REVIEW
Targeting JAK kinase in solid tumors: emerging opportunities and challenges

M Buchert¹,², CJ Burns¹ and M Ernst¹,²

Various human malignancies are characterized by excessive activation of the Janus family of cytoplasmic tyrosine kinases (JAK) and their associated transcription factors STAT3 and STAT5. In the majority of solid tumors, this occurs in response to increased abundance of inflammatory cytokines in the tumor microenvironment prominently produced by infiltrating innate immune cells. Many of these cytokines share common receptor subunits and belong to the interleukin (IL)-6/IL-11, IL-10/IL-22 and IL-12/IL-23 families. Therapeutic inhibition of the JAK/STAT3 pathway potentially offers considerable benefit owing to the capacity of JAK/STAT3 signaling to promote cancer hallmarks in the tumor and its environment, including proliferation, survival, angiogenesis, tumor metabolism while suppressing antitumor immunity. This is further emphasized by the current successful clinical applications of JAK-specific small molecule inhibitors for the treatment of inflammatory disorders and hematopoietic malignancies. Here we review current preclinical applications for JAK inhibitors for the treatment of solid cancers in mice, with a focus on the most common malignancies emanating from oncogenic transformation of the epithelial mucosa in the stomach and colon. Emerging data with small molecule JAK-specific adenosine triphosphate-binding analogs corroborate genetic findings and suggest that interference with the JAK/STAT3 pathway may suppress the growth of the most common forms of sporadic colon cancers that arise from mutations of the APC tumor suppressor gene. Likewise inhibition of cytokine-dependent activation of the JAK/STAT3 pathway may also afford orthogonal treatment opportunities for other oncogene-addicted cancer cells that have gained drug resistance.

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INTRODUCTION

Members of the Janus (JAK) family of protein tyrosine kinases were simultaneously discovered during the late 1980’s in the laboratories of Andrew Wilks, John Krolewski and John O’Shea and comprise JAK1, JAK2, JAK3 and TYK2.¹–³ Subsequent work established roles for JAKs in cytokine signal transduction during lymphocyte development, proliferation and differentiation, the host’s immune response during viral and bacterial infections and during acute and chronic inflammation.⁴ The interest in JAKs shifted sharply with the discoveries of widespread activating point mutations in JAK2, and later in JAK3, in myeloproliferative diseases,⁵–⁶ and the appreciation of inflammation as a hallmark of cancer.⁷ Although activating mutations in JAKs have not been found in solid tumors, JAKs are key participants in signaling networks fueled by an oversupply of cytokines secreted from both tumor cells and cells in the tumor microenvironment. In turn, this has spurred a strong interest to consider JAKs as potential drug targets for solid tumors. Here we provide an overview of recent findings and developments on therapeutic targeting of JAKs in solid tumors, with a focus on gastrointestinal and liver cancer.

The four JAK family members JAK1, JAK2, TYK2 and JAK3 associate with a variety of cytokine and growth factor receptors. Upon ligand binding to their cognate receptors and JAK activation afforded by reciprocal trans-phosphorylation, JAKs mediate intracellular signaling cascades principally through phosphorylation of STATs at a highly conserved tyrosine residue. Once tyrosine phosphorylated, STAT proteins form homo- or heterodimers, which are actively imported into the nucleus and bind to DNA consensus motifs to elicit specific transcriptional responses. In mammals, the receptor-specific pairing between four different JAKs and eight different STAT proteins allows for a high diversity of signaling outcomes from the limited number of proteins engaged (Table 1). Activation of JAK-dependent intracellular signaling is carefully regulated at various levels. Conformational restraint of the JH1 kinase domain through association with the pseudo-kinase (JH2) domain maintains the kinase in an inactive form prior to receptor engagement. Meanwhile, the action of tyrosine phosphatases and expression of specific inhibitors of JAKs limit the duration of JAK-STAT signaling. Although JAKs are constitutively associated with a large number of cytokine receptors, we will focus in this review on the four receptor families most relevant for cancer.

GP130 CO-RECEPTOR FAMILY

The glycoprotein receptor subunit GP130 (also known as IL6ST or CD130) is shared by the IL-6 family of cytokines comprising IL-6, IL-11, IL-27, OSM, LIF and others (see Table 1 and Figure 1) and is associated with JAK1, JAK2 and TYK2, which in turn activate STAT1, STAT3 and to a much lesser extent STAT5. Although IL-6 is a pro-inflammatory cytokine and enables the progression of many different tumor types by promoting proliferation and survival of neoplastic cells as well as stimulating angiogenesis,¹⁰–¹³ the far less-studied IL-11 is the dominant GP130-family cytokine driving
inflammation-associated gastrointestinal tumors. At least in vitro, IL-11 expression is induced by hypoxia in many human tumor cell types and promotes tumorigenesis via an autocrine mechanism. Meanwhile, in colorectal cancer (CRC) transforming growth factor β-stimulated cancer-associated fibroblasts secret IL-11 to trigger the GP130/JAK/STAT pathway in tumor cells to facilitate their survival and confer metastatic potential.

### IL-12/IL-23 RECEPTOR FAMILY

The dimeric IL-12 and IL-23 ligands activate heterodimeric transmembrane receptor complexes that share the common IL-12 receptor β1 chain and a ligand-specific IL-12 receptor β2 or IL-23 receptor subunit, respectively, and which are constitutively associated with JAK2 and TYK2. As the IL-23 receptor subunit binds to STAT3, whereas both IL-12 receptor subunits bind STAT4, only IL-23 signals through STAT3 and STAT4. In the tumor microenvironment, activated STAT3 favors transcription of the IL-23-specific p19 subunit over that of the IL-12-specific p35 subunit, which both compete for binding with p40 to form mature IL-23 and IL-12, respectively. IL-23 is upregulated in human CRC and promotes tumor growth and progression in mice. In this situation, IL-23 is produced by myeloid cells that become activated by exposure to microbial products through a TLR/Myd88-dependent pathway and which results from a defective epithelial cell barrier associated with oncogenic activation of the canonical WNT/β-catenin pathway. By contrast, dendritic cells and macrophages alongside naïve T cells can secrete IL-12 to induce T helper 1 (Th1)–mediated antitumor response mediated by interferon (IFN)γ. In addition, IL-23 is essential for the pathogenic maintenance of the Th17 population, which also requires STAT3-mediated induction of the transcription factors RorγT and Rora.

