Targeting the Interleukin-6/Jak/Stat Pathway in Human Malignancies

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ABSTRACT

The Janus kinase/signal transducer and activator of transcription (Jak/Stat) pathway was discovered 20 years ago as a mediator of cytokine signaling. Since this time, more than 2,500 articles have been published demonstrating the importance of this pathway in virtually all malignancies. Although there are dozens of cytokines and cytokine receptors, four Jak2s, and seven Stats, it seems that interleukin-6–mediated activation of Stat3 is a principal pathway implicated in promoting tumorigenesis. This transcription factor regulates the expression of numerous critical mediators of tumor formation and metastatic progression. This review will examine the relative importance and function of this pathway in nonmalignant conditions as well as malignancies (including tumor intrinsic and extrinsic), the influence of other Stats, the development of inhibitors to this pathway, and the potential role of inhibitors in controlling or eradicating cancers.

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INTRODUCTION

The Janus kinase/signal transducer and activator of transcription (Jak/Stat) signaling pathway was first discovered in a study of interferon signaling, identifying how a growth factor leads to the activation of a transcription factor.¹⁻³ Jak2 (tyrosine kinases) engage with cytokine receptors and mediate tyrosine phosphorylation of their associated receptors and recruited proteins, including Stats.

Tyrosine phosphorylated Stats are released from the receptors and form homodimers, which translocate to the nucleus where they bind canonical sequences and modulate transcription.⁴ In addition to tyrosine phosphorylation, Stats are serine phosphorylated within their transcriptional activation domain, influencing their transcriptional activation function, stability, and noncanonical functions.⁵⁻¹¹ Stats are also acetylated, methylated, sumoylated, and ubiquitinated, which alters their stability, dimerization, nuclear localization, transcriptional activation function, and association with histone acetyltransferases and histone deacetylases.¹²⁻²² Importantly, Jak/Stat activation is tightly regulated through the expression of positive (cytokines, receptors, tyrosine kinases) and negative regulators (tyrosine phosphatases, protein inhibitors of activated Stat, suppressor of cytokine signaling [SOCS] proteins).²³⁻³¹

The function of the Jak2s and Stats in normal cells were determined principally through the analysis of mice or tissues deficient for each of these

molecules.^{32,33} For example, Jak1-deficient mice die perinatally; it is required for leukemia inhibitory factor, interleukin-6 (IL-6), IL-10, interferon (IFN), and IL-2 signaling. Jak2 deficiency leads to profound anemia and mice die E12.5.³³⁻³⁵ Jak2 plays a critical role in signaling through the single-chain (erythropoietin, growth hormone, and prolactin receptors), IL-3 (IL-3, IL-5, and granulocyte macrophage colony-stimulating factor [GM-CSF] receptors), and IFN- γ receptor families and embryonic stem-cell maintenance.³⁶⁻³⁸ Interestingly, Jak2 can directly modify chromatin through tyrosine phosphorylation of histone H3 tyrosine 41 and histone arginine methyltransferase.³⁶⁻³⁸

Stat1 is the principal transcriptional mediator of IFN signaling and plays a central role in the regulation of innate and adaptive immune responses. Additionally, many other cytokines (eg, IL-6 family) can lead to its phosphorylation in conjunction with other Stats (notably Stat3 and Stat5). Stat1 is a positive regulator of Th1 differentiation and a negative regulator of regulatory T cells (Tregs).^{39,40} Gain of function Stat1 alleles was discovered in patients with chronic mucocutaneous candidiasis, which leads to enhanced production of IFNs and IL-27 and an imbalance between Stat1 and Stat3 activation in IL-17–producing T cells, resulting in impaired IL-17–dependent immunity.⁴¹

