaticus*, combined with AOM-induced mutagenesis	CAC
Il10$^{-/-}$	Colon and caecum	Adenocarcinoma	Loss of intestinal immune regulation, causing aggressive innate inflammation and T_H1 cell responses	CAC
Xenografts	Usually subcutaneous or orthotopic	Variable	Variable	NA
Experimental metastases	Lung (intravenous delivery) or liver (intrasplenic delivery)	Typically highly invasive	Variable	NA

AOM, azoxymethane; APC, adenomatous polyposis coli protein; CAC, colitis-associated cancer; CRC, colorectal cancer; DSS, dextran sodium sulfate; ETBF, enterotoxigenic *Bacteroides fragilis*; *Il10*, interleukin-10; NA, not applicable; *Trp53*$^{fl/fl}$, homozygous expression of floxed gene encoding p53; *Rag2*, recombination-activating gene 2; T_H, T helper.

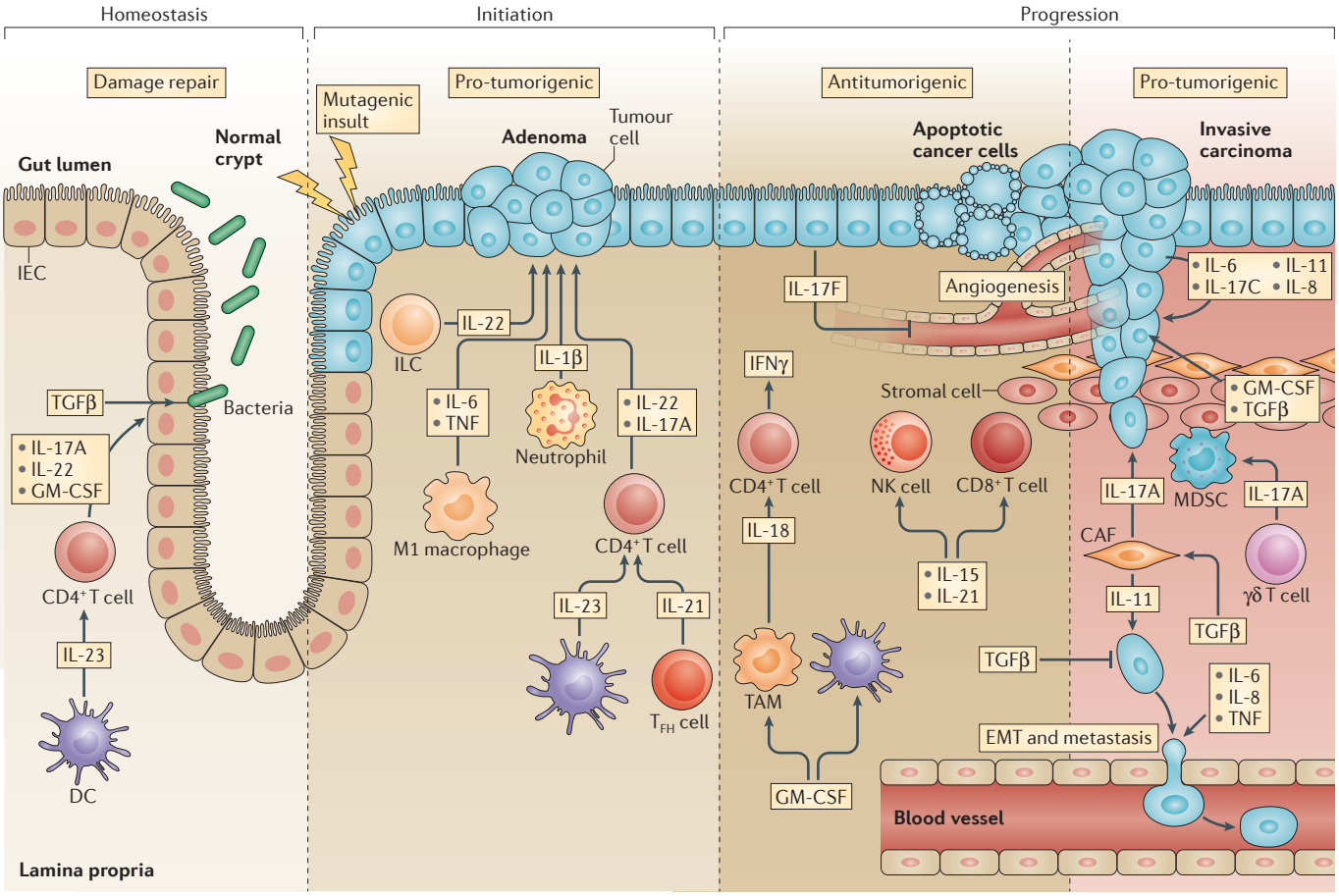
Recent data have demonstrated a novel protective role for TNF. Mice deficient in both TNF and IL-10 develop colitis and cancer with greater rapidity and severity than mice lacking IL-10 alone, and they develop high serum levels of IL-6, IFNγ and IL-17A[27]. Furthermore, the development of spontaneous colitis in these mice is prevented by antibiotic treatment, suggesting that inappropriate bacterial handling due to TNF deficiency can promote colitis and CAC[27]. By contrast, haematopoietic cell-specific deficiency of TNF dramatically attenuates spontaneous adenoma formation in the non-colitis-based *Apc*$^{\Delta 468}$ model of CRC (in which transgenic mice express a mutant form of *Apc*, which encodes adenomatous polyposis coli protein)[28]. Similarly, TNF is required for the tumour-promoting effect of obesity in both the dextran sodium sulfate (DSS)–azoxymethane (AOM) CAC model and human CRC xenograft settings[29]. TNF is also a critical pro-neoplastic factor associated with the stress-induced senescence-associated secretory phenotype[30]. Finally, TNF may support metastasis by inducing epithelial to mesenchymal transition (EMT) in CRC through increasing the activity of the transcription factor SNAI1, an effect mediated by AKT-dependent glycogen synthase kinase 3β (GSK3β) inactivation[31].

Epithelial to mesenchymal transition
(EMT). A reversal of the mesenchymal to epithelial transition that occurs during development. EMT or the de-differentiation of epithelial cells can have normal physiological roles (such as in wound healing) or can be associated with fibrotic pathologies and cancer.

TGFβ. TGFβ is a complex cytokine with important roles in tissue homeostasis, wound healing and cancer[32]. TGFβ activates the serine/threonine kinase receptors TGFβR1 and TGFβR2, which in turn activate the intracellular proteins SMAD2 and SMAD3, which interact with SMAD4 to regulate gene expression[33].

TGFβ is generally accepted to have tumour-suppressive roles in early-stage cancer[34–36], an effect that is consistent with a high frequency of mutations in TGFβ signalling components in CRC[37]. Rapid onset of CRC was recently reported in *Helicobacter bilis*-infected *Smad3*$^{-/-}$ mice, two-thirds of which develop inflammation-associated mucinous carcinomas as early as 6 weeks post-infection[38]. Consistent with a tumour-suppressive role, human CRC cells with high TGFβ sensitivity are weakly metastatic in orthotopic xenograft models[39]. Intriguingly, SMAD4 deficiency elevates the expression of CC-chemokine ligand 15 (CCL15) by human CRC, which is associated with poor prognosis and the recruitment of CC-chemokine receptor 1 (CCR1)$^+$ myeloid cells to liver metastases[40].

