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Commentary

The many faces of Janus kinase

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ABSTRACT

Janus kinases have proved to be essential for many immunological processes but there is growing evidence that they also play a critical role in pathogenesis of many diseases including inflammatory diseases and cancer where they promote multiple steps of tumorigenesis. Several companies are in late stage clinical programs for the development of JAK kinase inhibitors and the first small molecule JAK inhibitor, Jakafi® (ruxolitinib) has been just approved for treatment of myeloproliferative neoplasms. Several other molecules are on the rise to treat arthritis, psoriasis and multiple types of cancer. This commentary will provide a review of the JAK kinase field as it pertains to small molecule inhibition for the treatment of cancer and autoimmune diseases with an emphasis on JAK2. The use of experimental and clinical inhibitors of JAK will be discussed for solid tumor and hematological malignancies, lupus, arthritis, colitis, neurological disorders, pain, diabetes and cardiovascular disease. In addition, it will review current paradigms in the field and treatment programs which could be complemented by small molecule inhibitors of Janus kinase.

1. Introduction

Janus kinases (JAKs) were discovered in early 1990s using PCR-based strategies or low stringency hybridization and were often referred as "just another kinase" reflecting their uncertain function at the time when many other kinases were cloned. The JAK family in mammals consists of 4 members: JAK1, JAK2, JAK3 and TYK2. They all share similar structure which is characterized by the presence of seven JAK homology (JH) domains. The C-terminal JH1 domain is the main catalytic domain. Adjacent to JH1, is the second kinase domain JH2, which had been considered catalytically inactive and therefore designated a pseudokinase domain. However, the recent report [1] demonstrated that JH2 is a dual-specificity protein kinase that phopshorylates two negative regulatory sites in JAK2: Ser^{523} and Tyr^{570}. Inactivation of JH2 catalytic activity increased JAK2 basal activity and downstream signaling. It is of note that most naturally occurring JAK2 activating mutations including the V617F in myeloproliferative neoplasms and R683G in acute lymphoblastic leukemias are localized to the JH2 domain and function by releasing the autoinhibitory interactions between the JH1 and JH2 domains [2]. The presence of two kinase domains is unique to the JAK family and was a rationale for naming them Janus kinases, after the two-faced (like two kinase domains) Roman god.

The JAK/STAT pathway is the major signaling cascade downstream from cytokine, chemokine and growth factor receptors including growth hormone, prolactin and leptin. This signaling pathway consists of the JAK (Janus kinase) family of non-receptor tyrosine kinases and the STAT (signal transduction and transcription) family of transcription factors. Stimulation of cells with a cytokine or a growth factor results in activation of JAKs, which phosphorylate and activate STATs, promoting their dimerization and nuclear translocation where they regulate transcription of STAT-dependent genes. In addition, STAT can be activated by other kinases including SRC, ABL and EGFR. Under normal physiological conditions the ligand-dependent activation of the JAK/STAT signaling is transient and tightly regulated. In addition to a STAT-dependent signaling, activated JAK2 can epigenetically regulate expression of STAT-independent genes by a direct phosphorylation of histone H3 [3]. Such a JAK2 direct nuclear signaling promotes expression of important oncogenes like MYC and has been implicated in tumorigenesis of the primary mediastinal B-cell lymphomas (PMBL) and other hematopoietic cancers [4].

There is considerable interest in the JAK inhibitor arena with several companies reporting JAK inhibitors in various stages of clinical development (Phases I–III). The companies, inhibitors, specific target (JAK1, JAK2, JAK3 or some combination) and the current status of the respective inhibitors are highlighted in Table 1. Pfizer's tofacitinib and Incyte/Novartis ruxolitinib (recently approved; Jakafi®) are the two most advanced JAK

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Table 1
JAK inhibitors currently under development.

Company	Chemical series and/or lead compounds	Target	Indication	Status of compounds/project
Pfizer	Tofacitinib CP690550	JAK3	RA	Phase III
Incyte/Novartis	Ruxolitinib INCB18424	Pan JAK	MPN*	Approved Nov. 16, 2011; Jakafi®
AstraZeneca	AZD1480	JAK1/JAK2	MPN Cancer	Phase II Phase I
YM Biosciences	CYT387	JAK1/JAK2	MPN	Phase II
Incyte/Lilly	LY3009104 (INCB28050)	JAK1/JAK2	RA	Phase II
Onyx	ONX0803 (SB1518)	JAK2	Advanced myeloid and lymphoid malignancies	Phase I/II
TargeGen	TG101348	Pan JAK	Myelofibrosis MPN	Phase II Phase I
S*Bio	SB1518 SB1578 SB1317	JAK1/JAK2	Myelofibrosis myeloid and lymphoid malignancies	Phase I Phase II
Ambit Biosciences	AC-430	JAK2	Cancer	Phase I
AEgera	AEG41174	JAK2/Bcr-Abl	Cancer	Phase I
Eli Lilly & Co	LY 2784544	JAK2	MPN	Phase I
BMS	BMS-911543	JAK2	Myelofibrosis	Phase I/II
Exelixis	XL-019	JAK2	Myelofribrosis	Phase I

inhibitors currently in phase III clinical development for both oncological and non-oncological (inflammatory, autoimmune) indications. Additional companies have reported preclinical activities in the JAK inhibitor area including Novartis, Portola Pharmaceuticals, Abbott, Vertex, Rigel, Kyowa Hakko, SuperGen and SGX.

2. Targeting JAK2 kinase for cancer therapy

Persistent activation of STAT3 or STAT5 has been demonstrated in a wide spectrum of solid human tumors including breast, pancreatic, prostate, ovarian and hepatic carcinomas, as well as in the majority of hematopoietic tumors including lymphomas and leukemias [5,6]. In many cases constitutive STAT activation was correlated with a more malignant, refractory and relapsing disease. The widespread constitutive activation of STAT3 and STAT5 in tumors and tumorigenic cell lines suggests that these factors are critical drivers of tumor formation and progression. Direct evidence linking STAT transcription factors to oncogenesis comes from work with a spontaneously dimerizing, constitutively active variant of STAT3, which was able to transform normal fibroblasts and convert them into tumor-forming cells. Thus, STAT3 possesses intrinsic oncogenic potential and its constitutive activation can initiate genetic programs sufficient for tumorigenesis. In this context, inactivation of JAK/STAT signaling in many hematopoietic tumors resulted in inhibition of cell proliferation and/or induction of apoptosis. Although STAT3 in tumor cells can be activated by various kinases, JAK2 has been shown to be the most important upstream activator mediating STAT3 activation in 15/16 human tumor cell lines derived from various solid tumors [7]. These observations provide a molecular rationale for targeting JAKs, particularly JAK2 for cancer therapy (Fig. 1).