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**Table 1. JAK/STAT utilization**

<table>
<thead>
<tr>
<th>Receptor family</th>
<th>Ligand</th>
<th>JAK kinase</th>
<th>STAT</th>
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<tr>
<td>IL-2R</td>
<td>IL-2, IL-7, IL-9, IL-15, IL-21</td>
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<td>STAT1, STAT3, STAT5</td>
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<td>GP130</td>
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<td>STAT1, STAT2, STAT3, STAT4, STAT5</td>
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<td>JAK2, TYK2</td>
<td>STAT3, STAT4</td>
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<td>STAT6</td>
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<td>JAK2</td>
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<td>STAT1, STAT2</td>
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<td>GH</td>
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<td>CXCL12</td>
<td>JAK2, JAK3</td>
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Abbreviations: CD131, cytokine receptor common subunit beta; CT-1, ciliary neurotrophic factor; CXC, chemokine (C-X-C motif); EGF, epidermal growth factor; EPO, erythropoietin; GH, growth hormone; GMCSF, granulocyte-macrophage colony stimulating factor; GP130, glycoprotein 130; GPCR, G-protein coupled receptor; GCSF, granulocyte colony stimulating factor; IL, interleukin; IFN, interferon; JAK, janus kinase; LIF, leukemia inhibitory factor; OSM; oncostatin M; PDGF, platelet-derived growth factor; PRL, prolactin; STAT, signal transducer and activator of transcription; TPO, thrombopoietin; TSLP , thymic stromal lymphopoietic protein; TYK2, tyrosine kinase 2. This table summarizes the combinatorial use of janus tyrosine kinases and Stat proteins in cytokine/growth factor signaling. See text for further details.

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**Figure 1.** Schematic representation of the Jak/Stat-dependent cytokine receptor systems most relevant for regulating the progression of solid malignancies. The shared receptor subunits for the IL-6 family (gp130) and the IL-22 family (IL10R2) are indicated by identical coloring among the various receptors complexes that comprise each family. The depicted size of the Stat molecules indicates their relative importance for the transduction of cancer-relevant activities.
IL-10/IL-22 RECEPTOR FAMILY

The large family of cytokines utilizing the IL-10/IL-22 receptor includes IL-10, IL-19, IL-20, IL-22, IL-24 and IL-26 alongside IL-28A, IL-28B and IL-29 (also known as IFNα2, IFNα3 and IFNα1, respectively). Most of these cytokines share the common IL10R2 receptor subunit, except IL-19, IL-20 and IL-24, which utilize the related IL20R2. IL-10 signals through a tetrameric receptor complex comprising two IL10R1 and IL10R2 subunits, which associate with JAK1 and TYK2 and engage STAT3 as the sole transcription factor. The major role of IL-10 is to limit excessive inflammatory responses typically by downregulating T-cell functions.22 Accordingly, IL-10-deficient mice spontaneously develop inflammatory bowel disease as a consequence of CD4+ T-cell hyperactivation in response to antigen exposure from the intestinal microflora.23 Conversely, excessive IL-10 production by tumor cells and tumor-associated immune cells leads to an immunosuppressive microenvironment and is commonly observed in ovarian,24 breast,25 lung,26 colon27 and skin cancers.28–30 In stark contrast to its well-documented immunosuppressive functions, IL-10 was recently shown to be instrumental in activating tumor-resident CD8+ T cells to induce tumor rejection. Interestingly, this did not require de novo migration of activated CD8+ T cells from primary or secondary lymph nodes. Instead, IL-10 administration selectively increased proliferation of the autochthonous tumor-resident CD8+ T-cell population.31,32

IL-22 binds to a heterodimeric receptor comprising the ubiquitously expressed IL10R2 chain and IL22RA1, which is found on skin, pancreas, liver, kidney and the intestine. The IL-22 cytokine signals via JAK1 and TYK2 and activates STAT3 far more prominently than STAT1 and STAT5. Myeloid cells and lymphocytes are the major source of IL-22, which binds to various epithelial cells to induce host defense mechanisms and to promote tissue repair.33–35 The role of IL-22 in chronic inflammatory disease including psoriasis, rheumatoid arthritis and inflammatory bowel disease is complex with both pathogenic and beneficial effects being reported.36–41 Similarly, IL-22 supports both cancer-promoting and repressing functions in preclinical mouse models.42–44

IFN RECEPTOR FAMILY

IFNs are pleiotropic cytokines with antiviral, antiproliferative, anti-tumor and immunomodulatory properties. IFNα/β bind to IFNαR1 and IFNαR2 and signal predominantly via STAT1 and STAT2 via engagement of JAK1 and TYK2. IFNα/β-mediated activation of STAT1 in dendritic cells is involved in the activation of CD8+ T cells, and thus is important for the rejection of immunogenic tumor cells.35 Similarly, nuclear STAT1 expression in CRC predicts a better patient outcome and strongly correlates with accumulation of intratumoral T cells and major histocompatibility complex class I expression,46 and shifts the antitumor immune response from natural killer cells toward CD8+ T cells.47

Meanwhile, IFNγ utilizes the IFNγR1 and IFNγR2 receptor subunits and relays the intracellular signal via JAK1/JAK2 and STAT1. Several studies have underpinned the importance of the IFNγ pathway for the inhibition of tumor growth. Mice lacking either IFNγ, IFNγR or STAT1 show increased tumor incidence.48–50 In contrast to IFNα knockout mice where the antitumor response is orchestrated by bone marrow-derived cells, IFNγ asserts its effects directly on tumor cells by inducing cell cycle arrest, apoptosis and increasing antitumor immune recognition.51–54 Chronic IFNγ signaling, and associated STAT1 hyperactivation, in suppressor of cytokine signaling (SOCS1)-deficient mice, leads to spontaneous colorectal carcinoma development.55 Likewise, STAT1 overexpression is linked to more aggressive forms of triple-negative breast cancers49 and an IFNγ/STAT1 gene signature predicted poor survival for a subgroup of brain tumors.57