Despite restraining tumorigenesis at early stages, high levels of TGFβ in human CRC are associated with poor prognosis, particularly in patients with locally advanced disease[41]. Recent data may reconcile this paradox.



Cytokine	Cellular source(s)	Cellular responder(s)
TNF	Haematopoietic cells[27,28]	IECs[29] and cancer cells[31]
TGFβ	CAFs[43]	Cancer cells[43] and CAFs[41,42]
IL-1β	TAMs[46] and neutrophils[48]	IECs[48] and cancer cells[46,47]
IL-6	TAMs[54], CAFs[55] and mesenchymal stem cells[56]	IECs[57] and cancer cells[55–57]
IL-11	CAFs[41]	Cancer cells[41,58]
IL-23	DCs[69] and TAMs (CD11b$^+$GR1$^+$)[61]	Haematopoietic cells[61]
IL-17A	CD4$^+$ αβ T cells[59,67], CD8$^+$ Tc17 cells, γδ T cells[67], ILCs[66] and CAFs[70]	Cancer cells[63], CICs[70] and MDSCs[67,69]
IL-17C	IECs[72]	IECs[72] and cancer cells[72]
IL-17F	IECs[74]	Endothelial cells[74]
IL-22	ILC3s[66] and CD4$^+$ T cells[78,81]	Cancer cells[66,78,81]
IL-18	TAMs and IECs[86]	IECs[84] and T cells[85,86]
IL-8	CD44$^+$ cancer stem-like cells[93]	CD11b$^+$GR1$^+$ myeloid cells[94]
GM-CSF	Cancer cells[96–98]	Cancer cells[96], antigen-presenting cells[96] and monocytes[97]
IL-15	Cancer cells[102]	NK cells[99,100], CD8$^+$ T cells[99], B cells and T cells[102]
IL-21	CD4$^+$ T cells[105] and activated NK cells[105]	NK cells[104,105] and CD8$^+$ T cells[105]

Figure 2 | **Cytokines in the pathogenesis of colorectal cancer.** Cytokines produced by innate and adaptive immune cells, stromal fibroblasts and cancer epithelial cells have diverse and pleiotropic roles at different stages of colorectal cancer (CRC) progression. Interleukin-22 (IL-22) and IL-17A, expressed by CD4$^+$ T cells and innate lymphoid cells (ILCs) in response to IL-23 and IL-21 from dendritic cells (DCs) and T follicular helper (T_{FH}) cells, are important for mucosal tissue healing, microbiota management and host defence, but can also promote tumour formation. At early stages of tumorigenesis, cytokines such as IL-15 and IL-21 potentiate cytotoxic responses by CD8$^+$ T cells and natural killer (NK) cells; granulocyte–macrophage colony-stimulating factor (GM-CSF) activates macrophages and DCs; IL-18 induces the release of interferon-γ (IFNγ); IL-17F inhibits angiogenesis; and transforming growth factor-β (TGFβ) impairs cancer cell growth and dissemination. All of these cytokine-mediated effects inhibit tumour progression. However, in well-established tumours, which may have developed adaptations to resist impairment by specific cytokines, many cytokines can enhance tumour progression by promoting proliferation, inhibition of apoptosis, angiogenesis, stromal reorganization, epithelial to mesenchymal transition (EMT), metastasis and suppression of antitumour immunity (for example, via myeloid-derived suppressor cells (MDSCs)). Major cytokine producers and associated responder cells for specific cytokines are listed in the accompanying table. CAF, cancer-associated fibroblast; CIC, cancer-initiating cell; IEC, intestinal epithelial cell; TAM, tumour-associated macrophage; Tc17 cell, IL-17-producing cytotoxic T cell; TNF, tumour necrosis factor.

An elegant study demonstrated that gene expression signatures associated with poor outcome in CRC are driven by TGFβ-induced programmes in stromal cells and that TGFβ promotes metastasis by acting on the tumour microenvironment[42]. Indeed, TGFβ can induce IL-11 expression by cancer-associated fibroblasts (CAFs), which promotes STAT3 signalling in CRC and increases multi-organ metastasis[41]. Similarly, TGFβ induces fibroblasts to produce extracellular matrix-remodelling enzymes in co-cultures of CRC cells and fibroblasts[43]. Effective control of mouse CRC progression by the TGFβR1 inhibitor LY2157299 makes targeting TGFβ in patients with late-stage CRC a noteworthy therapeutic possibility[41,42].

IL-1β. IL-1β, a potent activator of NF-κB, is expressed at high levels in several cancer types and expression increases during progression of CRC[44,45]. IL-1β can activate the WNT–β-catenin pathway in CRC by inactivating GSK3β[46], and it induces mesenchymal and stemness features, including increased colony-forming capacity, expression of stemness genes such as *BMI1* and *NES* (which encodes nestin), and increased resistance to chemotherapy[47]. Zinc finger E-box-binding homeobox 1 (ZEB1), an important pro-mesenchymal transcription factor, has a crucial role in these processes[47]. In the DSS–AOM model, neutrophils deliver large quantities of IL-1β to the tumour microenviroment[48]. Blockade of IL-1β using soluble IL-1 receptor antagonist attenuates tumour infiltration by inflammatory cells, expression of IL-6 by mononuclear cells and tumour formation[48]. Very high levels of IL-1β are also evident in tumours from non-colitic $Apc^{\Delta 468}$ mice. Interestingly, this does not occur in the absence of nuclear receptor RORγt[28].

IL-6. IL-6 is produced by diverse cell types and is a crucial mediator of inflammation and immunity[49]. The IL-6 receptor transduces signals via gp130 (also known as IL-6Rβ), which is the shared receptor chain of the IL-6 family, and is a strong inducer of STAT3 activation.

IL-6 has several important roles in cancer progression, driving processes such as proliferation, migration and angiogenesis[49]. Mouse and human studies have emphasized its role in both CAC and sporadic CRC. IL-6-dependent STAT3 signalling is a critical promoter of cancer cell proliferation and survival in the DSS–AOM model[50,51]. Intriguingly, IL-6 production in this experimental system is dependent on sphingosine-1-phosphate (S1P) signalling through the S1P receptor, and tumorigenesis is strongly attenuated when mice are treated with a prodrug inhibitor of the S1P pathway (FTY720; also known as fingolimod)[52]. IL-6-induced STAT3 signalling in myeloid cells was recently described as a tumour recurrence mechanism following radiotherapy[53]. Notably, this effect was apparently mediated by Toll-like receptor 9 (TLR9) signalling in myeloid cells that were recruited to regressing tumours[53].

Macrophages are historically regarded as the primary source of IL-6 in CRC. Indeed, natural killer T (NKT) cells were recently shown to promote CAC by producing IL-13, which resulted in the polarization of macrophages to an M2 phenotype and substantial IL-6 production by tumour-associated macrophages[54]. New data, however, link IL-6 production with CAFs in human tumours[55]. IL-6 is produced by cancer-associated mesenchymal stem cells and has pro-tumorigenic effects in human CRC through the induction of Notch 1 and CD44 expression[56]. A novel potential driver of IL-6 production in human CRC was recently identified in the form of bacterial biofilms on the colonic mucosa of patients with CRC[14]. Although causality was not firmly established, biofilms were associated with increased IL-6 production in the lamina propria, epithelial STAT3 activation and loss of E-cadherin expression[14].