Stat3 is activated in response to the IL-6 and IL-10 family of cytokines, G-CSF, leptin, IL-21, and IL-27 as well as to receptor tyrosine kinases (MET

and epidermal growth factor receptor [EGFR]) and non-receptor tyrosine kinases (Abl, Src, Syk).⁴²⁻⁵² Stat3 deficiency is embryonic lethal (E6.5), underscoring its role in early development, whereas tissue-specific loss of Stat3 demonstrates its importance in regulating inflammation (Th17 cells, myeloid cells, Bregs, dendritic cells).^{33,53-60} IL-6, IL-23, and IL-21 through Jak-mediated phosphorylation of Stat3 are required for Th17-cell generation, essential for protective immunity against fungi, and participate in autoimmune diseases.⁶¹ The most significant negative regulator of immune-mediated inflammation is the IL-10 cytokine, which also signals through Jak1/Jak2/Tyk2 and Stat3. Ablation of the IL-10 receptor or Stat3 in Treg cells leads to fatal Th17-mediated colitis. The ability of different Stat3-activating cytokines (IL-6, IL-23, IL-10) to regulate Th17-cell functions (both activate and inhibit) remains an unanswered question, but possible/likely mechanisms involve the levels of cytokines, their corresponding receptors, the degree of Stat3 phosphorylation, SOCS3-dependent inhibition of glycoprotein 130 (gp130), and the interplay between Tregs and Th17 cells.⁶²⁻⁶⁵ Stat3 also plays a critical role in the development and function of myeloid cells. Mice deficient for Stat3 in myeloid cells develop chronic colitis (in a lymphocyte-dependent manner), phenocopying mice deficient for IL-10.^{66,67} Furthermore, macrophage-derived IL-10 is a critical regulator of Treg suppressive functions in models of colitis.⁶⁸ Stat3 has been shown to transcriptionally repress IL-12 and IL-23 through IL-10 signaling in myeloid cells.⁶⁹ Thus, Stat3 activation in different cell types through different receptors (IL-6 or IL-10 receptors) can regulate immune effector cells, leading to controlled inflammatory responses. Stat3 is required for G-CSF-mediated expansion of both immature and mature granulocytes.⁷⁰ The specific roles Stat3 plays in hepatic inflammation/damage/regeneration through its activation in myeloid cells and in hepatocytes are essential to prevent liver failure by attenuating a strong innate (Stat1-dependent) inflammatory response.⁷¹ Stat3 also plays a critical role in generating effector B cells from naive precursors in humans.⁷² Mutations in the DNA-binding domain of Stat3 (dominant negative) were shown to be a cause of hyperimmunoglobulin E syndrome, which is a primary immunodeficiency leading to recurrent infections (bacterial and fungal), elevated levels of immunoglobulin E through defective production of Th17 cells, and impaired generation of tolerogenic dendritic cells.⁷³⁻⁷⁷ Interestingly, these patients are predisposed to the development of B-cell lymphomas, which highlights the complexities of globally suppressing Stat3 activity.⁷⁸

CONSTITUTIVE ACTIVATION OF JAKS/STATS IN CANCER

In contrast to normal cells, in which Stat tyrosine phosphorylation occurs transiently, it has been determined that Stats 1, 3, and 5 are persistently tyrosine phosphorylated in most malignancies (particularly Stat3).⁷⁹⁻⁸¹ The mechanisms by which Stat3 is persistently or constitutively tyrosine phosphorylated in cancers include increased production of cytokines and cytokine receptors, which occurs in both an autocrine and paracrine manner (from the tumor microenvironment), a decrease in the expression of the SOCS proteins through promoter methylation, and loss of tyrosine phosphatases.^{52,82-86} A few genetic abnormalities have recently been discovered in malignancies, which lead to increased Stat3 or Stat5 tyrosine phosphorylation. For example, a subset of myeloproliferative disorders harbor a somatic activating mutation in the *Jak2* kinase (V617F