However, IL-10 administration to tumor-bearing mice induces strong activation of STAT1 in tumor-resident CD8+ T cells, most likely via IFNγ, and promoted tumor rejection.31 IFNγ therapy is used for treating hematological malignancies56 and patients with recurring melanoma receive IFNα2b.57 However, IFNγ also induces the expression of the PD1 ligand, which, upon interaction with the PD1 receptor on effector T cells reduces their activity, thereby not only limiting collateral damage during inflammation but also reducing the host’s antitumor response.58,59

ACTIVATION BY NON-CYTOKINE RECEPTOR

Activation of the JAK-STAT pathway can also occur downstream of other non-receptor tyrosine kinases including SRC and BCR-ABL, as well as receptor tyrosine kinases, which are often overexpressed or persistently activated in many solid tumors.60–62 The latter includes the human epidermal growth factor receptor (HER), the vascular endothelial growth factor receptor and the receptor for platelet-derived growth factor and insulin-like growth factor.63 Independent of JAK kinases, STAT3, STAT5A and STAT5B are also common targets for non-receptor tyrosine kinases including the c-ABL and the SRC-family kinases SRC, LYN, FYN, as well as some G-protein coupled receptor agonists.64–70 Accordingly, STAT3 or STAT5 are required for v-SRC-dependent cellular transformation.71–73 Often, activation of STAT proteins by the cellular homolog c-SRC occurs downstream of the HER family of receptors.74 Thus, c-SRC-dependent STAT3 and STAT5 activation appears to be necessary for tumorogenesis,74,75 and in human breast cancer HER family members and c-SRC are overexpressed in 60% and 70%, respectively.

The JAK-STAT signal transduction pathway is tightly controlled by at least three different mechanisms of negative regulators, comprising the SOCS proteins, the PIAS (protein inhibitors of activated STATs) and protein tyrosine phosphatases such as SHP-1, SHP-2, CD45 and PTP1B/TC-PTP. The physiological importance of these negative regulators is illustrated by their frequent impairment through mutations in many different pathologies. Accordingly, loss or reduced expression of SOCS1, PIAS3, PIASY or SHP-1, or mutations in PTPN11/SHP-2 and SOCS1, are frequently observed in myeloproliferative diseases.76–80 Epigenetic silencing of SOCS1 through promoter hypermethylation is observed in tumors of the cervix,81 ovaries,82 pancreases,83–85 and in melanoma86 and hepatocellular carcinomas (HCCs).87,88 and during the transformation of liver cirrhosis to HCC.86–88 SOCS1 and SOCS2 downregulation are a common feature in HCC,89–92 ovarian cancer,89 and melanomas,93,94 whereas SOCS3 downregulation is found in colon,100,101 lung,102 gliomas,103,104 and prostate105 cancer. Indeed, there is a striking correlation between SOCS expression and disease progression and prognosis.99,106,107

Aberrant CpG island methylation of the SOCS1 promoter is observed in 8% of primarily younger CRC patients, indicating that SOCS silencing may occur during early stages of cancer development.108 Moreover, SOCS2 promoter methylation is prevalent in up to 25% of patients and coincides with reduced survival.109 In gastric cancer, SOCS1 hypermethylation occurs in ~40% of patients and is more pronounced in malignant lesions than in normal gastric mucosas.110 Likewise SOCS1 methylation and reduced SOCS1 expression correlated with lymph node metastasis. Hypermethylation of the SOCS1 and SOCS3 promoters is also observed in Barrett’s esophageal adenocarcinoma and corresponding cell lines.111

EXCESSIVE JAK-STAT ACTIVITY IN THE TUMOR MICROENVIRONMENT

Many of the cytokines that are locally produced by cancer-associated fibroblasts, cancer-associated adipocytes and tumor-associated immune and endothelial cells fuel the growth of
tumors.\textsuperscript{112–114} Accordingly, excessive JAK-STAT signaling in cells of the tumor microenvironment underpins transcriptional programs that favor angiogenesis and neo-vascularization. Thus, STAT3 induces the expression of vascular endothelial growth factor, and hypoxia-inducible factor 1 alpha as potent transcription factors that coordinate angiogenesis and the responses to hypoxia.\textsuperscript{115–117} STAT3 also promotes immune cell infiltration, tumor invasion and metastasis. Moreover, excessive STAT3 activity in these cells also dampens antitumor responses by, for instance, increasing the number of immunosuppressive and tumor-promoting myeloid-derived suppressor cells and by blocking dendritic cell differentiation and expansion.\textsuperscript{118} STAT3 also favors Th17 cell differentiation, which in turn promotes a pro-inflammatory microenvironment.\textsuperscript{118,119} On the other hand, effector T-cell proliferation is effectively blocked through STAT3-mediated IL-23p19 expression, whereas the production of pro-tumorigenic IL-10-producing type 1 regulatory T cells is promoted by the IL-27-mediated activation of STAT3.\textsuperscript{117,120} Likewise, STAT3 further curbs the capacity of the adaptive immune system to mount an efficient antitumor response through increased PD-L1 expression on antigen presenting cells.\textsuperscript{121}