Possibly the most novel function attributed to IL-6 in recent years is its ability to induce microsatellite instability (MSI) in CRC[57]. Elevated microsatellite alterations at selected tetranucleotide repeats (EMASTs) are the most common MMR defect in CRC, and they are associated with both inflammation and poor outcome. Treatment of CRC cell lines and non-transformed colonic epithelial cells with IL-6 (but not TNF, IL-1β, IFNα or IFNγ) leads to relocalization of the human MMR mediator MSH3 from the nucleus to the cytoplasm, resulting in abundant frameshift mutations at EMAST loci. Active STAT3 is both necessary and sufficient for this process, suggesting that other STAT3 drivers, such as IL-11 or IL-22, could have similar effects[57]. These data elegantly demonstrate how a clinically distinct phenotype of cancer defined by cell-intrinsic features (namely, EMASTs) may be aetiologically driven by cell-extrinsic factors from the tumour microenvironment.

New players in the CRC cytokine milieu

IL-11. IL-11, a member of the IL-6 family[49], is highly expressed in human CRC and is a stronger correlate of phosphorylated STAT3 than is IL-6 (REF. 58). In both DSS–AOM and $Apc^{Min/+}$ models, $Il11ra1^{-/-}$ mice (which lack IL-11 receptor subunit-α) have profoundly reduced tumour formation irrespective of IL-6 signalling[58]. Similarly, IL-11RA1 blockade can attenuate CRC xenograft growth. Based on this study and the recent report that TGFβ promotes metastasis by inducing IL-11 production by CAFs[41], IL-11 has emerged as an important new STAT3-inducing cytokine in CRC.

The IL-17 family (IL-17A, IL-17C and IL-17F). A relationship between IL-17A and CRC was first identified in spontaneous intestinal tumorigenesis models. IL-17A blockade significantly reduced tumour burden in $Apc^{Min/+}$ mice infected with enterotoxigenic *Bacteroides fragilis*[59]. *Il17a* deficiency had a similar effect in the standard $Apc^{Min/+}$ model[60]. In another CRC model driven by loss of APC in the colon (CPC-APC), $Il23^{-/-}$ and $Il17ra^{-/-}$ mice display reduced STAT3 activity and tumorigenesis[61]. $Il17a^{-/-}$ mice are also resistant to CAC in the DSS–AOM model, possibly due to diminished levels of colonic IL-6, TNF, IFNγ and STAT3 activation[62]. Reciprocal bone marrow chimaera experiments have shown that intact signalling through IL-17RA in epithelial cells (in which it activates NF-κB and extracellular signal-regulated kinase (ERK) signalling), but

WNT–β-catenin pathway
A signalling pathway that regulates cell fate determination, proliferation, adhesion, migration and polarity during development. In addition, WNT proteins and their downstream signalling molecules have been implicated in tumorigenesis and have causative roles in human colorectal cancers. WNT signalling activates TCF–LEF family transcription factors by stabilizing their co-activator, β-catenin, and mobilizing this factor from the cytoplasm to the nucleus. Adenomatous polyposis coli protein (APC), the most commonly inactivated protein in colorectal cancer, is a negative regulator of this signalling pathway.

Stemness
An imprecise term referring to the possession of qualities normally found in stem cells, such as the capacity for self-renewal.

Microsatellite instability
(MSI). A DNA hypermutation process that is indicative of defects in DNA mismatch repair. MSI is detectable as differences in the number of repeats in microsatellite loci relative to the repeat number found in the inherited genome.

not in haematopoietic cells, is required for tumorigenesis[63]. Notably, high expression of a functional T helper 17 (T_H17) cell mRNA signature is associated with poor prognosis in human CRC[64,65].

Although it is generally accepted that $CD4^+$ αβ T cells (specifically, T_H17 cells) are the dominant source of IL-17A in the tumour microenvironment, $CD8^+$ CTLs (Tc17 cells), γδ T cells (γδT17 cells) and innate lymphoid cells are also key IL-17A producers[59,66–68]. Tumour-infiltrating dendritic cells (DCs) can promote γδT17 cell production of IL-17 via IL-23 and, similar to studies in mouse models[69], human intratumoural γδT17 cells reportedly promote the recruitment and expansion of myeloid-derived suppressor cells[67]. Therefore, IL-17A may indirectly contribute to immune silencing in the tumour microenvironment. Beyond haematopoietic cells, CAFs can express IL-17A in response to chemotherapy in human CRC and in xenograft models[70], and IL-17A blockade in the CPC–APC model potentiates chemosensitivity to 5-fluorouracil[63]. Furthermore, IL-17A may educate tumour stromal cells to promote resistance to anti-angiogenic therapies[71].

IL-17C, a ligand of IL-17RE, is expressed in response to the microbiota in a myeloid differentiation primary response protein 88 (MYD88)-dependent manner in intestinal epithelial cells during early-stage CRC[72]. In both a spontaneous CRC model ($Apc^{Min/+}$) and a CAC model (DSS–AOM), $Il17re^{-/-}$ mice show a reduced tumour burden and reduced tumour cell expression of the anti-apoptotic proteins B cell lymphoma 2 (BCL-2) and BCL_{XL} (REF. 72). IL-17C levels are also elevated in human cancers[72].

IL-17F, which is very similar to IL-17A and signals through the same receptor, may have opposing effects to IL-17A in certain settings[73]. IL-17F expression is significantly reduced in human CRC[74], and diminishes tumorigenesis in the DSS–AOM model, in which it acts indirectly as a negative regulator of vascular endothelial growth factor (VEGF) expression and angiogenesis[74]. The disparity between the functions of IL-17A and IL-17F in CRC warrants careful consideration in the context of immunomodulatory therapies.

IL-22. IL-22 is an IL-10 family cytokine that is produced by innate lymphoid cells, T_H17 cells, T_H22 cells and possibly neutrophils, predominately at barrier surfaces[75,76]. By enhancing epithelial cell proliferation, survival and barrier function, IL-22 has a crucial role in the resolution of tissue damage induced by microbial insult, reactive oxygen species and other harmful stimuli[75]. Although necessary for damage repair, IL-22 can promote colitis in some settings by triggering pro-inflammatory cytokine secretion and perpetuating the inflammatory response[75,77].

Colonic innate lymphoid cells ($IL\text{-}17^+IL\text{-}22^+$) are a dominant source of IL-22 in a CAC model in which 129*SvEv.Rag2*$^{-/-}$ mice are infected with *Helicobacter hepaticus* and treated with AOM, causing colitis and invasive CRC in 3–5 months[66]. IL-22 is required for both the induction and maintenance of tumours in this system, probably via STAT3 (REF. 66). Similarly, $Apc^{Min/+}$ mice deficient in IL-22-binding protein (IL-22BP; also known as IL-22RA2), a negative regulator of IL-22, develop a greater tumour burden than IL-22BP-sufficient mice, whereas $Apc^{Min/+}$ mice lacking IL-22 show impaired tumorigenesis[78].