and exon 12) or in the thrombopoietin receptor (*MPL*), resulting in hyperactivation of Jak2 or thrombopoietin signaling (both Stat5 and Stat3 phosphorylation).⁸⁷⁻⁹¹ Somatic in-frame deletions in the *gp130* receptor can lead to hyperactivation of the receptor and activation of Stat3 and the development of inflammatory hepatocellular adenomas in mice.⁹² The receptor protein tyrosine phosphatase delta is frequently inactivated in glioblastoma (GBM) multiforme, head and neck, and lung cancers and has been shown to dephosphorylate Stat3.²⁹ LNK is a regulator of hematopoiesis through direct binding and inhibition of Jak2.⁹³ In one report, myeloproliferative disorders expressing mutations in the *LNK* gene led to enhanced Jak2/Stat3/5 activation and myeloproliferative neoplasms.⁹⁴ Sphingosine-1-phosphate receptor-1 upregulates Jak2 activity, leading to enhanced Stat3 phosphorylation.⁹⁵ Stat3 transcriptionally regulates itself, sphingosine-1-phosphate receptor-1, and the *IL-6* gene, leading to a positive feed-forward loop in a number of epithelial tumors.⁹⁵⁻⁹⁷ Aberrant EGFR and Ras signaling can lead to increased cytokine production, resulting in enhanced Stat3 phosphorylation, demonstrating both redundancies and crosstalk between seemingly parallel pathways.^{82,98-100}

DEPENDENCE ON JAK/STAT3 SIGNALING IN MALIGNANCIES

Numerous studies have revealed Stats (particularly Stat3) to be required in many aspects of tumorigenesis, including differentiation, proliferation, apoptosis, increased sensitivity to cytotoxic agents, angiogenesis, recruitment of immune cells, and metastasis.^{3,81,101} To explore the specific functions of each signaling component and to determine their mechanisms of action, a number of tools and approaches have been used, including murine knockout and knock-in models, shRNA knockdown approaches, introduction of dominant-negative forms (lacking specific residues or domains), inhibitors of tyrosine kinases (natural products or designed), inhibitors of Stat3-DNA binding (platin derivatives), molecular inhibitors of Stat3 dimerization (SH2 [Src homology 2] –domain mimetics), decoys (optimal DNA binding sites), and blocking antibodies to receptors.^{3,102-104}

For example, mice deficient for Stat3 in specific cell types do not develop oncogene- or mutagen-induced cancers and/or develop less aggressive cancers.^{57,105-109} Conversely, the generation of mice expressing mutant forms of the gp130 receptor can lead to hyperactivation of Stat3, resulting in gastric adenomas and lymphopoiesis.¹¹⁰⁻¹¹² Inducible expression of a constitutively activated form of Stat3 in the lung leads to chronic inflammatory changes and increased cytokine production, eventually resulting in *de novo* tumorigenesis.¹¹³ Introduction of sh/siRNA to Stat3 in cancer-derived cell lines leads to a marginal effect on *in vitro* growth but results in a significant effect on *in vivo* tumor growth, principally through decreased angiogenesis and invasion, possibly through a reduction in the Stat3-mediated production of tumor-secreted pro-inflammatory and angiogenic factors (IL-8, IL-6, hypoxia-inducible factor 1 alpha, vascular endothelial growth factor [VEGF]).^{114,115}

Stat3 Activation in Tumor-Associated Cells

Stat3 activation in nontumor cells plays a significant role in tumor progression, particularly in those tumor subtypes associated with chronic inflammation (eg, colitis-associated colorectal cancers,