IL-22 has a protective role in the intestine by promoting epithelial proliferation and maintaining the integrity of the epithelial barrier. IL-22, produced mainly by ROR\gammaT\textsuperscript{+} intestinal lymphoid cells, is a potent inducer of antimicrobial peptides and mucins, which regulate bacterial colonization of the intestinal epithelial surface and associated lymphoid tissues and thus contributes to normal intestinal physiology by maintaining the homeostatic balance between immune system and the colonic microbiota.\textsuperscript{122} In line with these observations, IL-22-deficient mice develop dysbiosis and are more susceptible to colitis,\textsuperscript{123} whereas IL-22 binding protein deficiency, which counter balances the activity of free IL-22, protects against colitis and sustains colitis-associated tumorigenesis.\textsuperscript{124} However, a more detrimental role of this cytokine has recently been uncovered where it helped pathogenic bacteria out-competing the host's microflora.\textsuperscript{125} As would be expected from excessive STAT3 activation in neoplastic cells that express IL-22 receptors, unabated IL-22 expression by Th17, Th22 and innate lymphoid cells promotes colon tumorigenesis.\textsuperscript{126}

Excessive JAK-STAT activity in neoplastic cells

In malignant cells, STAT3 simultaneously induces survival genes, such as Bcl-xL, Bcl-2, Mcl-1 and Survivin,\textsuperscript{127,128} as well as cell cycle progression and proliferation genes, including c-Myc, CyclinD1, Cdc2 and others.\textsuperscript{129–131} At least in vitro this is sufficient for constitutive active STAT3 to transform fibroblasts, suggesting that STAT3 may function as a proto-oncogene.\textsuperscript{132} Furthermore, IL-6-mediated STAT3 activation promotes epithelial-to-mesenchymal transformation and metastasis, thereby functionally linking the IL-6-rich tumor microenvironment with tumor progression.\textsuperscript{133,134} Meanwhile, the survival and proliferation functions of JAK/STAT3 signaling facilitate colonization of metastasizing tumor cells at distant sites. In CRC and lung cancer, for instance, IL-11/GP130/STAT3 signaling promotes invasion and colonization of the liver and other distant organs.\textsuperscript{16,135} At least in CRC, IL-11 is expressed by cancer-associated fibroblasts in response to tumor-derived transforming growth factor \(\beta\), whereas transforming growth factor \(\beta\) in lung cancer cells also induces the expression of a long non-coding RNA, which binds to and stabilizes IL-11 mRNA.

Although less well studied in solid malignancies, the JAK2/STAT5 axis appears to be relevant for cancers of the prostate,\textsuperscript{136} breast\textsuperscript{137} and CRC\textsuperscript{138} and is activated by growth hormone, epidermal growth factor and many chemokines.\textsuperscript{139} Survey of the literature indicates that STAT5 can have tumor promoting as well as suppressing functions, depending on tissue and cellular context. Excessive STAT5 activation in human CRC or prostate cancer, for instance, correlates with shorter survival.\textsuperscript{140,141} STAT5 also induces malignant transformation of mammary epithelial cells,\textsuperscript{142} and the STAT5b isoform specifically promotes hepatocellular carcinoma progression and epithelial-to-mesenchymal transition.\textsuperscript{143} In endothelial cells, CCR2/JAK2/STAT5 signaling aids extravasation of CCL2-positive CRC cells, and therefore pharmacological blockade of this pathway with a JAK2 inhibitor greatly reduced the number of lung metastasis in a corresponding xenograft model.\textsuperscript{144} Conversely, STAT5 inhibition blocks CRC cell proliferation and their invasive capacity.\textsuperscript{145} However, activated STAT5 is a predictor for good prognosis in breast cancer,\textsuperscript{146} and loss of STAT5 promotes liver fibrosis and increased liver tumor formation.\textsuperscript{147,148}

Although most cellular functions attributed to STAT proteins have been linked to their canonical functions as tyrosine phosphorylated transcriptional regulators, emerging evidence also suggest functions outside the nucleus. The switch from oxidative to glycolytic glucose metabolism, for instance, is facilitated by STAT3 through upregulation of lactate dehydrogenase.\textsuperscript{149} Aerobic glycolysis is an important characteristic of tumor cells and allows cancer cells to switch from normal aerobic glucose metabolism in favor of glycolysis by bypassing the oxidative phosphorylation process and instead converting pyruvate to lactate.\textsuperscript{150} The recent realization that serine-phosphorylated STAT3 functions within the mitochondria\textsuperscript{151,152} and that MEK-dependent mitochrondrial STAT3 is required for cellular transformation by activated Ras oncogenes reveals an important mechanism for STAT3 in cellular transformation independent of JAKs.\textsuperscript{153} Moreover, at least in leukemic T cells, tyrosine phosphorylated STAT5 is able to translocate to mitochondria as well, where it may be involved directly in regulation of mitochondrial DNA expression and induction of a metabolic shift to aerobic glycolysis and thus favoring tumor cell metabolism.\textsuperscript{154} Likewise, unphosphorylated STAT3 may also have an important, cytokine-independent role in melanoma, breast and head and neck cancers where its overexpression promotes expression of the RAS and MET oncogenes.\textsuperscript{155–158}

Although there is overwhelming genetic and phenomenological evidence that excessive tumor-intrinsic STAT3 activity promotes survival and proliferation of malignant cells, some molecular scenarios suggest that reduced STAT3 expression may also promote tumor formation. For instance, STAT3 deficiency promoted K-RAS-dependent lung adenocarcinoma formation,\textsuperscript{159} reminiscent of findings showing that the overexpression of a constitutively active STAT3 reduced tumor cell growth in a K-RAS-driven HCC model of p14/p19ARF-deficient cells.\textsuperscript{160} The former observations have been reconciled by the capacity of STAT3 to sequester nuclear factor-\(\kappa\)B, thereby reducing IL-8 expression and accumulation of myeloid cells in the developing tumors.\textsuperscript{159} Meanwhile, it has been suggested that complete STAT3 deficiency in the intestinal epithelium of Apc\textsuperscript{Min} mice may promote tumor development through compensatory STAT1 activation and associated downregulation of the cell adhesion protein CAECAM-1.\textsuperscript{161} However, a caveat with all of these models is that the tumor-promoting activity appears contingent on the complete STAT3 ablation in tumor cells, an outcome unlikely achieved by therapeutic interference with STAT3 activation, including targeting of JAK kinases. Strikingly, genetic reduction of STAT3 expression, as an approximation of a therapeutic impact, has not been associated with a reduction of the aforementioned ‘tumor suppressor’ activities of STAT3.