In humans, elevated serum levels of IL-22 are associated with chemotherapy resistance in patients with CRC, and IL-22 mediates chemotherapy resistance *in vitro*[79,80]. Kryczek *et al.*[81] recently reported that IL-22 promotes CRC stemness. In this study, T cell-derived IL-22 activated STAT3 in human CRC cells, causing expression of the H3K9-specific *N*-methyltransferase DOT1L. DOT1L in turn activated expression of core stem cell genes (*SOX2*, *NANOG* and *POU5F1*) that enhanced tumorigenicity; expression levels of these genes negatively correlated with patient prognosis[81]. Given the importance of IL-22 in maintaining the viability of normal intestinal stem cells in mice[82], regulation of the cancer stem cell niche may be a critical element of IL-22 biology in CRC.

IL-18. Consistent with its role in promoting protective T_H1 cell and CTL responses, recent studies of IL-18 suggest a protective effect in CAC. IL-18 levels were dramatically reduced in DSS–AOM-treated mice deficient in components of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome — for example, *Pycard* (which encodes pyrin domain- and CARD-containing protein), *Casp1* (which encodes caspase 1) or *Nlrp3*[83,84]. $Il18^{-/-}$ and $Il18r^{-/-}$ animals are highly susceptible to tumour formation in the DSS–AOM model, whereas exogenous IL-18 protects $Casp1^{-/-}$ mice against DSS–AOM-induced neoplasia[84,85]. MYD88-deficient animals are similarly susceptible to CAC, an effect that is attributed to impaired IL-18R signalling[85]. Notably, IL-18 produced downstream of inflammasome activation in the intestinal epithelium attenuates experimental colitis through direct stimulation of T_{Reg} cells, suggesting that IL-18 could protect against oncogenic inflammatory processes[86]. Beyond the inflammasome, CAC is reportedly suppressed by IL-18 produced downstream of butyrate, a bacterial metabolite with key tolerogenic functions in the intestine[87].

IL-8. Genetic variation in the genes encoding IL-8 and its receptor CXC-chemokine receptor 2 (CXCR2) are associated with CRC progression, therapeutic resistance and tumour recurrence[88,89]. *IL8*-transfected HCT116 and Caco2 cells exhibit enhanced xenograft growth and vascularization[90]. Similarly, transgenic expression of *IL8* in mouse skin promotes the outgrowth, vascularity and metastasis of subcutaneous CRC xenografts[91]. Antagonism of CXCR2 with the small molecule SCH-527123 impairs cell proliferation, motility, invasiveness, survival and sensitivity to oxaliplatin in CRC xenografts[92]. The EMT-inducing transcription factor SNAI1 can induce *IL8* expression in $CD44^+$ cancer stem-like cells, which may be important for maintenance of stemness and tumorigenicity[93]. Furthermore, CD44, SNAI1 and IL-8 are co-expressed in human CRC[93]. *In vivo* studies of IL-8 have been hampered by the absence of an orthologue in mice. To address this issue, Asfaha *et al.*[94] generated mice carrying a bacterial

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous group of myeloid cells related to macrophages, granulocytes and dendritic cells. These cells are produced in response to various inflammatory and/or tumour-derived cytokines and are thought to inhibit tumour-specific immune responses.

artificial chromosome encompassing the entire human *IL8* gene and its regulatory elements. When these mice were subjected to DSS–AOM treatment or crossed to $Apc^{Min/+}$ animals, increased tumorigenesis, $CD11b^{+}GR1^{+}$ myeloid cell recruitment and tumour angiogenesis were observed relative to control mice[94]. As SCH-527123 has proven to be safe in early-phase clinical trials, targeting IL-8 in human CRC should be feasible.

Granulocyte–macrophage colony stimulating factor. Granulocyte–macrophage colony-stimulating factor (GM-CSF) is currently used as an adjuvant in cancer vaccine strategies to potentiate antitumour immunity via activation of antigen-presenting cells[95]. Intriguingly, GM-CSF is highly expressed in more than one-third of CRCs, and patients whose tumours concomitantly express GM-CSF and both subunits of the GM-CSF receptor have excellent 5-year survival rates[96]. These results are apparently independent of enhanced antitumour immunity, as GM-CSF correlates with favourable prognosis even in patients with poor $CD8^{+}$ T cell infiltration[97]. In mice, however, DSS–AOM-induced tumours feature high GM-CSF levels that drive VEGF release, angiogenesis and adenoma formation[98]. Aside from work in human CRC cell lines[98], a link between GM-CSF and angiogenesis has not been made in clinical CRC samples.

Members of the IL-2 family — IL-15 and IL-21. The potential of IL-15 as an immunotherapeutic agent is due to its crucial role in promoting the proliferation and activation of NK cells and $CD8^{+}$ T cells[99]. In mouse syngeneic lung metastasis models, mice treated with IL-15 and an agonistic CD40-specific antibody (the actions of which promote the *trans*-presentation of IL-15–IL-15Rα complexes by DCs) displayed prolonged survival and improved NK cell cytotoxicity relative to mice treated with either agent alone[100]. In another study, IL-15 treatment increased the expression of programmed cell death protein 1 (PD1) on $CD8^{+}$ T cells and stimulated IL-10 production[101]. However, combined treatment with IL-15 and antibodies specific for PD1 ligand 1 (PDL1) and cytotoxic T lymphocyte antigen 4 (CTLA4) increased the survival of mice with metastatic CRC[101]. Notably, *IL15* deletion or low *IL15* expression in human CRC confers a high risk of relapse and is associated with poor antitumour immunity[102]. These findings highlight the potential for immunotherapeutic use of IL-15 in human CRC.

Similarly to IL-15, IL-21 is considered to be host protective due to its capacity to enhance NK cell and $CD8^{+}$ T cell cytotoxicity. IL-21 expression is associated with beneficial immune responses and good prognosis in CRC[65], and it has shown promise in clinical trials when combined with standard therapies. For example, IL-21 enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) elicited by cetuximab in patients with oesophageal squamous cell carcinoma[103]. Recombinant IL-21 was administered in combination with cetuximab in a Phase I clinical trial in patients with stage IV CRC and showed some evidence of immune-stimulatory activity[104]. However, in the DSS–AOM model, $Il21^{-/-}$ animals display attenuated tumour formation, decreased levels of IL-6 and IL-17A, and reduced numbers of intratumoural lymphocytes[105]. Furthermore, IL-21 neutralization after the last DSS cycle reduced tumour burden in wild-type animals despite a marked reduction in NK cells and $CD8^{+}$ T cells[105]. By contrast, Jauch *et al.*[106] reported enhanced $CD8^{+}CD103^{+}$ T cell cytotoxicity in $Il21^{-/-}$ mice in the same experimental system. In the few tumours that did form in $Il21^{-/-}$ mice, the balance between IL-17A and IFNγ was skewed dramatically towards IFNγ, which was necessary for efficient cytotoxic activity. Therefore, during DSS–AOM-induced tumorigenesis, IL-21 may promote T_H17 cell polarization, resulting in poor cytolytic responses[106].