hepatocellular carcinoma, and pancreatic cancer).^{57,116-121} Activation of Stat3 by VEGF, platelet-derived growth factor, IL-6, or IL-10 is responsible for various immunosuppressive activities, such as the blockade of dendritic-cell maturation and the release of IL-10, which inhibits T-cell and macrophage activation and downregulated HLA expression in cancer cells. The association between angiogenesis and immunosuppression may be the result of hypoxia, which induces production of hypoxia-inducible factor 1 and VEGF in a Stat3-dependent manner, which also leads to the recruitment and differentiation of Tregs and myeloid-derived suppressor cells (MDSCs).¹²² Analysis of circulating myeloid cells in patients with metastatic melanoma revealed high levels of phosphorylated Stat3 (pStat3) in MDSCs (as defined by suppression of CD8 T cells), which was abrogated by pretreatment with a Jak inhibitor.¹²³ Deletion of Stat3 in myeloid cells or in vivo targeting of Stat3 in toll-like receptor 9-positive cells (eg, tumor-associated dendritic cells, MDSCs, and B cells) by synthetically linking a Stat3 siRNA to a CpG oligonucleotide agonist of toll-like receptor 9 targets resulted in enhanced CD8 T-cell responses and activation of tumor-associated dendritic cells and monocytes, leading to an antitumor immune response.^{59,124} Conversely, Stat3 activation in myeloid cells led to increased IL-6 expression and IL-6 trans-signaling in models of pancreatic cancer, which mediates Stat3 activation in epithelial cells promoting tumorigenesis.¹¹⁸ Thus, silencing or inhibition of Stat3 activity may be a strategy to overcome MDSC function and enhance the immune response to cancer.

Heterogeneous Stat3 Activation in Cancers

Immunohistochemical approaches on tumor microarrays are the most common manner by which to examine the relative levels of Stat proteins. Although this approach is preferable to most other methods, a number of important limitations should be mentioned. First, a majority of tumor samples express variable levels of pStats in terms of signal intensity, distribution, and cell types involved (Figs 1A, 1B). Specifically, we have determined that levels of pStat3 are highest on the leading edge of tumors in association with stromal, immune, and endothelial cells. This is not surprising given the evidence that paracrine sources of IL-6 (eg, from cancer-associated fibroblasts or myeloid cells) can induce autocrine production of

IL-6 and pStat3 expression in tumor cells, leading to heterogeneous levels of pStat3.^{51,57,100,125,126} Thus, a proper examination of tumor samples should include the complete tumor section, including the leading edge, because the cores obtained for tumor microarrays are typically centrally located.

In addition to the individual roles each Stat may play in promoting or inhibiting tumorigenesis, the relative roles that individual Stats play when coactivated in cancers are only beginning to be explored. For example, Stats 1, 3, and 5 are simultaneously tyrosine phosphorylated in a number of human cancers including breast, lung, and head and neck tumors. The presence of pStat5 (in addition to pStat3) in head and neck tumors can enhance tumor growth and invasion and may contribute to resistance to EGFR inhibitors and chemotherapy.¹²⁷⁻¹²⁹ Interestingly, in these squamous cell carcinomas, erythropoietin is felt to be mediating Stat5 phosphorylation, in contrast to breast cancers, whereby prolactin is the likely ligand. Of note, both of these growth factors lead to Jak2 activation. The functional interplay between activated Stat3 and Stat5 has also been described in breast cancers. Activated Stat3 and IL-6 are preferentially found in triple-negative breast cancers or in high-grade tumors and are associated with poor response to chemotherapy.^{100,130-133} In human tumors, the presence of pStat5 is found predominantly in well-differentiated estrogen receptor (ER)-positive tumors and is associated with favorable prognosis.¹³⁴ Furthermore, the presence of pStat5 is a predictive factor for endocrine therapy response and strong prognostic molecular marker in ER-positive breast cancer.¹³⁵ A recent study examined the consequences of simultaneous activation of Stat3 and Stat5 and determined that compared with tumors only expressing Stat3, tumors expressing both were more likely to be ER positive and human epidermal growth factor receptor 2 negative and of a lower stage.¹³⁶ Furthermore, Stat5 activation affects the transcriptional profile of tumors expressing activated Stat3. For example, prolactin-mediated Stat5 activation in breast cancers can lead to the transcriptional repression of B-cell lymphoma 6 (regardless of pStat3 status), overexpression of which is associated with high grade and metastatic disease.^{136,137} These studies suggest that examination of the levels of multiple Stats in a tumor sample may be required before determining the optimal treatment regimen. Understanding the relationship between the Jak/Stat

Fig 1. Heterogeneity in phosphorylated signal transducer and activator of transcription 3 (pStat3) expression. (A) Immunohistochemical analysis of pStat3 levels in a primary invasive ductal carcinoma. Nuclear pStat3 is detected in stromal (blue arrow) and endothelial cells (red arrow) and with variable levels in tumor cells (black arrow, strong staining; gray arrow, weak staining). (B) Schematic demonstrating this variability in pStat3 expression.

pathway and other aberrantly regulated signaling pathways and the roles they play in tumors, as well as how this relates to responsiveness to therapies, is an active area of clinical investigation in the attempt to personalize treatments.