2.1. Mechanism of constitutive STAT3/STAT5 activation in tumors: elevated cytokine levels

Activating mutations in either STAT3 or STAT5 have not been identified, and in most solid tumors constitutive activation of the JAK/STAT signaling is due to increased levels of activating cytokines and growth factors secreted in an autocrine or paracrine

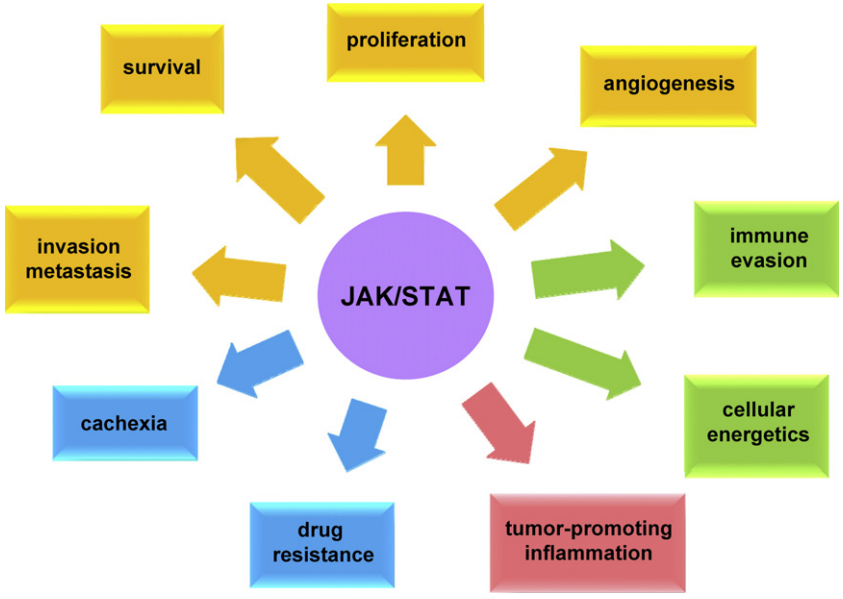

Fig. 1. Involvement of JAK/STAT signaling in multiple pathways of carcinogenesis and cancer metastasis. Model highlights and summarizes the various roles JAK/STAT signaling pathways play in the hallmarks of cancer including tumor cell survival, metastasis, drug resistance and most importantly the TME response prompted by tumor-driven inflammation, also inflammation that can lead to tumorigenesis. The model summarizes the various roles JAK/STAT signaling pathways play in tumorigenesis. The color code highlights an overlap between the JAK-mediated signaling and hallmarks of cancer as defined by Hanahan and Weinberg: yellow boxes represent the original hallmarks (Cell 2000; 100:57–70); new hallmarks (green) and tumor-enabling activities (pink) were added in 2011 (Cell 2011; 144:646–74). Blue boxes indicate additional clinical features of cancer which are promoted by the JAK/STAT signaling.

manner by tumor and stromal cells. In particular, high levels of IL-6 have been demonstrated in many solid tumors including breast, lung, gastric, and pancreatic carcinomas and in circulation and often correlate with more malignant phenotype and a poor prognosis [8–10]. JAK2 is the major mediator of the IL-6- and IL-11-dependent signaling. Likewise, high levels of IL-4 have been found in thyroid carcinomas. The JAK/STAT activating cytokines can be secreted by both tumor and stromal cells, in particular by tumor-promoting immune cells like macrophages and neutrophils [11]. Recent studies have strongly implicated STAT3-driven chronic inflammatory conditions in tumorigenesis of many cancers including hepatic, colon, breast and pancreatic carcinomas [9,11].

2.2. Mechanism of the constitutive STAT3/STAT5 activation in tumors: inactivation of endogenous suppressors of JAK/STAT signaling

In normal cells JAK/STAT signaling is transient and tightly regulated by multiple negative regulatory mechanisms, including activity of the SOCS (suppressors of cytokine signaling) family of endogenous JAK inhibitors. In addition, SOCS proteins facilitate proteosomal degradation of activated JAKs. Furthermore, activation of the JAK/STAT pathway is controlled by phosphatases which dephosphorylate and inactivate JAKs and STATs including SHP-1, and proteins belonging to the PIAS family that block binding of STATs to DNA. Analysis of expression of members of the SOCS family in tumors revealed a widespread epigenetic inactivation of SOCS1 and SOCS3 in multiple tumors, including hepatocellular carcinomas (65%), HNSCC (90%), pancreatic carcinomas (25%) and MM (62%). In primary mediastinal B-cell lymphoma (PMBL) and classical Hodgkin lymphoma (cHL), the SOCS1 locus was frequently (50% of patients) deleted resulting in an increased half-life of activated JAK2 [12]. Finally, SHP-1 expression is frequently inactivated by promoter methylation in lymphomas [13]: 95% of follicular lymphoma patients, 75% of mantle lymphoma and 100% of MALT lymphoma. In a separate study, 93% of all hematopoietic tumors exhibited promoter methylation of SOCS1 and/or SHP-1, resulting in an aberrant JAK2 activation [14]. Recently, a report also demonstrated that a slow, progressive methylation of SOCS1 led to activation of JAK2/STAT signaling in dormant tumors and mediated immune evasion and drug resistance [15]. Restoration of SOCS1 expression inhibited growth of hepatocellular carcinoma cells in vitro, confirming a critical role of SOCS inactivation in tumorigenesis [16]. The widespread inactivation of endogenous suppressors of the JAK/STAT pathway suggests a genetically driven selective pressure indicating that constitutive activation of the JAK/STAT signaling confers a significant growth advantage to multiple types of tumors.