Tumour adaptations to cytokines

As cytokines can have beneficial or deleterious effects on cancer in a context-dependent manner, it is logical that cancers develop adaptations to maximize the benefits that they receive from cytokine signalling (FIG. 3). In this section, we discuss recently described examples of this process in CRC.

p53: more than just a guardian of the genome. *TP53* (known as *Trp53* in mice) encodes the p53 tumour suppressor protein and is mutated in approximately one-third of primary sporadic CRCs, more than 50% of sporadic CRC metastases and up to 85% of CACs[107–109]. *TP53* mutations occur very early during CAC development, and they are even detectable in the mucosa of patients with ulcerative colitis before the onset of neoplasia[110]. Although the classical role of p53 is to control genome integrity and prevent the outgrowth of mutated cells, it is also an important regulator of inflammatory pathways[111].

Evidence that p53 controls inflammation at an organ level was recently reported using mice with p53 deletion restricted to intestinal epithelial cells[112]. When given AOM, these mice develop CRCs capable of local metastasis. Notably, relative to non-invasive *Trp53*-wild-type tumours that formed with AOM administration alone, *Trp53*-deficient tumours featured barrier defects, expression of EMT markers, elevated levels of NF-κB-dependent chemokines and cytokines, and a pronounced myeloid cell infiltrate that was required for efficient tumour invasion and metastasis[112]. The specific mechanism by which p53 controls barrier function and inflammation is not clear; however, data from breast cancer suggest that p53 may control tight junction integrity and thereby regulate barrier function in the intestinal epithelium[113,114]. Loss of p53 or its transcriptional target p21 (a critical cell cycle suppressor) in stressed intestinal organoids allows enterocytes to exploit senescence-associated autocrine TNF signalling and acquire malignant properties[30]. Loss of p53 also augments IL-6 signalling in CRC via reduced expression of the p53 transcriptional target microRNA-34a (miR-34a), which represses the expression of IL-6R and reduces IL-6-mediated EMT[115].

TP53 mutations also promote cytokine signalling through gain-of-function effects. One such mutation (R248W) results in stronger responses to low-level TNF stimulation by prolonging NF-κB activation and

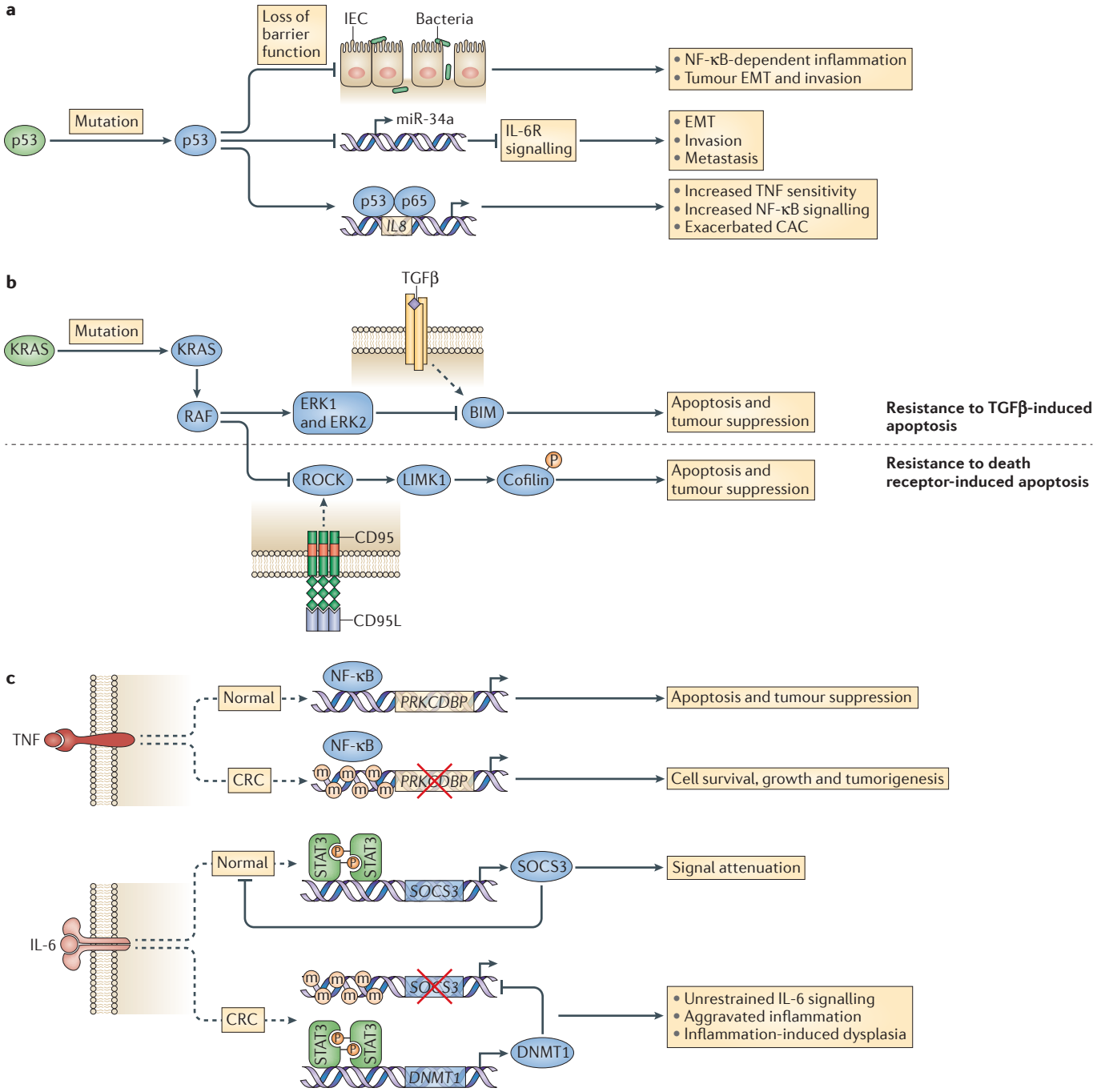


Figure 3 | **Adaptive mechanisms of cytokine exploitation in colorectal cancer.** Several adaptations that allow colorectal cancer (CRC) cells to maximize the benefits that they receive from immune interaction have been identified, examples of which are shown here. **a** | Mutations in *TP53* (which encodes p53) have several effects including: disrupted barrier integrity in the intestinal epithelium and consequent inflammation-associated tumour progression; enhanced interleukin-6 (IL-6)-induced signal transducer and activator of transcription 3 (STAT3) signalling via mRNA stabilization and increased surface expression of IL-6 receptor α-subunit (IL-6Rα) as a result of reduced expression of microRNA-34a (miR-34a); and enhanced tumour necrosis factor (TNF) sensitivity through direct interaction with the nuclear factor-κB (NF-κB) subunit p65 (also known as RELA) and its stabilization on target gene promoters (*IL8* shown here). **b** | Oncogenic *KRAS* mutations enhance resistance to the pro-apoptotic effects of transforming growth factor-β (TGFβ) and CD95–CD95 ligand (CD95L) signalling. TGFβ resistance occurs via the KRAS–RAF–extracellular signal-regulated kinase (ERK) cascade, which inhibits expression of the pro-apoptotic protein BIM. Resistance to CD95L is bestowed by KRAS–RAF-mediated suppression of RHO-associated protein kinase (ROCK) and LIM domain kinase 1 (LIMK1). **c** | Several homeostatic components of cytokine signalling pathways are repressed in CRC through aberrant gene methylation (indicated by circles labelled 'm'). Expression of pro-apoptotic protein kinase Cδ-binding protein (PRKCDBP), which is normally induced by TNF–NF-κB signalling, is frequently suppressed in this way. The gene encoding suppressor of cytokine signalling 3 (SOCS3), a critical negative feedback regulator of cytokine-induced STAT3 signalling, is methylated during the development of inflammation-associated dysplasia, possibly via IL-6-mediated expression of DNA methyltransferase 1 (DNMT1). CAC, colitis-associated cancer; EMT, epithelial to mesenchymal transition; IEC, intestinal epithelial cell.

renders mice more susceptible to CAC formation[116]. Finally, amino-terminally truncated isoforms of p53 and other p53 family members also regulate inflammatory networks in cancer, although their role in CRC remains unknown[117].