JAK in gastrointestinal homeostasis and disease

The fruit fly Drosophila melanogaster has been a fertile ground to gain insights into mechanisms by which JAK/STAT signaling underpins homeostasis and regeneration of the intestinal epithelium. Infection by pathogenic bacteria or tissue damage,
for instance, leads to the secretion from intestinal epithelial cells (IECs) of the ligand Upd (the sole fly orthologue for mammalian IL-6 family cytokines), which activates the JAK/STAT pathway in neighboring intestinal stem cells to induce their division.\textsuperscript{162,163} Indeed, the overexpression of Upd is enough to drive intestinal stem cell hyper-proliferation\textsuperscript{164} and constitutive activation of the WNT/β-catenin pathway, in response to loss of the tumor suppressor Apc in intestinal stem cells, triggers their hyperproliferation through non-cell autonomous activation of the JAK/STAT pathway.\textsuperscript{155–157}

Several lines of evidence suggest that the role of the JAK/STAT pathway in intestinal regeneration and intestinal stem cell homeostasis is conserved from fly to mammals. The mucosa of the small intestine in mice, for instance, shows better recovery from cytoablative therapies following systemic administration of IL-11, and this was associated with the preservation of the clonogenic potential of IECs.\textsuperscript{168–171} Mechanistically, IL-11 treatment afforded the protection of IECs by a partial suppression of apoptosis and an increased rate of their proliferation.\textsuperscript{172} Others reported activation of the JAK2/STAT3 pathway during the transition from normal to a hyper-proliferative colonic epithelium.\textsuperscript{173} In line with this, Epstein–Barr virus-positive gastric adenocarcinomas show amplifications of the JAK2 locus in humans.\textsuperscript{174}

In mice, and most likely in humans, IL-6 and IL-11 are required for sporadic CRC,\textsuperscript{175} and we have shown that IL-11 acts as the dominant GP130 cytokine over IL-6 in driving CRC in mouse models.\textsuperscript{14} Surprisingly, the tumor-promoting activity of IL-11 is not mediated by hematopoietic cells, but likely through binding of IL-11 directly to IECs.\textsuperscript{176,177} Accordingly, the growth of APC-mutant tumors can be inhibited by therapeutic administration of IL-11 Mutein, a mutant version of IL-11 with a 20-fold higher binding affinity to IL-11, but which prevents subsequent binding to GP130 and hence GP130 activation.\textsuperscript{14} Likewise in a murine colitis-associated colon cancer model, IL-11 Mutein decreased STAT3 activation and tumor growth, which coincides with re-expression of the BCL-2 protein antagonist BIM. A reduced tumor load is also observed after therapeutic administration of the JAK1/2-specific inhibitor AZD1480 or the JAK2-specific inhibitor CEP-33799 in the colitis-associated colon cancer model.\textsuperscript{138,178} As both compounds were effective, it is unclear whether inhibition of JAK1 is necessary in this experimental setting or whether other JAKs require simultaneous inhibition. Although AZD1480 also blocked STAT3 activation, genetic experiments suggest that STAT3 is the predominant driver of tumorigenesis in the colitis-associated colon cancer model.\textsuperscript{179}

Excessive, IL-11-dependent STAT3 activation is also the principal driver of tumorigenesis in the GP130\textsuperscript{177,179} mouse model of inflammation-associated gastric cancer.\textsuperscript{180} Similar to the colitis-associated colon cancer model, IL11Ra deficiency rather than ablation of IL-6 or STAT1 prevented gastric tumor formation in this model, whereas therapeutic IL-11 inhibition slowed it down.\textsuperscript{14,180} This effect was phenocopied following inhibition of the JAK/STAT pathway by administration of the JAK inhibitors AZD1480 or WP1066.\textsuperscript{138,181} Indeed, genetic deletion of STAT3 in gastric tumor cells was sufficient to reduce tumor burden, suggesting that the JAK/STAT3 effect was at least in part tumor cell intrinsic and not mediated by cells of the tumor stroma.\textsuperscript{138}

Although the effectiveness of blocking JAK kinase activity appears expected in light of the strong inflammation-driven nature of the aforementioned cancer models, we recently also documented a tumor cell intrinsic requirement for the JAK/STAT3 pathway for the most common forms of sporadic CRC.\textsuperscript{182} These arise in >80% of cases from aberrant activation of the WNT/β-catenin pathway, which primarily results as a consequence of homozygous loss or inactivation of the APC tumor suppressor gene. Given that the WNT/β-catenin pathway underpins the continuous renewal of the intestinal epithelium and ensures tissue homeostasis throughout adult life, treating APC-mutant tumors with WNT/β-catenin pathway inhibitors is likely to cause on-target toxicity. Surprisingly, the growth of APC-mutant neoplastic epithelium, but not of normal intestinal epithelium, also requires continuous signaling through the GP130/JAK/STAT3 pathway. Indeed, genetic reduction of GP130 activity or of IEC-specific STAT3 expression limits proliferation of APC-mutant tumors and corresponding CRC cell lines.\textsuperscript{182} Remarkably, this occurs without interfering with excessive WNT/β-catenin pathway activation, suggesting that the GP130/JAK/STAT3 pathway becomes rate-limiting for the proliferation of IECs exposed to high WNT/β-catenin signaling.\textsuperscript{182} Consistent with this, mucosal wound-healing as a physiological situation of the intestinal epithelium being exposed to high WNT/β-catenin signaling, is also impaired in mice carrying impairment mutations in the GP130/JAK/STAT3 pathway, whereas homeostatic renewal of the uninjured epithelium is unaffected. These observations suggest that GP130/JAK/STAT3 pathway activation may help to expedite IEC proliferation specifically at sites of epithelial injury, which are characterized by localized tissue inflammation. Thus, the GP130/JAK/STAT3 pathway may provide an evolutionary conserved rheostat mechanism, whereby the extent of epithelial denudation, and hence commensal bacteria-elicited inflammation, modifies the capacity of WNT/β-catenin signaling-dependent mucosal barrier regeneration. This response remains self-limiting in light of subsiding inflammation as a consequence of a reestablished mucosal barrier.