2.3. JAK: activating mutations and locus amplifications

Aberrant activation of the JAK2 kinase has been implicated directly in several hematopoietic malignancies. Chromosomal translocations resulting in constitutively active TEL-JAK2, PCM-JAK2 and BCR-JAK2 chimeric proteins have been identified in several patients with various leukemias and lymphomas and shown to be oncogenic, thus providing the initial clinical proof-of-concept for targeting JAK2 in cancer. Discovery of the activating V617F JAK2 mutation in a majority of myeloproliferative neoplasias (MPN) and its role in disease development further confirmed the oncogenic potential of JAK2 [17,18]. Experimental evidence from mouse models and clinical samples indicated a causative role of V617F JAK2 in polycythaemia vera (PV) and progression to myelofibrosis [19]. Expression of the V617F JAK2 in BaF3 cells conferred supersensitivity to various cytokines which use JAK2 as a signaling partner, suggesting a more general mechanism of cellular response to overexpression or overactivation of JAK2. The discovery of the V617F mutation prompted a search for additional JAK2 mutations in various tumors, particularly of hematopoietic origin. To date, JAK2-activating mutations (I682F and R683G) have been identified in a fraction of BCP-ALL (B-cell precursor ALL) patients [20]. These JAK2 mutations cosegregate genetically (>85%) with translocation and increased expression of the CRLF2 receptor that uses JAK2 for signaling, suggesting that JAK2 plays a critical role in leukemogenesis of BCP-ALL [21]. Patients with the JAK2/CRLF2 signature have an abysmal clinical prognosis. Genetic amplification represents another way to increase activity of JAK2 in tumors. In cHL and PMBL, amplification of the 9p24 locus occurs in approximately 35–50% of patients resulting in JAK2 overexpression and constitutive JAK2/STAT signaling [22,23]. In this regard, JAK2 has been shown to be a critical driver of PMBL and cHL tumorigenesis [4].

2.4. JAK2-mediated STAT-independent epigenetic regulation of gene expression

Genome-wide expression profiling revealed that many genes regulated by the JAK2 activation do not have STAT binding sites in their regulatory regions, suggesting a STAT-independent signaling pathway. Recent reports demonstrated that activated JAK2 can translocate to the nucleus where it directly phosphorylates histone H3 tail on tyrosine 41, blocking recruitment of the heterochromatin protein HP1 [3]. Since HP1 protein mediates formation of the repressive chromatin, JAK2-mediated H3 phosphorylation alters chromatin structure promoting expression of multiple genes in a STAT-independent manner. The nuclear activity of JAK2 could be suppressed by JAK2 inhibitors similarly to inhibition of the JAK/STAT pathway [3]. The role of nuclear JAK2 signaling in oncogenesis has been recently confirmed in cHL and PMBL, both of which are characterized by a frequent amplification of the 9p24 locus. An RNA interference study of amplified genes identified JAK2 and the histone demethylase JMJD2C as drivers of tumorigenesis [4]. Both activities cooperated in promoting heterochromatin formation and expression of specific genes including oncogenes like cMYC. JAK2 inhibitors could reverse tumor promoting activities of JAK2, including inhibition of cMYC expression. Hence, in cHL and PMBL, JAK2 drives tumor formation at least in part by epigenetic modification of chromatin structure. Global epigenetic changes have been associated with multiple malignancies and represent emerging targets in cancer research.

2.5. JAK2/STAT-mediated tumor immune evasion

The immune system functions as an extrinsic tumor suppressor and there is growing evidence that the quality and quantity of immune infiltrates in tumor strongly contribute to the clinical course [24] and responsiveness to therapies. Constitutive activation of JAK/STAT signaling in tumor and stromal cells mediates suppression of tumor immunosurveillance and dendritic cell activation [25,26]. STAT3/5-dependent cytokines secreted by tumor and stromal cells (e.g., TGFβ, IL-10, IL-23) also promote proliferation and recruitment of T regulatory (Treg) cells and myeloid-derived suppressor cells (MDSC), the two major cell types that suppress anti-tumor immunity [6]. STAT3 is persistently activated in various tumor-infiltrating immune cells where it drives expression of tumor-promoting cytokines such as IL-6, IL-23 and VEGF and inhibits expression of tumor suppressing factors such as IL-12 [6,27]. In addition, the JAK2/STAT3 signaling pathway plays a critical role in transmitting signals initiated by these cytokines. Inactivation of STAT3 in a hematopoietic cells triggered intrinsic anti-tumor immunity including activation of dendritic cells, T cells and natural killer (NK) cells in tumor bearing animals leading to suppression of tumor growth and metastasis in multiple syngeneic tumor models [28]. Thus, inhibition of the JAK2/STAT3

signaling can redirect immune responses toward anti-tumor immunity. Adoptive T cell therapy aims to improve natural anti-tumor immune responses and shows promise for treating tumors; however the immunosuppressive environment of tumor stroma limits this therapeutic approach. Recent studies [29,30] demonstrated that STAT3 inactivation in either T or in myeloid cells drastically augmented effector functions of adoptively transferred $CD8^+$ T cells resulting in increased anti-tumor efficacy.

2.6. JAK2 inhibitors: targeting tumor stroma and tumor associated inflammation

Tumor stroma has been now recognized as a critical contributor to cancer pathogenesis and a major driver of tumor progression. Although in most cases the initial oncogenic event leads to an oncogenic transformation of normal cells, the subsequent growth and progression of tumors are critically dependent on interactions with tumor stroma, which provides growth and angiogenic factors and generates an immunosuppressive environment. The reciprocal heterotypic interactions between neoplastic and stromal cells facilitate progressive changes in both compartments which will eventually transform normal tissues into high-grade malignancies. There is a growing recognition that interference with these tumor-promoting interactions might represent an attractive therapeutic approach. Although recent reports demonstrated a potential role for tumor-associated fibroblasts in tumorigenesis [31], most of the research has focused on functions of various immune cells [9]. The most frequently found immune cells are tumor-associated macrophages (TAM) and T cells. TAMs primarily promote tumor growth and may be obligatory for angiogenesis, invasion and metastasis [32]; moreover, a high TAM content correlates with poor prognosis [33]. TAMs and other immune cells are recruited by tumor cell-derived signals and, in turn become critical sources of tumor promoting cytokines such as IL-1, IL-6, IL-23, TNF, VEGF and proinvasive factors, including specific metalloproteinases (MMP-9) and cathepsins. Infiltrating T cells, in particular, Th2 $CD4^+$ stimulates tumor progression either directly or by educating TAMs [34]. The high ratio of $CD4/CD8^+$ T cells in tumors is associated with a poor prognosis [35]. The critical role of inflammatory cells in tumorigenesis is further underscored by strong association of some tumors such as colon or pancreatic carcinomas with chronic inflammation. Furthermore, recent reports indicate that many known oncogenes such as RAS or SRC facilitate tumorigenesis by activating NF-κB and JAK/STAT pathways and recruiting immune cells early during tumor development [9,11,36]. Most of the tumor-promoting functions of inflammatory cells and tumor–stromal interactions are mediated by the JAK2/STAT pathway, which can upregulate expression of many cytokines and/or serve as a downstream signaling effectors (e.g. IL-6, IL-11), thus placing JAK2 as an important target for anti-tumor therapies. Indeed, recent reports using KRAS-driven mouse models of PDAC (pancreatic ductal adenocarcinoma) confirmed a critical role of STAT3 and JAK/STAT signaling in tumor initiation and progression. STAT3 inactivation suppressed PDAC formation through multiple mechanisms affecting tumor cells (inhibition of proliferation and apoptosis) and stroma [11]. The latter also included inhibition of macrophage recruitment, decrease in total inflammatory infiltrates, and the expression of proinflammatory cytokines including IL-6 and LIF. Similar results were obtained in colon cancer models [37]. The role of JAK2 in shaping a tumor-promoting stromal compartment has been recently confirmed pharmacologically. AZD1480, a JAK2 inhibitor, suppressed growth of multiple solid tumor xenografts but had no effects on growth of any of the xenograft cell lines at doses that fully inhibited STAT3 phosphorylation [7]. Immunocytochemistry revealed that activated STAT3 was present in tumor and stromal cells and that AZD1480 inhibited both signals. These results, in accordance with our own observations (Dobrzanski, unpublished data), suggested that inhibition of tumor growth was achieved through effects of JAK2 inhibition on the tumor microenvironment. These studies further underscore the notion that a JAK/STAT signaling role in cancer extends beyond its well-documented effects on cell proliferation and survival of tumor cells. Its critical involvement in shaping tumor microenvironment and orchestrating tumor-promoting inflammatory responses suggests that JAK2 inhibitors should have significant therapeutic effects in a variety of cancers.