Inflammatory modulation by the KRAS oncogene. Another frequently perturbed gene in CRC is *KRAS*, which harbours constitutively activating mutations in 25–40% of primary tumours and metastases[109,118]. Among tumours with MMR proficiency, *KRAS* mutations are associated with significantly worse clinical outcome, and *KRAS* mutation is strongly associated with resistance to epidermal growth factor receptor inhibitors such as cetuximab[118–120]. Little is known about the influence of KRAS on cytokine signalling in CRC, but among cancers in general, oncogenic RAS (which can be generalized to include HRAS and NRAS) contributes directly to the production of several pro-inflammatory cytokines and chemokines such as IL-6, IL-8, IL-11, GM-CSF and CXC-chemokine ligand 1 (CXCL1)[121–124]. Intriguingly, *KRAS* or *NRAS* mutations in microsatellite-stable CRCs are associated with reduced expression of genes associated with T_H1-type immunity, antigen presentation and checkpoint blockade targets[125].

A recent report demonstrated that oncogenic KRAS may be critical for resisting the tumour-suppressive effects of TGFβ. Intriguingly, constitutively active KRAS suppresses TGFβ-induced expression of the pro-apoptotic protein BIM (also known as BCL2L11) in mouse adenomas via ERK1 and/or ERK2 activation, rendering adenoma cells resistant to TGFβ toxicity[126]. These findings were replicated in organoids derived from human CRC[126]. The ability of KRAS signalling to selectively inhibit tumour-suppressive functions of TGFβ may thus be an important mechanism by which TGFβ evolves from tumour suppressor to tumour promoter during CRC progression[127]. Mutant KRAS can also act as a molecular switch that determines the toxicity of death receptor signalling via the TNF family members TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) and CD95 ligand (CD95L; also known as FASL and TNFSF6)[128]. In both human and mouse CRC cells, KRAS blocks the apoptotic effects of TRAIL and CD95L but leaves their pro-invasive properties intact, effectively switching this pathway from a death-inducing signal to a pro-metastatic one[128]. Further evidence that KRAS shapes tumour–microenvironment interactions can be gleaned from pancreatic tumours, more than 90% of which feature activating *KRAS* mutations[129]. In two recent studies, KRAS was shown to promote pancreatic cancer by inducing the expression of IL-17RA and intercellular adhesion molecule 1 (ICAM1) on epithelial cells and by promoting the development of a microenvironment enriched in T_H17 cells and inflammatory macrophages[130,131].

Silencing of CRC tumour-suppressor genes. Although *TP53* and *KRAS* are modified through mutation in CRC, the expression levels of several other genes relevant to cytokine signalling are modified epigenetically. For example, the gene encoding pro-apoptotic protein kinase Cδ-binding protein (PRKCDBP) is silenced by promoter hypermethylation in more than one-third of primary CRCs[132]. Intriguingly, PRKCDBP expression is induced by TNF in an NF-κB-dependent manner and promotes efficient TNF-induced apoptosis *in vivo*[132]. The gene encoding suppressor of cytokine signalling 3 (SOCS3), an important negative regulator of cytokine-induced STAT3 signalling, is similarly silenced through methylation in dysplastic colonic epithelium of patients with ulcerative colitis, which also feature high expression of the DNA methyltransferase DNMT1 (REF. 133). IL-6 was independently shown to induce DNMT1 protein expression in human CRC[134], suggesting that chronic inflammation may perpetuate a feedforward loop of cytokine signalling in a dysplastic setting. The gene encoding the receptor for oncostatin M (OSMR), another member of the IL-6 family, is methylated and silenced at a high frequency in CRC (up to 90%) but not in patients with ulcerative colitis or those with a normal colonic mucosa. Unlike IL-6, OSM seems to be cytostatic and therefore have tumour-suppressive effects in CRC[135,136].

Deletion of genes that support antitumour immunity. Selective deletion or amplification of cytokine and chemokine genes is a novel mechanism used by transformed cells to manipulate the cytokine environment. A surprising 21 chemokine genes were shown to be perturbed in this manner in more than 15% of human CRCs based on comparative genomic hybridization analysis[65]. One such aberration, *CXCL13* deletion, was associated with reduced numbers of tumour-infiltrating B cells and T follicular helper cells, and poor clinical outcome. Furthermore, both CXCL13 and its receptor, CXCR5, were important for controlling the outgrowth of syngeneic orthotopically transplanted tumours in mice[65]. Numerous cytokine and cytokine receptor genes are similarly amplified or deleted in CRC[102]. Notably, *IL15* deletion is enriched in metastatic CRC and associated with low *IL15* expression[102]. Reduced *IL15* expression is in turn associated with weak tumour infiltration by CTLs, low frequencies of proliferating B cells and T cells, and poor clinical outcome. Given the well-accepted role of IL-15 in supporting $CD8^+$ T cell expansion and viability in the periphery[99], this represents a novel mechanism of T cell attrition-based immune evasion.

Collectively, a growing literature demonstrates that CRCs are capable of directly altering the composition of the immune microenvironment by selectively augmenting the production of inflammatory cytokines with pro-tumorigenic effects or by depleting cytokines that favour immune surveillance. Furthermore, CRCs acquire mutations or epigenetic adaptations that maximize the oncogenic potential of inflammatory cytokines. This implies that significant clinical gains could be made by manipulating immune pathways in specific groups of patients whose tumours possess a relevant molecular signature; for example, neutralization of TNF in tumours with *TP53* mutations or strategies to bolster immunity against *IL15*-deletion mutants.

Cytokine networks and therapy for CRC

Although cytokines are largely studied as discrete variables, emerging data highlight the importance of elucidating the regulation and functional consequences of complex cytokine networks. Generally speaking, it is clear that tumour infiltration by T_H1 cells and CTLs is associated with favourable patient prognosis, whereas T_H17 cells and their products correlate with increased rates of disease recurrence[64,65,137]. Enrichment of IL-17$^+$ T cells, many of which are pro-inflammatory forkhead box P3 (FOXP3)$^+$ cells, has been repeatedly demonstrated in human CRC[28,73,138–140]. Consistent with this observation, human colon tumours exhibit high levels of T_H17 cell-derived cytokines (IL-17A, IL-17F and IL-22), T_H17 cell-polarizing cytokines (IL-1β, IL-6, IL-21 and TGFβ) and core pro-inflammatory cytokines such as TNF[44,48,138,139]. The abundance of several of these cytokines correlates with advanced disease stage and poor histological grade[44]. Interestingly, cytokines associated with immune regulation (such as IL-10) or T_H1 cell responses (such as IFNγ) are not highly enriched in CRC[138,139].