IL-6/JAK/STAT SIGNALING IN TUMORIGENESIS: INHIBITING THE PATHWAY

IL-6 Blockade

IL-6 was determined to have pleiotropic functions activating numerous cell types expressing the gp130 receptor and the membrane-bound IL-6 receptor (classical IL-6 signaling). In addition, a soluble form of the IL-6 receptor (sIL-6 receptor) binds to IL-6 and interacts with gp130. This so-called IL-6 *trans*-signaling represents an alternative to classical IL-6 signaling and permits IL-6 to modulate a broad spectrum of target cells including epithelial cells, neutrophils, macrophages, and T cells.¹³⁸ Given the importance of aberrant IL-6 signaling in driving Stat3 activation in cancers, IL-6 blockade using IL-6 ligand-binding antibodies and IL-6R blocking antibodies have been tested preclinically, demonstrating tumor growth inhibition either alone or in combination with cytotoxic chemotherapies. Clinically, an IL-6 ligand-blocking antibody (CNTO-328) is being tested in a number of phase I/II clinical trials in transplant-refractory myeloma and castrate-resistant prostate cancer.¹³⁹⁻¹⁴¹ An IL-6R blocking antibody (tocilizumab) was recently approved for Castelman's disease and rheumatoid arthritis and will likely be tested in cancers (Table 1; Fig 2).^{142,143}

Jak Inhibitors

The study of Jak inhibitors for targeting cancers began in 1996 with the use of the pan-Jak inhibitor AG490, which led to inhibition of both in vitro and in vivo growth of relapsed B-cell leukemias.¹⁴⁴ Since then, a number of natural products (eg, curcumin, resveratrol, flavopiridol, and piceatannol) have been tested preclinically and demonstrated to inhibit a myriad of pathways involved in inflammation, including inhibition of Stat3 phosphorylation, principally through a decrease in cytokine production or as a direct inhibitor of the Jaks.^{104,145} In addition, more potent and orally bioavailable Jak inhibitors have been developed by many pharmaceutical companies since the discovery of the *Jak2* mutation in myeloproliferative disorders. Preclinically, these inhibitors are extremely effective in abrogating disease in myeloproliferative models, which has led to their clinical testing.^{91,146-148} The best

studied is the Jak1/2 inhibitor INCB018424 (Incyte, Wilmington, DE), which is in phase III clinical trials and has shown significant clinical improvements (reduction in splenomegaly, fatigue, discomfort, night sweats), correlating with a decrease in circulating pro-inflammatory cytokines.¹⁴⁸ However, only partial reductions in the mutant *Jak2* allele burden were observed. Perhaps this is because of inadequate inhibition of the mutant *Jak2* kinase or because another driver is responsible for the disease. Similar observations have been made with the other Jak inhibitors (CEP-701, XL019, TG101348), although they have their unique sets of adverse effects including thrombocytopenia, anemia, neutropenia, transaminitis, GI intolerance, and neurotoxicity.¹⁴⁸ These Jak inhibitors differ in their specificity (Jak1/2 v Jak2), potency, and half-life, which may be the reason for differences in adverse effects. Importantly, Jak1/2 is required for normal hematopoiesis, and therefore, these drugs will lead to anemia and thrombocytopenia unless different dosing schedules or lower doses are administered. The role of Jak inhibition in solid tumors was examined preclinically in models of IL-6–driven breast, ovarian, and prostate cancers using the Jak1/2 inhibitor AZD1480, which led to the suppression of tumor growth (Table 2).¹¹⁴ These compounds are now being tested in phase I clinical trials for solid tumors.