2.7. JAK/STAT signaling and modulation in the treatment of inflammatory bowel disease (IBD) and colitis-driven colorectal cancer (CAC)

As discussed above, there is a growing recognition that inflammation plays a critical role in tumor initiation and progression [9,38] and many tumors including pancreatic, hepatic and colon carcinomas have a strong inflammatory component Elevated levels of inflammatory cytokines like IL-6, IL-11, and GM-CSF have been demonstrated in a wide spectrum of human and experimental tumors, in plasma of cancer patients and often correlated with a more malignant and refractory disease [39]. Most inflammatory signals promote tumorigenesis by activating NF-κB and JAK/STAT signaling pathways, both in tumor and stroma cells. Among the common cancers with a strong inflammatory input are gastrointestinal tumors including colorectal carcinomas (CRC), for which the critical role of JAK/STAT signaling in tumorigenesis has been well established and is reviewed below.

Colorectal cancer is one of the most fatal malignancies worldwide and develops spontaneously or as a long-term complication of chronic bowel inflammation such as in Crohn's disease (CD) or ulcerative colitis (UC) [40]. Patients with CRC have often elevated levels of IL-6 in the serum [41], and constitutively activated STAT3, which is expressed in the majority of colorectal tumors [42,43] and is associated with significantly higher mortality. Constitutive STAT3 activation in CRC-derived cell lines can promote proliferation and invasiveness of tumor cells in culture [44] and growth of tumor xenografts [42] in an IL-6-dependent manner [45].

The critical role of inflammation and JAK/STAT signaling in CRC was confirmed and further elaborated in an azoxymethane/dextran sodium sulfate (AOM/DSS) induced colitis-associated cancer model [8,46]. These types of models demonstrate that immune cells infiltrating preneoplastic lesions and tumors secrete multiple cytokines which promote a localized inflammatory response and mediate proliferation and survival of premalignant intestinal epithelial cells that can result in tumorigenic transformation [47]. The cytoprotective and protumorigenic activity of IL-6 in models of CAC was shown to be mediated by STAT3 as its genetic ablation in intestinal epithelial cells (IEC) inhibited tumor induction and growth [8,37]. Importantly, the phenotype of mice deficient in STAT3 and IL-6 knockout mice were remarkably similar in the AOM/DSS model indicating a critical role of the IL-6/JAK2/STAT3 signaling pathway in tumorigenesis of CAC. Conversely, exogenous administration of IL-6 or genetic hyperactivation of STAT3 resulted in accelerated tumor growth and increased tumor burden [8] further establishing the significance of IL-6 and STAT3 activation in CAC. These data confirmed previous reports which demonstrated that IEC-specific inactivation of SOCS3, an endogenous inhibitor of JAK2/STAT3 signaling, led to increased STAT3 activation and higher tumor burden of CAC [48]. Similarly, gp130 mutations that prevent binding of SOCS3 resulted in hyperactivation of STAT3 and growth of spontaneous gastric tumors [49,50]. These results provide a strong rationale for testing JAK inhibitors in CAC models. Indeed, we have demonstrated that CEP-33779, a

highly selective JAK2 inhibitor [51,52] exhibited a marked anti-tumor efficacy in the AOM/DSS model [46]. Reduction of tumor burden was associated with suppression of STAT3 activation and decreased IL-6 tumor levels. Furthermore, CEP-33779 significantly decreased levels of activated NF-κB in tumors. Inhibitory effects of JAK/STAT suppression on recruitment and activation of immune cells which express high levels of activated NF-κB like myeloid cells and T-cells [47] would provide a cell non-autonomous mechanism of JAK/STAT inhibitor-mediated repression of NF-κB activity. The reversible acetylation of RelA/p65 regulates the duration of its nuclear activation [53] and STAT3 has been shown to directly interact with RelA in the nucleus and to recruit p300 acetylase which in turn acetylates RelA extending its transcriptional activity in an IKK-independent manner [54]. Indeed, STAT3-driven cell-autonomous nuclear retention of NF-κB represents an important mechanism of NF-κB activation in cancer [55]. Inhibition of STAT3 activation by a JAK2 inhibitor would reduce the nuclear pool of STAT3 resulting in a suppressed NF-κB activation. Among genes regulated by STAT3 and NF-κB, either synergistically or individually, are many coding for anti-apoptotic proteins like Bcl-2, Bcl-xL, Mcl-1, survivin and mediators of cell proliferation like cyclin-D and cyclin-B. Indeed, inactivation of STAT3 in epithelial cells suppressed tumor formation in a mouse model of CAC, in part, by attenuating expression of Bcl-xL and survivin [8,37]. Downregulation of NF-κB activity by a JAK2 inhibitor might be a significant contributor to its anti-tumor efficacy. Thus, preponderance of clinical and preclinical evidence demonstrates that the IL-6/JAK2/STAT3 signaling plays a critical role in development and progression of CAC and provides a rationale for pharmacological targeting of this pathway in inflammatory tumors.