The overlapping functions and unpredictable interactions of cytokines in such diverse networks make it challenging to identify effective targets for intervention (FIG. 4). For example, haematopoietic cell deficiency of

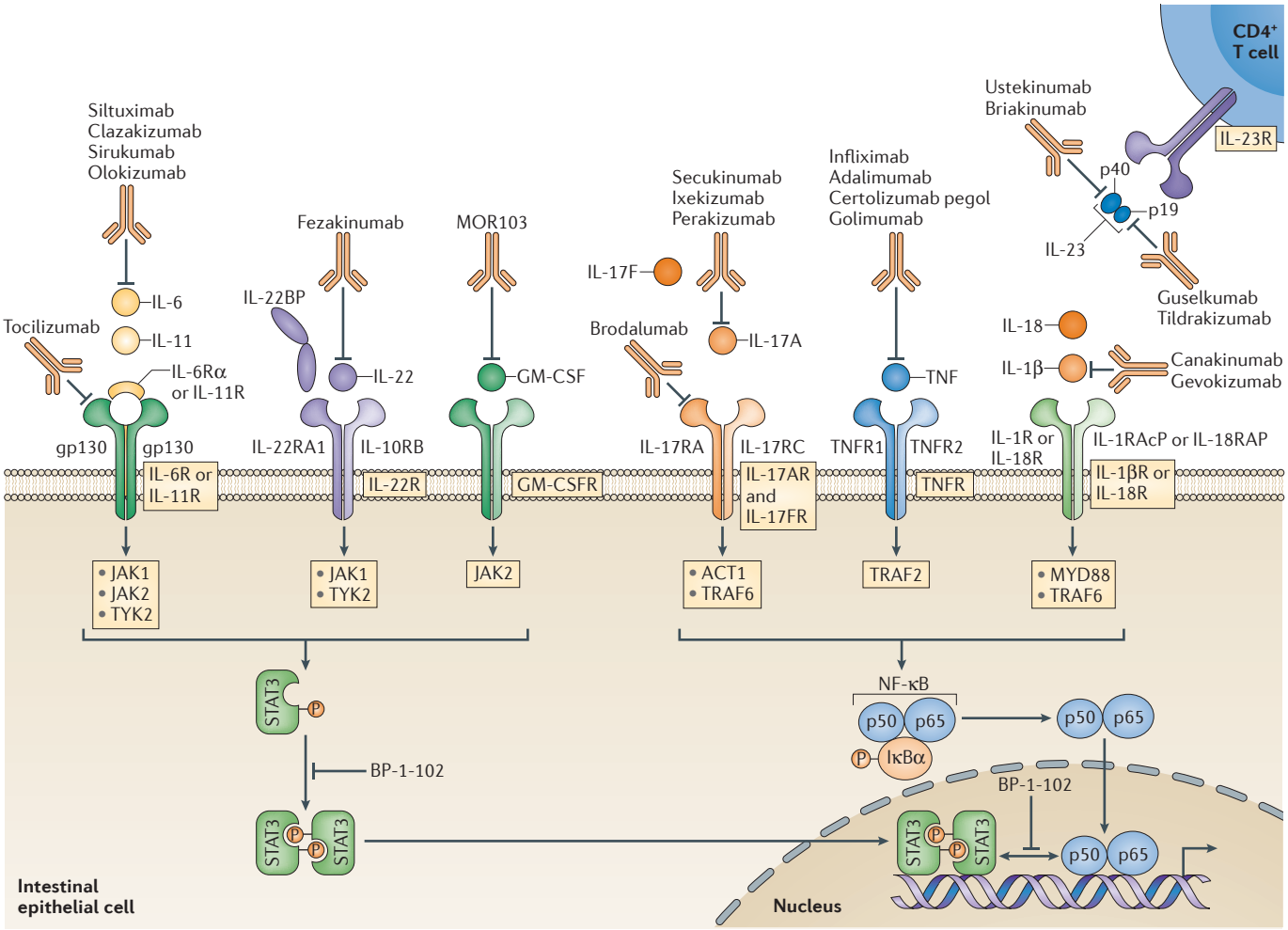


Figure 4 | **Cytokine signalling in colorectal cancer — opportunities for therapeutic intervention.** Major cytokines and their corresponding receptors expressed on intestinal epithelial cells (IECs) drive two critical intracellular signalling pathways, mediated by signal transducer and activator of transcription 3 (STAT3) and nuclear factor-κB (NF-κB), which together have numerous oncogenic effects. Receptor engagement activates Janus kinases (JAKs) and tyrosine kinase 2 (TYK2) in the STAT3 pathway or engages other downstream adaptor molecules in the NF-κB pathway. Interleukin-23 (IL-23) signalling in CD4$^+$ T cells induces the expression of IL-17A and IL-22, which are capable of activating STAT3 and NF-κB proliferative pathways in epithelial cells, respectively. The pro-tumorigenic or antitumorigenic effects of each cytokine are context dependent and influenced by crosstalk in the complex cytokine milieu. STAT3–NF-κB crosstalk can be driven by a number of cytokines with similar biochemical functions. A large number of monoclonal antibodies targeting cytokines or their receptors exist, but most have not been clinically tested for colorectal cancer (CRC). Combinatorial therapies that involve the use of cytokine or cytokine receptor blockade or small-molecule inhibitors such as BP-1-102 (which prevents STAT3 dimerization and STAT3–NF-κB interactions) may prove to be efficacious in the clinic. ACT1, adaptor protein CIKS; GM-CSF, granulocyte–macrophage colony-stimulating factor; IκBα, NF-κB inhibitor-α; IL-1RAcP, IL-1R accessory protein; IL-18RAP, IL-18R accessory protein; IL-22BP, IL-22-binding protein; MYD88, myeloid differentiation primary response protein 88; TNF, tumour necrosis factor; TNFR, TNF receptor; TRAF, TNFR-associated factor.

RORγt in the *Apc*$^{\Delta 468}$ model largely abolishes tumorigenesis along with the production of IL-1β, IL-6, IL-17A, IL-23 and TNF[28]. Although this suggests a strong role for T_H17 cell-derived cytokines, TNF is the most important cytokine driving tumorigenesis in this setting, whereas IL-6, IL-17A and IL-23 have lesser roles[28]. Similarly, in a CAC model with a mixed type 1 and type 17 cytokine signature, blockade of IL-22 strongly suppresses tumorigenesis, but blockade of IL-17A or IFNγ has only modest effects[66]. In the CPC-APC model of CRC, IL-17 and IL-23 signalling are clearly pro-tumorigenic but are not required for polyp formation, suggesting the involvement of additional pathways[61,63]. Consistent with these *in vivo* findings, supernatants from activated human tumour-infiltrating leukocytes (TILs) were shown to promote the proliferation of CRC cells, but this effect could not be abolished through neutralization of individual cytokines[139]. Rather, only combinatorial blockade of both STAT3 drivers (IL-6 and IL-22) and NF-κB inducers (TNF and IL-17A) could effectively block the mitogenic effect of TIL-derived products[139]. Intriguingly, treatment with the STAT3 inhibitor BP-1-102 was able to suppress TIL-induced activation of STAT3 and NF-κB, CRC cell proliferation and tumorigenesis when orally or intravenously administered to *Apc*$^{Min/+}$ mice[139]. The ability of BP-1-102 to inhibit the activation of both STAT3 and NF-κB, despite being a specific ligand of the STAT3 SH2 domain, may be due to the ability of STAT3 to enhance NF-κB signalling through direct physical interaction with NF-κB subunits[141–143].