As a corollary to the concept of cancers sharing hallmarks of ‘never closing wounds’, neoplastic CRC cells with driver mutations in the WNT/β-catenin signaling components APC or CTNNB1 (encoding β-catenin) retain their dependence on GP130/JAK/STAT3 pathway activation.

Building on these genetic insights, we recently observed that systemic administration of the JAK inhibitor AZD1480 also effectively blocked tumor growth in two models of CRC, which are driven by APC mutations and arise in the absence of overt inflammation.\textsuperscript{182} Importantly, this included the Apc\textsuperscript{min} mouse model of the familial adenomatous polyposis syndrome, where AZD1480 administration also delayed the formation of new adenomas.\textsuperscript{182} Systemic AZD1480 administration also blocked tumor growth of human CRC xenografts in immuno-compromised mice as hosts, suggesting that this effect was not mediated by re-activation of the host’s antitumor immunity associated with STAT3 inhibition in dendritic cells and other immune-regulatory cells.\textsuperscript{182} Our collective observations in complementing mouse models and human colon cancer cell line xenografts suggest that the growth of tumors is inhibited most effectively when cells harbor mutations in the tumor suppressor gene APC or the transcriptional cofactor β-catenin,\textsuperscript{182} which collectively result in aberrant activation of the canonical WNT pathway. As these lesions also show a gene expression profile that is consistent with persistent STAT3 activation,\textsuperscript{182} such gene signatures may serve as one of the guides for stratifying patients that are likely to respond to treatment with JAK inhibitors.

The JAK/STAT3 pathway can act as a tumor promoter in many other solid tumors. For instance, the substantially higher incidence of HCC observed in males was attributed to gender-specific difference in carcinogen-induced IL-6 production by Kupffer cells (liver macrophages).\textsuperscript{183} Subsequently, activating mutations in the GP130 receptor were identified in ~60% of human inflammatory hepatocellular adenomas.\textsuperscript{184} In the same tumor cohort, a further 12% of adenomas without GP130 mutations harbored activating mutations in STAT3.\textsuperscript{185} At least in vitro, the activity of these mutant GP130 receptors depended on JAK1 rather than JAK2 or TYK2 as it was efficiently blocked by the JAK1/2 inhibitor Ruxolitinib but not by AG490, a weak and non-selective tyrosine kinase inhibitor.\textsuperscript{186} JAK inhibitors or negative regulators of JAK activity indeed confer antiproliferative and pro-apoptotic effects on HCC cell lines in vitro and in vivo.\textsuperscript{186–189}
In lung tumors, autocrine IL-6 signaling acts downstream of mutant HER through the JAK/STAT3 pathway and promotes cellular transformation and tumor growth. Meanwhile, autocrine stimulation of mammary tumor cells by IL-6 leads to induction of the Notch pathway components Notch-3 and Jagged-1 to promote cell survival, increased resistance to hypoxia and an enrichment of tumor progenitor cells. Likewise, in squamous cell carcinomas of the skin an autocrine IL-6/JAK/STAT3 pathway is responsible for setting up a cytokine network promoting tumor proliferation and invasion. Thus, the above evidence alongside that from breast carcinoma models reiterates that a role of IL-6 as a tumor promoter.\(^{194,195,196,199,199}\)

Somatically occurring mutations that result in constitutive JAK activation are expected to be rare, although the JAK2 locus is amplified in gastric cancer\(^{173}\) and activating mutations in JAK1 have been discovered in human HCC.\(^{192}\) Even so, the JAK/STAT3 pathway is often activated in both tumor cells and the tumor microenvironment due to paracrine and autocrine feed-forward loops mediated and driven by an oversupply of primarily GP130-family cytokines.

EGFR (epidermal growth factor receptor) overexpression, which commonly occurs in solid malignancies, not only leads to a SHP-2 phosphatase-mediated inactivation of STAT1 causing the suppression of the antigen-processing machinery and associated immune surveillance,\(^{193}\) but also activates STAT3, thereby promoting the survival, proliferation and dissemination of cancer cells.\(^{194,195}\) Indeed, escape from immune surveillance is further promoted by the establishment of an immunosuppressive tumor microenvironment brought about by IL-6, IL-10, transforming growth factor beta1 and vascular endothelial growth factor, which are secreted upon induction of the antigen-processing machinery and associated immune surveillance.\(^{194,195}\) Many of these factors also activate STAT3 in tumor-infiltrating immune cells to establish a positive feed-back circuitry in a STAT3-dominated tumor microenvironment. Furthermore, STAT3 is often excessively activated independently of EGFR, as tumor cells also often overexpress the two components of the functional IL-6 receptor activated independently of EGFR, as tumor cells also often overexpress the two components of the functional IL-6 receptor activated independently of EGFR, as tumor cells also often overexpress the two components of the functional IL-6 receptor activated independently of EGFR, as tumor cells also often overexpress the two components of the functional IL-6 receptor activated independently of EGFR, as tumor cells also often overexpress the two components of the functional IL-6 receptor.\(^{194,195,196}\) As EGFR as well as GP130/IL6R alpha promote STAT3 phosphorylation by engaging JAK kinases, their inhibition is predicted to disrupt the positive feed-forward loop and to reverse immunosuppressive activity of STAT3.