2.8. JAK inhibitors as adjuvant therapies

Although JAK2 inhibitors would likely be effective as a monotherapy, particularly for multiple hematopoietic cancers, they seem to be well suited for combinatorial therapies. Among genes directly regulated by the JAK2/STAT signaling are anti-apoptotic members of the Bcl-2 family, including Bcl-2 and Bcl-xL. In addition, other antiapoptotic genes such as Mcl-1, IAPs and survivin are also targets of JAK2 signaling. JAK2 inhibitors have been shown to decrease expression of these genes resulting in induction of apoptosis. High levels of expression of antiapoptotic proteins increase the apoptotic threshold and contribute to drug resistance of cancer cells to standard therapies. Thus JAK2 inhibitors might increase the effective potency of standard chemotherapeutics targeting tumor cells. It has been reported that treatment with cytotoxic drugs such as doxorubicin or paclitaxel and radiation therapy induced IL-6 expression in tumors, and signaling through JAK/STAT pathways [56] potentially attenuating efficacy of the therapy. Based on these observations, a rational combinatorial therapy including JAK2 inhibitors as an adjuvant can be designed. Some of our preliminary studies confirmed increased cytotoxicity of a JAK2 small molecule inhibitor, CEP-33779 [52,57], and doxorubicin in 2 human tumor cell lines (Dobrzanski et al., 2011, unpublished data). The increased efficacy correlated with suppression of the doxorubicin-mediated IL-6 induction. In addition to inducing adaptive responses in tumors (e.g. IL-6 expression), most cytotoxic drugs induce necrosis of tumor and stromal cells. In contrast to apoptosis, necrotic cell death is associated with the release of cellular contents into the microenvironment and generation of proinflammatory signals. As a consequence, necrotic cells can recruit tumor-promoting inflammatory cells that facilitate angiogenesis, tumor cell proliferation, invasiveness and metastasis. Adjuvant JAK2 therapy may attenuate such an inflammatory response and increase overall efficacy of other drugs. Finally, many current targeted drugs, for example kinase inhibitors, are designed to target tumor cells with a specific oncogenic mutation; such therapies almost invariably lead to a generation of resistant tumor clones [58,59]. Because JAK2 inhibitors target, at least in part, stromal cells and their interactions with tumor cells, they may represent attractive partners for combination with specific targeted therapeutics like kinase inhibitors.

2.9. JAK2 signaling in cancer metabolism

Proliferating cancer cells display vastly different metabolic requirements than normal cells and their metabolic pathways must accommodate biosynthetic needs of rapidly proliferating cells and sufficient energy production to support tumor cell growth and survival [60,61]. Balancing such divergent processes requires major alterations of metabolic pathways and there is growing evidence that such a metabolic rewiring represents a common step in oncogenic transformation. Many major oncogenic signals including Myc [62], Kras [63] and Rho GTPases [64] must reprogram cell metabolism for a productive tumorigenesis. Recent report [65] demonstrated that a constitutively active mutant of JAK2 (V617F), which is present in majority of patients with myeloproliferative neoplasm (MPNs), promotes expression of multiple metabolic enzymes including glucose transporter (GLUT1) and an inducible bifunctional 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PKKFB3). The latter is a rate-liming enzyme which controls glycolytic flux through 6-phosphofructo-1-kinase. Inactivation of PFKFB3 in V617F JAK2-dependent cells resulted in reduced cell proliferation and tumor growth in vivo indicating a critical role of JAK2-mediated metabolic changes in oncogenic transformation. Importantly, PFKFB3 expression in granulocytes from MPN patients was 9-fold higher than in granulocytes from healthy donors confirming results from preclinical studies. Our own data demonstrated that JAK2/STAT3 signaling pathway upregulates expression of glutamine transporter ASCT-2 in tumor xenografts and anti-tumor efficacy of a highly selective JAK2 inhibitor was associated with suppression of ASCT-2 expression in vivo (Dobrzanski, unpublished data). These data indicate that similarly to other oncogenic pathways, constitutive JAK2 signaling can reprogram metabolic pathways to facilitate efficient tumorigenesis. Thus, such a metabolic switch might be a part of JAK2-mediated oncogenic signaling.

2.10. Summary of the therapeutic rationale for targeting JAK2 in cancer

Widespread constitutive activation of the JAK/STAT pathway in tumor and stromal cells suggest its critical role in tumor growth and progression. Frequent inactivation of endogenous repressors of the JAK/STAT pathway and high levels of cytokine signaling through the JAK/STAT pathway typically present in tumors provide a molecular explanation for the constitutive activity of JAK2 and a rationale for developing JAK2 inhibitors. The critical contribution of JAK2 signaling to various mechanisms mediating tumor growth and metastatic dissemination suggest that inhibition of JAK2 would provide multiple clinical benefits including: direct inhibition of proliferation and induction of apoptosis in tumor cells (cHL, PMBL, BCP-ALL), inhibition of stromal cell proliferation, inhibition of tumor-promoting interaction between tumor cells and stroma, inhibition of tumor-associated angiogenesis, reactivation of anti-tumor immune responses (suppression of immune evasion), suppression of tumor-promoting inflammation, suppression of chemotherapy induced adaptive (resistance) responses (adjuvant therapy), and decreased apoptotic threshold in tumor cells (adjuvant therapy). The simultaneous co-targeting of multiple

hallmarks of cancer (see Fig. 1 and [38]) by JAK2 inhibitors would likely result in more effective and durable therapeutic responses while decreasing a possibility of tumors developing acquired drug resistance.

3. JAK/STAT signaling and modulation in the treatment of rheumatoid arthritis (RA)

Epidemiological studies indicate that the prevalence of rheumatoid arthritis in developed countries is between 0.5% and 1% of the adult population and the incidence is between 20 and 50 cases per 100,000 population. The disease is three times more common in women than men, and women tend to develop a more aggressive form of the disease. Treatments for RA have included mainly disease-modifying antirheumatic drugs (DMARDs) and have been more prolific than for the more difficult to treat rheumatic diseases (e.g. lupus). Approved treatments for RA include NSAIDs, anti-metabolites such as methotrexate and leflunomide, various corticosteroids and glucocorticoids, sulfasalazine, and various biologics including abatacept (Orencia®), adalimumab (Humira®), etanercept (Enbrel®), infliximab (Remicade®), golimumab (Simponi®), and rituximab (Rituxan®). Other molecules implicated in the pathogenesis of RA include BLyS/BAFF, APRIL, p38/MAPK and the BCR protein tyrosine kinase, Syk kinase. The recent FDA approval of tocilizumab (anti-IL-6R) (Actemra®) further demonstrates the power of targeting cytokines and associated receptors to treat chronic inflammatory diseases [66].