Stat3 Inhibitors

To target Stat3 as a DNA-binding protein, an optimal Stat3-binding site (double-stranded DNA) was synthesized and administered to cells in culture, injected intravenously or intratumorally, leading to sequestration of dimeric Stat3 away from its endogenous targets and onto this decoy.¹⁴⁹⁻¹⁵³ Preclinically, the Stat3 decoy was tested in head and neck squamous cell carcinomas expressing high levels of tyrosine phosphorylated Stat3, which led to apoptosis of cancer cells, resulting in decreased tumor growth. Synergy between the decoy and other therapies was also demonstrated. Clinically, the Stat3 decoy is presently being tested in patients with head and neck cancer, because this disease is locally invasive and readily accessible to local injection (Table 1).

Attempts to find direct inhibitors of Stat3 has focused on developing agents that target the SH2 domain, preventing either Stat3 phosphorylation and/or dimerization. These include peptidomimetics and designed small molecules. Results from an in vitro DNA-binding assay would suggest that possible modes of inhibition by peptidomimetics in vivo include disruption/dissociation of preexisting constitutively active Stat3:Stat3 dimers.¹⁵⁴ Additionally, peptidomimetics might associate with nonphosphorylated Stat3 monomer proteins through pY-SH2 interactions (the peptide or mimetic contains the pY motif, and the Stat3 monomer has an SH2 domain) to form a heterocomplex. This in turn would decrease the levels of free nonphosphorylated Stat3 monomers available for de novo phosphorylation and activation. A number of these agents have been shown to inhibit cancer growth in multiple preclinical cancer models.¹⁵⁴⁻¹⁵⁶ Although these compounds have shown reasonable specificity to disrupting Stat3 function, they have not been developed clinically in part because of the high concentrations required to impart their effects.¹⁰⁴

Using a chemical library of clinically established and well-tolerated compounds in a luciferase cell-based assay led to the identification of inhibitors of Stat3- and Stat5-dependent transcription.¹⁵⁷⁻¹⁵⁹ For example, nifuroxazide, a drug used for the treatment of diarrhea, could inhibit Jak2, and Tyk2 effectively reduced pStat3 levels in multiple

Table 1. IL-6/Stat3 Inhibitors

Drug	Function	Indication	Status
Tocilizumab	Humanized mAb to IL-6 receptor	Castelman's disease RA	FDA approved
CNTO-136, CNTO-328	Fully human mAbs to IL-6	Lupus Multiple myeloma Prostate cancer	Phase II
REGN-88	Fully human mAb to IL-6 receptor	RA	Phase I
Stat3 decoy	Stat3 DNA competitor	Head and neck cancer	Phase 0

Abbreviations: FDA, US Food and Drug Administration; IL-6, interleukin-6; RA, rheumatoid arthritis; Stat3, signal transducer and activator of transcription 3.

Fig 2. Signal transducer and activator of transcription 3 (Stat3) signaling. Canonical: Stat3 is tyrosine phosphorylated by Janus kinase (Jak) kinases in response to cytokine/growth factor activation of cell surface receptors (eg, receptor tyrosine kinases [RTKs], glycoprotein 130 [gp130] with either interleukin-6 receptor [IL-6R] or soluble IL-6R [sIL-6R]). On tyrosine phosphorylation (PY), Stat3 dimerizes and localizes to the nucleus, where it binds to Stat3 responsive elements. Stat3 is also serine phosphorylated (PS). Soluble factors that activate Stat3 include the IL-6 family of cytokines. Noncanonical: Unphosphorylated Stat3 can bind to either nuclear factor κ B (NF κ B) or CD44 in the cytoplasm; the complexes translocate into the nucleus, where they bind NF κ B (IKE) and Stat3 DNA-binding elements. Acetylated Stat3 is required for association with CD44. PS Stat3 and PY Stat3 can also localize into the mitochondria, where they modulate ATP production. Agents to inhibit the Stat3 canonical pathway include targeting Jak (CEP-701, XL019, INCB018424, AZD1480) and the IL-6/sIL-6R interaction (tocilizumab, CNTO-328). HAT, histone acetyltransferase; HDAC, histone deacetylase; ISRE, interferon stimulated response element; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PIAS, protein inhibitors of activated Stat; SOCS, suppressor of cytokin signaling.