JAK inhibitors

The first bona fide JAK inhibitor reported was pyridone 6, a compound described by Merck in 2002 and shown to possess nanomolar activity against all members of the JAK family in biochemical assays.\(^{197}\) Although pyridone 6 has been used extensively to explore the effect of JAK inhibition in vitro, its poor pharmacokinetic profile precludes the use of pyridone 6 in animals. The crystal structure revealed that pyridone 6 binds to the adenosine triphosphate (ATP) pocket of the JH1 kinase domain of the activated (for example, phosphorylated) active conformation of JAK2.\(^{198}\) Although most JAK inhibitors subsequently described (vide infra) also bind to the JH1 domain in this conformation, at least one compound is able to bind to JAK2 in the inactivated form.\(^{199}\)

Although the ATP-binding site in the JH1 domain is highly conserved among the four JAK kinases, some of the corresponding ATP-competitive compounds show differential binding capacity and have entered clinical trial (Table 2). The design of JAK inhibitors has largely focused on two different indications, namely in hematology/oncology (ostensibly JAK2 or dual JAK1/2 inhibitors) and inflammatory diseases (selectively targeting JAK1 or JAK3, or dual JAK1/3 inhibitors). Although the initial clinical experience with JAK inhibitors was variable, the approvals of Tofacitinib (Xeljanz) and Ruxolitinib (Jakafi) clearly demonstrate that safe and effective JAK inhibitors can be developed.

Given the prevalence of activating mutations in JAK2 in myeloproliferative disorders, current clinical trials with JAK inhibitors have focused on hematological malignancies and chronic inflammation (arthritis, psoriasis, inflammatory bowel disease)\(^{6–8}\) (Table 2). The general findings from trials with compounds targeting primarily JAK2 indicate anemia and thrombocytopenia as rate-limiting toxicities, consistent with the prominent role of JAK2 in transducing signals from the receptors for erythropoietin and thrombopoietin. In the context of myelofibrosis, however, the JAK2 inhibitor CYT387 revealed a much reduced need for blood transfusions in a subset of patients.\(^{200}\) Meanwhile, some JAK1/2 inhibitors were associated with headache, nausea and neurotoxicity, resulting in the discontinuation of clinical studies with AZD1480 and XL019.\(^{201}\) As the latter side effects are only apparent with some JAK

<p>| Table 2. Selective JAK inhibitors in clinical trials for treatment of solid tumors and treatment of inflammatory bowel disease |</p>
<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Status</th>
<th>Tumor type</th>
<th>Clinical trials identifier</th>
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<tr>
<td><strong>A. Selective JAK inhibitors in clinical trials for treatment of solid tumors</strong></td>
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<td>AZD1480 JAK1, JAK2</td>
<td>Discont’d</td>
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<td>Ruxolitinib JAK1, JAK2</td>
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<td></td>
<td>Phase I/I</td>
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<td>NCT00638378, NCT02119650</td>
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<td>Phase I/I</td>
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<td></td>
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<td></td>
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<td>NCT02206763, NCT02244489</td>
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<td></td>
<td>Phase I</td>
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<td>INCB-39110 JAK1</td>
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<td>Solid tumor</td>
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<tr>
<td><strong>B. Selective JAK inhibitors in clinical trials for treatment of inflammatory bowel disease</strong></td>
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<tr>
<td>Peftacitinib JAK1, JAK3</td>
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<td>NCT01959282</td>
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<td>Tofacitinib JAK3, JAK2, JAK1</td>
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<td>Ulcerative colitis, Crohn’s disease, Ulcerative colitis</td>
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Abbreviation: NSCLC, non-small-cell lung carcinoma.
inhibitors, they are unlikely to result from specific, on-target toxicity; however, differences in drug permeability across the blood brain barrier may also account for these disparate central nervous system effects. Clinical studies with compounds directed toward inflammatory indications, and mainly targeting JAK1 and/or JAK3, typically show on-target rate-limiting toxicity with respect to increased infection rates. These inhibitory profiles for some of the JAK inhibitors are also associated with an increase in low- and high-density lipoprotein.

Given the high similarity of the ATP-binding pockets among the ~500 distinct kinases in humans, new targeting approaches have been pursued to increase selectivity and specificity of JAK inhibitors. However, whether more selective JAK inhibitors will demonstrate improved therapeutic benefit remains to be determined, as for instance selective JAK3 inhibitors appear to be less effective inhibitors of IL-2R signaling than pan-JAK inhibitors. Kershaw and colleagues, for instance, discovered in the crystal structure of a complex comprising SOCS3, the kinase domain of JAK2 and a fragment of GP130, that SOCS3 inhibits JAK2 kinase activity by binding to its substrate-binding groove in the JH1 domain. As this groove determines substrate specificity, this region has considerably more variability between different kinases than the ATP-binding pocket. However, it remains to be shown whether the substrate-binding pockets can serve as druggable pockets to which more selective and less toxic inhibitors can be targeted.

Synergistic activities of JAK inhibitors

JAK and most other protein tyrosine kinases require chaperone proteins to keep the kinases in a functionally active conformation, and interference with this process results in reduced kinase activity. Accordingly, combining the JAK2 inhibitor TG101209 with an inhibitor of heat shock protein 90 reduced JAK2 signaling and induced apoptosis in myeloproliferative neoplasms in a synergistic manner. This was attributed to misfolding and polyubiquitylation of JAK2 and its subsequent degradation by the 26S proteasome. Surprisingly, however, when tested on multiple myeloma cells, the JAK inhibitor CYT387 synergized with the proteasome inhibitor Bortezomib. Synergism with histone deacetylase inhibitors has also been observed in vitro and for the treatment of Hodgkin lymphoma, and the combination of the JAK inhibitor Ruxolitinib with the pan-deacetylase inhibitor Panobinostat showed complementary effects on disease biology in mouse models of myeloproliferative neoplasms. Others have found coactivation of the JAK/STAT3/5, ERK1/2, MEK1/2 and PI3K/AKT pathways in acute lymphoblastic leukemia xenografts harboring activating JAK mutations, and associated synergistic treatment effect between JAK inhibition and a dual ERK/MEK inhibitor. At least in myeloproliferative neoplasm this may occur through JAK-dependent, aberrant engagement of the thrombopoietin receptor, which can activate the SHC/RAS/ MEK/ERK signaling cascade. Probably, the most striking synergy is observed between JAK and PI3K inhibitors, owing to a mechanism whereby excessive STAT5 yields interaction with the regulatory p85 subunit of PI3K. Synergistic effects were also observed in preclinical studies of myeloproliferative neoplasms and lymphoma when combining JAK inhibitors with BH3 mimetics, and associated with antagonizing STAT3-dependent expression of the antiapoptotic proteins BCL-2, BCL-XL, Survivin and XIAP. Another report demonstrated synergistic suppression of bladder tumor cell growth in vitro and in vivo by combining AG490 with the natural sulfur compound methylsulfonylmethane, which may reduce the DNA-binding activity of STAT3 and STAT5B. It is, therefore, likely that combining JAK inhibitors with other compounds that either target commonly mutated signaling pathways in solid cancers (for example, EGFR, RAS, PI3K), or pathways that affect the tumor stroma (for example, angiogenesis, inflammation) may confer synergistic benefits.