It is well documented that JAK kinases play a pivotal role in cytokine receptor signaling to phosphorylate and activate signal transducer and activator of transcription (STAT) proteins. Several of these JAK-controlled cytokine receptor pathways are intimately involved in the initiation and progression of RA disease pathogenesis. Cytokines involved in these diseases (e.g., IL-2, IL-6, IL-12, IFNγ, and GM-CSF) are also essential to a proper functioning immune response to infectious agents. JAK1, JAK2, and TYK2 kinases are ubiquitously expressed, whereas JAK3 is limited to the lymphoid lineage as JAK3 associates with the common gamma-chain. Therapeutic benefit from JAK kinase inhibition has already been established in RA with the use of CP-690,550, a pan-JAK inhibitor originally intended for organ transplantation immunosuppression as it is a potent inhibitor of JAK3, but has also shown to have activity against JAK1 and JAK2 [67]. More recently, the selective JAK1/JAK2 inhibitor, INCB028050, has demonstrated efficacy in various rodent models of RA, further demonstrating the central role JAK kinases plays in this disease [68]. The mechanistic rationale of targeting cytokine pathways central to the pathogenesis of RA, but without the immunosuppressive side-effects of several of the TNF-alpha blockade biologics, has been demonstrated in the successful use of tocilizumab (Actemra®) for RA [69]. We have demonstrated the efficacy of a novel, orally active, selective JAK2 inhibitor, CEP-33779, in two mouse models of RA—the collagen antibody induced arthritis (CAIA) and spontaneous, more natural, collagen induced arthritis model (CIA) [57]. CEP-33779 reduced levels of local and circulating proinflammatory cytokines like TNFα, IL-1β, IL-6, IFNγ, IL-12 and GM-CSF, in addition, reduced several histopathological manifestations, anti-collagen type II specific Th1 cells and clinical disease manifestations such as paw swelling and clinical paw scores [57]. These results demonstrate that JAK2 plays an important role in RA and provide rationale for using a highly selective JAK2 inhibitor for RA therapy.

4. JAK/STAT signaling and modulation in the treatment of systemic lupus erythematosus (SLE)

The prevalence of lupus varies widely from one country to another, with the highest rates reported in Italy, Spain, Martinique and among Afro-Caribbean subpopulations of the United Kingdom [70]. According to the Centers for Disease Control and Prevention (CDC), the disease affects approximately 1.4 million individuals in the US alone. Approximately 90% of all cases of lupus occur in women, although the disease may be more severe when it occurs in males. The highest incidence is among women of childbearing age (between 15 and 45 years), although 10–20% of patients have pediatric onset of disease (mean age 11–12 years) [71]. Both adult and pediatric-onset SLE are more common in African Americans, Asian Americans and Hispanics than in non-Hispanic whites [71]. In general, both prevalence and severity of SLE are two to four times greater among nonwhite populations around the world [72].

Symptoms of systemic lupus erythematosus vary widely from one patient to another. They can range from mild to severe and may come and go over time, with disease flares alternating with periods of remission [73]. Common symptoms of lupus include painful or swollen joints and muscle pain; unexplained fever; red rashes, most commonly on the face; chest pain upon deep breathing; unusual loss of hair; Raynaud's phenomenon; photosensitivity; edema in legs or around the eyes; swollen glands; extreme fatigue; accelerated atherosclerosis and anemia. Some patients express neurological symptoms such as cognitive dysfunction, headache, seizures, fatigue and vertigo. Mood and anxiety disorders, including major depressive disorder, are found at a higher frequency among women with SLE as compared to the general population. Inflammation of the kidneys (nephritis), one of the most serious symptoms of lupus, can lead to kidney failure. In addition to the symptoms of the disease itself, the side effects of many drugs used to treat lupus range from poorly tolerated to organ- or life-threatening. In general, patients with severe involvement of the lungs, heart, kidney and brain have the worst prognosis. Infection is the most common cause of morbidity and mortality among SLE patients, and may contribute to disease exacerbations.

Autoantibody production, immune complex/complement deposition and leukocytic infiltration of target organs are key immunopathogenic events in systemic lupus erythematosus. SLE is best characterized by the presence of activated T and B cells in conjunction with the development of many different autoantibodies and chronic inflammation that can affect various parts of the body including the joints, skin, kidneys, CNS, cardiac tissue and blood vessels. Although the cause of SLE remains unknown, manifestations of the disease have largely been linked to genetic polymorphisms, environmental toxins and pathogens [74]. In addition, gender, hormonal influences and cytokine dysregulation have also been tightly linked to the development of SLE in humans [75,76].

Multiple cytokines signaling through the JAK/STAT pathway have been implicated in playing key roles during the initiation, progression and development of SLE including (Fig. 2), but not limited to, interleukin IL-6 [75,77], IL-12 [78] and type I IFN (alpha/beta) [79]. All three cytokines signal via receptors controlled by JAK kinases. Most important is the role of IL-6 as this cytokine has been implicated in multiple autoimmune diseases and directly contributes to plasma cell survival in bone marrow niches [80].

Several novel therapies are in clinical development that target key cytokine pathways or cell types thought to be involved in SLE progression [81]. Current treatments for SLE are limited to toxic and immunosuppressive agents with severe side-effects such as high dose glucocorticoids and/or hydroxychloroquine. Severe SLE disease or patients that have signs of renal involvement require more aggressive drugs including mycophenolate mofetil (MMF), azathioprine (AZA) and/or cyclophosphamide (CTX). Immunosuppressive drugs such as CTX, AZA and MMF are very toxic and only 50% of treated patients enter complete remission, with relapse rates up to 30% over a 2-year period. B-cells are thought to be one of the key cells types involved in the pathogenesis of SLE. However, depletion of these cell types in three separate, placebo-controlled,