myeloma.¹⁶⁰ Pyrimethamine, an antimalarial compound, was identified as an inhibitor of Stat3 and myeloma growth and is presently in clinical trials for the treatment of chronic lymphocytic leukemia and small lymphocytic leukemia.¹⁵⁸

Introduction of mutant (dominant-negative forms) Stats has allowed for delineation of the specific function of domains or residues and has led to the discovery of novel or noncanonical functions for

the Stats as mediators of tumorigenesis.^{161,162} Interestingly, both canonical and noncanonical roles of individual Stats have been determined to play a critical role in mediating both tumor initiation and promotion. The canonical pathway is defined as tyrosine phosphorylated Stats functioning as transcription factors. The noncanonical pathway includes the many roles of nontyrosine phosphorylated Stats, Stats as mediators of DNA methylation, Stats activating focal

Table 2. Jak Inhibitors

Drug	Function	Indication	Status
CEP-701	Jak2 inhibitor	Myelofibrosis Thrombocytopenia Polycythemia vera	Phase I/phase II
XL019	Jak2 inhibitor	Myeloproliferative disorders Myelofibrosis Polycythemia vera Thrombocytopenia	Phase I
INCB018424	Jak1-2 inhibitor	Hematologic malignancies Leukemia Myeloproliferative disease	Phase III Phase I
AZD1480	Jak1-2 inhibitor	Solid tumors Hematologic malignancies	Phase I

Abbreviation: Jak, Janus kinase.

adhesions, and Stats as regulators of mitochondrial function.^{96,163-169} Examples of the noncanonical pathway include nontyrosine phosphorylated Stat3 activating transcription in conjunction with nuclear factor κ B or CD44, localizing to the mitochondria and regulating ATP synthesis, and interacting with the microtubule-associated protein stathmin modulating the motility of cells.^{12,96,168-170} Interestingly, microtubule-targeting agents (eg, paclitaxel) can disrupt Stat3 tubulin interactions.¹⁷¹ Furthermore, serine phosphorylated but nontyrosine phosphorylated Stat3 can regulate transcription in chronic lymphoid leukemia cells.¹⁷² The roles of Stat acetylation, ubiquitylation, and sumoylation are presently being explored in regulating tumor formation and metastatic progression. Thus, both nontyrosine and tyrosine phosphorylated Stat3 play important functions in cancer cells, which should be taken into consideration in the choice of inhibitory agents to be used in preclinical and clinical settings (Fig 2).

DEPENDENCY ON JAK/STAT SIGNALING FOR TUMORIGENESIS

A number of important questions arise when we consider how to predict whether a particular tumor will be dependent on the Jak/Stat signaling pathway. Will tumors with the highest, most homogeneous expression of tyrosine phosphorylated Stat be predictive of dependence on this signaling protein? Alternatively, will tyrosine phosphorylated Stats in the microenvironment determine response to anti-Stat therapies? What is the contribution of the other Stats in determining responsiveness to targeted therapy? For example, if we use Jak inhibitors in the treatment of breast cancer, we will inhibit Stat5 phosphorylation, which may thwart the benefits of inhibiting Stat3.