Interactions between STAT3 and NF-κB are well recognized as critical processes in cancer, but a detailed account of these is beyond the scope of this Review[141]. Nevertheless, it should be emphasized that STAT3–NF-κB crosstalk is likely to be central to the complex pro-tumorigenic properties of the CRC cytokine milieu. Further intricacies were revealed by recent reports showing that SRC family kinases have key roles in mediating intestinal epithelial cell growth and regeneration, and that this can occur downstream of IL-6 family cytokines in a STAT3-independent manner[144,145]. Although IL-6 and TNF are important players in CRC, the tumour microenvironment is enriched in additional cytokines that have overlapping biochemical functions, including IL-1β, IL-11, IL-17A, IL-17F and IL-22. Therapeutic targeting of these pathways is therefore challenging, as exemplified by the failure of the IL-6-specific antibody siltuximab to elicit clinical responses in a recent Phase I/II trial[146]. Similarly, although siltuximab had detectable biological effects in a Phase II clinical trial for ovarian cancer, little clinical activity was observed[147]. Given the volume of data supporting a pro-tumorigenic role for IL-6, it is notable that only one intervention trial of IL-6 blockade in CRC has been conducted thus far (TABLE 2).

Clinical trials of other cytokine-targeting agents are similarly sparse: etanercept (a recombinant TNFR) was used in a Phase III clinical trial to treat cancer-related cachexia but showed no appreciable efficacy[148]. Two clinical trials of anakinra (a recombinant IL-1 receptor antagonist) in advanced CRC are currently recruiting.

Table 2 | **Clinical trials of cytokine-modulatory therapies for colorectal cancer**

Cytokine target	Agent	Phase	ClinicalTrials.gov identifier	Group responsible	Status
IL-1α	MABp1 (Xilonix, XBiotech)	I	NCT01021072	XBiotech	• Completed • Well tolerated and some indication of disease control[149]
		III	NCT01767857	XBiotech	• Ongoing • Not recruiting
IL-1β	Anakinra (Kineret, Sobi)	I	NCT01624766	M.D. Anderson Cancer Center, Texas, USA	Recruiting
		II	NCT02090101	Centre Georges François Leclerc, Dijon, France	Recruiting
IL-6	Siltuximab (CNTO-328 and Sylvant, Janssen-Cilag)	I/II	NCT00841191	Centocor	• Completed • Well tolerated but no objective responses observed[146]
IL-10	AM0010 (PEG-IL-10)	I	NCT02009449	ARMO BioSciences	Recruiting
IL-21	Recombinant human IL-21	I	Unknown	CRUK and Novo Nordisk	• Terminated • Some evidence of immune stimulation[104]
TNF	Etanercept (Enbrel, Amgen)	III	NCT00046904	Mayo Clinic and National Cancer Institute, USA	• Completed • No efficacy observed for control of cancer-related anorexia or cachexia[148]

CRUK, Cancer Research UK; IL, interleukin; PEG-IL-10, pegylated IL-10; TNF, tumour necrosis factor.

Intriguingly, a dose-escalation trial of the IL-1α-specific antibody MABp1 in metastatic cancer demonstrated safety and some evidence of disease control[149]. Finally, several Phase I/II trials of the small-molecule multi-kinase inhibitor sorafenib (which inhibits, among other things, Janus kinase 2 (JAK2)–STAT3 signalling) are currently in various stages of completion in CRC. Numerous other agents against cytokines relevant to CRC have either been approved for therapeutic use in inflammatory diseases or are currently in clinical trials including, to name but a few, infliximab (a TNF-specific antibody), canakinumab (an IL-1β-specific antibody), secukinumab (an IL-17A-specific antibody), ustekinumab (IL-12p40-specific antibody), tocilizumab (IL-6R-specific antibody), fezakinumab (an IL-22-specific antibody) and CAM3001 (a GM-CSF receptor α-subunit-specific antibody)[150–155]. None of these agents is currently in clinical trials for CRC.

It is too early to say whether targeting single cytokines in CRC will be clinically effective, but if CRCs prove refractory to standard mono-specific modalities, neutralizing signals from multiple cytokine pathways may prove fruitful. Small-molecule JAK inhibitors (particularly those that target JAK1 and JAK2) are such an option and have shown promising results in various cancer settings[156–160]. Cytokine families could also be broadly inhibited by targeting shared receptor subunits. For example, blockade of gp130 would inhibit the signalling of several cytokines including IL-6, IL-11, oncostatin M and leukaemia inhibitory factor[161]. Similarly, blockade of IL-22RA1 would impair not only IL-22-induced signalling but also that of IL-20 and IL-24 (REF. 162). Therapy with multiple mono-specific agents is another option and would allow for inhibition of mechanistically distinct pathways. By extension, the use of bispecific antibodies to target distinct cytokine pathways using a single therapeutic agent is an intriguing yet unexplored option for CRC[163]. Finally, both mono-specific and combinatorial approaches will probably achieve their full potential when coupled with careful molecular stratification of patients with CRC. For example, DNA methyltransferase inhibitors such as decitabine may be useful for treating tumours in which *SOCS3* has been epigenetically silenced, thereby restoring negative feedback on gp130 signalling[133,164]. Similarly, based on the augmented TNF and IL-6 signalling observed in CRC cells with *TP53* mutations, blockade of these cytokines may be most beneficial for treating p53-mutant tumours[30,115,116].

Conclusion

Intestinal cytokine networks are critical mediators of tissue homeostasis, inflammation and tumorigenesis. In both CAC and sporadic CRC, established cytokines such as IL-6 and TNF are being joined by a growing catalogue of novel players with similar biochemical functions, such as IL-17A and IL-22. Collectively, these cytokines promote several key hallmarks of cancer, including resistance to apoptosis; aberrant growth and proliferation; induction of genetic instability; angiogenesis; and invasiveness and metastasis. The multitude of pro-tumorigenic cytokines in CRC implies that combinatorial or broad-spectrum approaches to anti-cytokine therapies may be advantageous. Importantly, we have only begun to understand how specific molecular features of tumours dictate aberrant cytokine responses, and it is our opinion that exploiting these links for precise patient stratification will be essential for the successful application of immunomodulatory therapies in CRC.

Finally, this Review has revealed an area of very limited exploration: namely, how cytokine networks operate in metastatic sites. Most patients who undergo surgery for CRC never experience local relapse, but a substantial proportion of patients nevertheless develop deadly metastases in organs such as the liver and lung, indicating that the precursors of these lesions had probably emigrated from the primary intestinal tumour by the time of surgery. Although cytokine signalling in primary tumours is clearly important, targeting cytokine pathways that promote relapse by acting directly within the metastatic niche may be critical for preventing life-threatening disease progression in patients.

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Competing interests statement
The authors declare no competing interests.