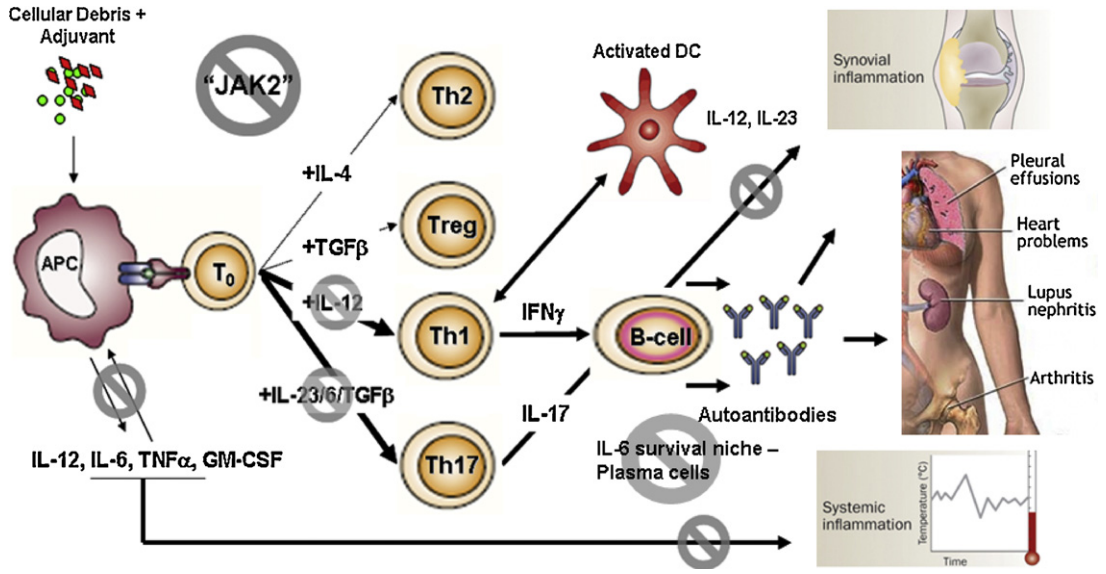

Fig. 2. Therapeutic rationale for targeting JAK2 for the treatment of systemic lupus erythematosus. Model shows the various immunopathological components of a SLE providing a few examples of common disease manifestations to the far right. Starting from self antigen accompanied by external PAMPs (e.g., LPS, CpG) or endogenous DAMPs (e.g., HMGB1, dsDNA, ATP) in combination with polymorphisms or mutations in key peripheral tolerance mechanisms (e.g., CTLA-4, PD-1), antigen presenting machinery (e.g., HLA-DR3/4), PAMP-sensing molecules or activators (e.g., TREX-1, LINE-1, LL37, IRAK-1/4, TLRs and NODs) or dead-cell clearance mechanisms (e.g., C1q, FcγRI/III) can lead to a break in tolerance to self-tissues licensing autoreactive T cells to license autoreactive B cells to make autoantibodies and cytokines. Cytokines the contribute to SLE include IL-12, IL-6, IFNα/β and TNFα, many of which are controlled by JAK kinases, more specifically JAK2 kinase, making this target important in the fight against SLE progression.

Phase III trials using anti-human CD20 monoclonal antibodies, rituximab and ocrelizumab did not result in significant disease treatment and safety was compromised as opportunistic infections increased with treatment over time [82].

The rationale for targeting the survival of LL-PCs in the treatment of SLE using proteasome inhibitors, anti-BAFF/BLyS monoclonal antibodies and anti-IL-6 or IL-6R antibodies has emerged as alternative therapy for the treatment of SLE [83]. As described above, JAK2 play a critical role in transducing signals from the IL-6R, and IL-6 is involved in both SLE and the maintenance of the pool of potentially autoreactive PCs. Therefore, inhibition of JAK2 signaling using a selective and potent JAK2 inhibitor should weaken the supportive effects of IL-6 on sustaining autoreactive plasma cells in SLE. We have evaluated a selective oral inhibitor of JAK2, CEP-33779, in two mouse models of SLE—the Fas-deficient mutant MRL/lpr mouse model and the spontaneous (NZB × NZW) F1 hybrid mouse model, to ablate autoantibody producing long lived plasma cells and treat active and fatal lupus nephritis in diseased animals [51]. CEP-33779 reduced circulating lupus-associated cytokines and chemokines including CXCL10/IP-10 and CXCL9/MIG, IL-17A, IL-12 and IL-10 [51]. In addition JAK2 inhibition lead to a decrease in renal kidney disease, mortality, lymphomegaly, splenomegaly, lupus-supporting, autoantibody producing short and long lived plasma cells in both the spleen and bone marrow and a rapid decline in proteinuria and anti-chromatin, smith antigen and dsDNA antinuclear antibodies (ANAs) [51]. Most important, JAK2 inhibition resulted in near equivalent disease efficacy as compared to our orally active proteasome inhibitor, Delanzomib/CEP-18770 (Seavey et al., unpublished data), and the more toxic proteasome inhibitor, bortezomib/Velcade [84].

5. JAK/STAT signaling and modulation in other autoantibody-driven autoimmune diseases—Graves's disease and Sjogren's syndrome

There is some evidence that the JAK/STAT pathway plays a role in mediating some of the disease manifestations of Graves's disease [85]. Graves's disease is an antibody driven autoimmune disorder that leads to the over activity of the thyroid gland (hyperthyroidism). The primary mechanism of action is thought to be anti-self antibodies directed against the Thyroid stimulating hormone (TSH) receptor driving the over expression of several thyroid hormones responsible for many metabolic pathways. A frequent autoimmune component of Graves' disease includes the development of thyroid-associated ophthalmopathy (TAO). Orbital fibroblasts when activated by IL-1β and other proinflammatory cytokines produce excess prostaglandin E2 (PGE2) and hyaluronan. Cytokines IFNγ and IL-4 attenuate IL-1β mediated PGE2 and hyaluronan production. Blockade of JAK2 using the pan-JAK inhibitor AG490 could block IFNγ and IL-4 signals (both receptors are controlled by JAK kinases) potentially enhancing Graves' disease pathogenesis however, orbital fibroblasts were found to also express CD40 which is upregulated by IFNγ in TAO. Hence blockade of JAK kinase signaling and thus actions of IFNγ on orbital fibroblasts in TAO could ameliorate disease in combination with dexamethasone [85,86]. There is also some evidence that the JAK/STAT pathway could play a role in the pathogenesis of Sjogren's syndrome (SS). Sjogren's syndrome is a systemic autoimmune disease very similar to SLE but more localized to the exocrine glands that produce tears and saliva (salivary glands). Primary SS is associated with the over expression of cytokines such as IL-10 which signals via JAK1 and TYK2 and is usually involved in immunosuppression or Th2-helper cell functions such as B cell help. One study found that STAT3 was constitutively activated in $CD3^+$ lymphocytes from a primary SS patient population but that neither JAK1 nor TYK2 were activated suggesting that constitutive activation of STAT3 may be via an inherent defect in STAT3 protein or in one of the regulatory proteins such a PIAS or PTP [87]. The V617F JAK2 mutation has also been postulated to have some role in SS due to B cell clonality described in the bone marrow of patients with RA and from glandular biopsies from patients with SS, however this has yet to be further investigated [88,89].