Resistance to JAK inhibitors

Similar to observations with most other kinase inhibitors, long-term efficacy of JAK inhibitors is challenged through the emergence of acquired drug resistance. For instance, ATP-binding cassette drug efflux transporters efficiently reduce accumulation of the JAK inhibitor CYT387 in the brain, suggesting a role for these transporters in controlling drug distribution across the blood–brain barrier. As ATP-binding cassette transporters are expressed in a wide range of tissues such as the intestine and liver, they may also mediate resistance to JAK inhibitors in these organs. Meanwhile, re-activation of the JAK/STAT pathway through heterodimerization between activated JAK2 and JAK1 or TYK2, and most likely resulting in activation of JAK2 in trans by other JAK kinases, has been observed in preclinical models and patients treated with JAK2 inhibitors. However, this effect was reversible as the resistant cells regained their sensitivity to JAK2 inhibition following intermittent withdrawal of the compound.

Given the multiple pathways that can activate STAT3 (and other STATs) suggests potential situations where JAK inhibition may confer only limited benefit. For instance, resistance to JAK inhibition in chronic myeloid leukemia patients may be conferred by mutation or amplification of the BCR–ABL gene fusion product encoding a constitutive active version of the ABL tyrosine kinase, which can activate STAT3. Likewise, STAT3 activation may occur by membrane-associated SRC-family kinases, and transduce motility of hepatocellular carcinoma cells through SRC-mediated activation of RAC1 via STAT3. Indeed, epistasis between SRC and STAT3 may be phylogenetically conserved, as cellular overgrowth in the fruit fly is also dependent on STAT3 activity triggered by excess SRC expression.

To predict mutations within JAKs that would overcome the inhibitory activity of ATP-binding pocket inhibitors, Barber et al. used a random in vitro mutagenesis screen with a constitutively active TEL-JAK2 fusion protein as the driver. Intriguingly, all resistance conferring mutations found were in the kinase domain of JAK2 and resulted in sustained activation of STAT, ERK and AKT pathways in the presence of inhibitor. Whether resistance to JAK inhibitors could also be conferred by gene amplification of JAK kinases or (epi-)genetic inactivation of SOCS and other negative regulators remains unknown, although both mechanisms are observed in some solid cancers already prior to treatment.

OUTLOOK

Notwithstanding the likely nature of excessive JAK activity accounting for the bona fide tumor driver events in some hematological malignancies, we predict that the inhibition of JAK/STAT3 signaling may show its biggest clinical relevance as adjuvant therapy. Indeed, it is conceivable that anti-JAK therapy may, for instance, serve together with surgery in the management of multiple adenoma formation in familial adenomatous polyposis patients akin to the use of the angiogenesis inhibitor Bevacizumab and the EGFR inhibitor Erlotinib alongside chemotherapy to extend survival of stage IV CRC patients. However, as the c-MET/JAK/STAT3 signaling cascade also mediates resistance to MEK inhibitors in KRAS mutant CRC, administration of JAK1/2 inhibitors was also able to inhibit metastatic growth through blocking of this ADAM17-dependent escape mechanism. Indeed, JAK inhibitors may show a wider range of utility, owing to the observation that acquisition of resistance to MEK inhibitors in a wide variety of RAS/ERK kinase-addicted tumors involves excessive STAT3 activation via FGF and IL-6. In these situations, JAK inhibitors overcome this drug resistance and combined treatment of tumor cells with
MEK and JAK inhibitors blocked tumor cell proliferation more potently than either drug treatment on its own. Over the last decade seminal papers have illustrated the importance of the JAK/STAT pathway in promoting tumor cell intrinsic cancer hallmarks including survival and proliferation, alongside differentiation and cellular stemness. These observations were complemented by an appreciation that excessive JAK/STAT pathway activation within the tumor microenvironment also confers cancer-enabling characteristics, including angiogenesis and suppression of antitumor immunity. Although excessive activation of STAT3 and STAT5 in hematological malignancies often results from activating somatic mutations in JAKs, the oversupply of inflammatory cytokines within the tumor microenvironment underpins the predominant mechanism in solid tumors. Irrespective of whether these events account for the driver mutation or for the enabling characteristic that ensures growth and metastatic dissemination, many tumor cells become highly additive to continuous activation of the JAK/STAT pathway. This affords immediate therapeutic opportunity that can be readily tested and exploited with an increasing diversity of small molecule JAK inhibitors either already approved for some indications or in advanced clinical trials. However, the opportunities arising from targeting the JAKs as the kinases shared by many inflammatory cytokines also provides the challenge to maximize the therapeutic window and controlling dose-limiting on-target toxicities. Although the latter may be circumvented by antibody-mediated interference with individual cytokines and their receptors (for example, the anti-IL-6 receptor antibody Tocilizumab), the large number of tumor-promoting cytokines may limit efficacy of therapeutic targeting of a single one. Thus, targeting JAK1 as the converging intracellular signaling nodes becomes achievable with compounds that more selectively target individual JAKs and, in particular, better distinguish between JAK1 and JAK3 to minimize immunosuppression as the most prominent concern associated with long-term JAK inhibition.

CONFLICT OF INTEREST
Matthias Ernst and Michael Buchert have filed a patent application on the use of JAK inhibitors for the treatment of colon cancer, and Chris Burns is an inventor on several patents describing JAK inhibitors.

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