Perhaps the most significant effect of inhibiting IL-6/Jaks and Stat3 will be on its role in modulating the tumor-associated immune microenvironment. The complex relationship between tumor-associated Tregs and Th17 cells (both dependent on Stat3), dendritic cells, Th1, Th2, Bregs, MDSCs, neutrophils, and activated macrophages may result in unexpected findings on inhibition of this pathway. For example, Stat3 has been shown to enhance the expression of IL-23 (leading to the expansion of Tregs) while conversely suppressing the expression of IL-12 within the tumor milieu.¹⁷³ In the context of colitis, IL-10 signaling in Tregs can suppress pathogenic Th17 responses.⁶⁵ Tumor-evoked Bregs express activated Stat3 and induce

transforming growth factor- β conversion of Tregs from resting T cells.¹⁷⁴ Thus, depending on what agent is used to block, these signaling molecules are likely to give rise to markedly different effects. For example, inhibition of Jak2 during encounters between human T cells and allogeneic monocyte-derived dendritic cells induced T-cell tolerance, preserving Treg numbers and impairing expansion of Th1 and Th17 cells.¹⁷⁵ In contrast, IL-6 receptor blockade had no effect on dendritic-cell maturation, T-cell proliferation, Treg expansion, or Th1/Th17 responses in vitro.¹⁷⁶ Inhibition of Jak1/2 was shown to decrease CD11b/Gr1-positive cells in a murine model of breast cancer, and although effects on T-cell subsets/activity were not reported, it seems likely that they would have a significant impact.¹⁷⁷ These few examples are meant to highlight the importance of examining these critical immune cell types while inhibiting these pathways in the hopes of correlating clinical responses to changes in the immunophenotype of the tumor.

Will nontyrosine phosphorylated Stats play a critical role in promoting tumorigenesis? If so, inhibiting phosphorylation of the protein may prove to be ineffective. Attention to the biology of the cancer should be considered when choosing a particular regimen. For example, prostate cancers (androgen receptor [AR] positive) are usually responsive to androgen blockade. However, when castration-resistant disease develops, tumors often express higher levels of the AR, possibly through activated Stat3, which can transcriptionally regulate AR.¹⁷⁸⁻¹⁸¹ Thus, we may consider combining antiandrogens with anti-Stat3 drugs rather than with chemotherapy in hormone-refractory metastatic prostate cancer. It is also important to recognize that Stats may act as tumor suppressors in certain tumor contexts. For example, Stat3 can inhibit tumorigenesis in phosphatase and tensin homolog-null GBMs. While in EGFRvIII expressing GBMs Stat3 is required for tumorigenesis,^{182,183} There are conflicting data in murine APC models of intestinal cancer where one publication demonstrated that Stat3 was required for tumorigenesis while another suggested that Stat3 loss promoted tumor progression and invasion of intestinal tumors.^{57,107,109}

Inhibitors of Jaks, Stat3, or IL-6 signaling induce growth arrest, inhibit angiogenesis, and block recruitment of immune cells to the tumor but rarely lead to complete abrogation or regression of tumor formation. Although many in vitro studies have demonstrated that concomitant inhibition of the IL-6/Jak/Stat3 pathway with cytotoxic chemotherapies can promote apoptosis in a variety of cancer-derived cell lines, few in vivo studies have been performed to confirm or support these in vitro findings. Identifying appropriate Jak/Stat inhibition-containing regimens preclinically is essential for optimizing the sequence in which combination therapies should be administered. Furthermore, with the development of small-molecule inhibitors of tyrosine kinases with relatively short half-lives, we may wish to consider developing and testing regimens using pulsatile high doses, which may result in more effective targeting, fewer adverse effects, and less acquired resistance.^{184,185} Importantly, what is difficult and lacking in many clinical trials are the means of determining whether our targeted therapies are indeed hitting the appropriate targets. Thus, determination of the optimal correlative studies is essential. These questions and examples are meant to point out that when we design clinical trials to test inhibitors of the Jak/Stat pathways, we are fully cognizant of the potential pitfalls and difficulties in interpreting the outcomes of these trials. Nevertheless, we are extremely optimistic that inhibition of this pathway will prove to be beneficial in the treatment of a number of malignancies. We hope that

an awareness of the complexities of this pathway will lead to informed and careful clinical trial design, resulting in an understanding of how these therapies induce tumor regression and cures.

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