6. JAK/STAT signaling and modulation in neuroinflammatory diseases

Evidence for the role for Janus kinase in neuroinflammation has been murky to say the least. The involvement of inflammation in central nervous system (CNS) related disorders such as multiple

sclerosis (MS), Alzheimer's disease (AD) and Parkinson's disease has been brewing in the field for more than 30 years. What is known is that the JAK/STAT pathway is activated in the brain during times of stress and injury and that the cytokine IL-6 (which also signals via the JAK2-related co-receptor complex gp130) may play both an inflammatory as well as neuroregenerative role [90,91]. Neuropathic pain after peripheral nerve injury, which is usually associated with local neuroinflammation in the spinal cord, is a severe incapacitating condition with which clinical treatment remains challenging. Peripheral nerve lesion leads to rapid activation of the JAK/STAT pathway in dorsal spinal cord microglial with up regulation of IL-6 [90]. Blockade of such JAK/STAT signaling via the expression of SOCS3 or inhibition using pan-JAK inhibitor, like AG490, can prevent microglial cells expression of IL-6 and CCL2 further dampening resulting peripheral inflammation [90]. Links between signaling of gonadotropin-releasing hormone (GnRH) neurons and female reproduction has been shown to be JAK2 dependent as JAK2-deficient GnRH-expressing neurons in mice leads to a delay in puberty, first estrus, abnormal estrous cycle and impaired fertility all with estrogen-negative feedback remaining intact [92]. Sensory axons in the adult spinal cord do not regenerate after injury, but if the sciatic nerve is axotomized before injury of the dorsal column, injured axons regenerate and time-dependent phosphorylation of STAT3 in the dorsal root ganglion (DRG) neurons are observed [93]. Inhibition of STAT3 activation via JAK2 inhibition using AG490 on the proximal nerve stump can reduce neurite outgrowth [93]. Hence the role for JAK in neuroinflammation and regeneration may be double pronged as JAK/STAT signaling pathways play both a role in promoting nerve damaging inflammation as well as nerve end regeneration after resulting inflammation has receded.

Point mutation in TYK2 from a GC to GG genotype, typically referred to as variant rs34536443, has been strongly associated with the development of MS in the human population and has repeatedly been shown to be a major risk factor in the development of this disease [94–96]. The use of IFNβ in the treatment of MS is to primarily induce IL-10 from innate cell types as an anti-inflammatory cytokine. JAK1 activity is required for IFNβ to activate PI3K and Akt which results in the repression of GSK3β activity in a mouse model of MS (i.e., experimental autoimmune-induced encephamyelitis (EAE)) [97]. In addition, many of the MS-promoting cytokines such as IL-1β, TNFα and especially that of IL-6 and IL-12 either signal through or induce JAK/STAT signaling molecules [98]. Blockade of JAK2 and TYK2 using AG490 was shown to treat EAE in diseased mice and was strongly associated with a decrease in Th1, NK and microglial activity with a decrease in IL-12 cytokine levels [98].

Janus kinase has also been shown to play a role in neuropathic pain via JAK2-STAT3 in RA and pain hypersensitivity evoked by innocuous stimuli [99,100]. Blockade of JAK using AG490 could attenuate both mechanical allodynia and thermal hyperalgesia in rats that have had ligation of the L5-L6 spinal nerves which was consistent with an anti-IL-6 antibody intrathecal injection [101]. Tyrosine kinase TYK2-STAT3 signaling has been implicated in beta-amyloid-induced neuronal cell death in a mouse model of Alzheimer's disease and activity of JAK1 has been implicated in Down syndrome [102,103]. For Parkinson's disease, inhibitors of JAK2 can suppress the neuroprotective effect of standard of care drugs donepezil and galantamine, both acetylcholinesterase (AChE) inhibitors [104].

7. JAK/STAT signaling and modulation in metabolic and cardiovascular diseases

There is a clear role for JAK/STAT in cardiovascular disease (CVD) however to what extent and under what conditions these pathways should be modulated is still under debate. The major cytokine players in early CVD are IL-6 and the small peptide hormone Leptin, both signal through JAK2 controlled receptors. Leptin is an adipose tissue-derived hormone implicated in atherosclerosis, obesity and metabolism [105]. This 16-kDa hormone acts as a key factor for the maintenance of energy homeostasis in central and peripheral tissues. In most obese individuals, serum leptin levels are increased and correlate with an individual's body mass index [105]. Blockade of JAK signaling using tool inhibitor AG490 has shown in several studies to reduce incidence and severity of atherosclerosis in susceptible mouse populations (e.g. ApoE−/− mice) and block the activity of oxidized lipid products reducing oxidative stress burden [106,107]. Blockade of JAK2 signaling using AG490 has also been shown to reduce resulting mouse liver damage (fibrosis) in a model of ischemia and reperfusion [108]. Even the MPN JAK2 mutation V617F has been associated with spleen and cerebral vein thrombosis in essential thrombocythemia and accompanying myeloproliferative syndromes [109,110]. The JAK/STAT pathway also plays a role not only in the development of autoimmune type-1 diabetes (T1D) but also the more metabolically driven type-2 diabetes which is also now evolving as an inflammatory type disease [111]. Erythropoietin (EPO) protects against diabetes through direct effects on pancreatic beta cells and JAK2 is an essential intracellular mediator of the EPO receptor and SOCS-1/3 have shown to influence the growth and apoptosis of these cell types [112].

8. Conclusions

Targeting Janus kinases as a therapeutic modality for the treatment of multiple indications is quickly approaching with the recent approval of Novartis/Incyte's ruxolitinib and soon to arrive, Pfizer's tofacitinib and Lilly/Incyte's JAK1/JAK2 dual inhibitor, INCB28050. Multiple companies are in mid- to late-stage clinical trials for the small molecule targeting of JAKs for cancer and inflammation. It is possible that the link between inflammation, pain, cancer, metabolic disorders and CNS complications would allow for the dissemination of off label use of these recently approved JAK inhibitors. Due to the central role JAK-controlled cytokines play in disease remodeling, severity and duration, tumor–stromal interactions and basic immunity, JAK inhibitors may find themselves helping the fight against Malaria (i.e., the cytokines storm associated with malarial infection), combinations with cancer vaccines or immunotherapy (to reduce Tregs and MDSCs in the TME) or topical skin applications for use in eczema or psoriasis. The future holds many opportunities for JAK inhibitors, something which has eluded scientists for a long time but has now only begun to emerge from the ashes of failed cytotoxic and sometimes expensive therapeutic agents.

Conflict of interest

All authors on this manuscript are employees of Cephalon, Inc.

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