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Therapeutic modulators of STAT signalling for human diseases

Gabriella Miklossy*, Tyvette S. Hilliard*, and James Turkson

Cancer Biology, and Natural Products and Experimental Therapeutics Programs, University of Hawaii Cancer Center, 701 Ilalo Street, Honolulu, Hawaii 96813, USA

Abstract

The signal transducer and activator of transcription (STAT) proteins have important roles in biological processes. The abnormal activation of STAT signalling pathways is also implicated in many human diseases, including cancer, autoimmune diseases, rheumatoid arthritis, asthma and diabetes. Over a decade has passed since the first inhibitor of a STAT protein was reported and efforts to discover modulators of STAT signalling as therapeutics continue. This Review discusses the outcomes of the ongoing drug discovery research endeavours against STAT proteins, provides perspectives on new directions for accelerating the discovery of drug candidates, and highlights the noteworthy candidate therapeutics that have progressed to clinical trials.

The signal transducer and activator of transcription (STAT) protein family is composed of seven members: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6. Structurally they share five domains, which are an amino-terminal domain, a coiled-coil domain, a DNA-binding domain, an SH2 domain and a carboxy-terminal transactivation domain (FIG. 1). The transactivation domain contains one or two amino acid residues that are crucial for the activity of the STAT protein; that is, phosphorylation of a particular tyrosine residue promotes dimerization, whereas phosphorylation of a particular serine residue enhances transcriptional activation^{1–3}.

The STAT proteins were discovered as cytoplasmic transcription factors that mediate cellular responses to cytokines and growth factors^{1,2} (FIG. 2). Once a ligand interacts with its receptor, STAT activation is induced by the phosphorylation of the key tyrosine residue in the STAT transactivation domain by growth factor receptors, Janus kinases (JAKs), SRC family kinases and other tyrosine kinases. This leads to numerous events including STAT–STAT dimerization through a reciprocal phospho-tyrosine (pTyr)-SH2 domain interaction, nuclear translocation, DNA binding and the transcriptional induction of genes in the nucleus. Physiological negative regulators, such as suppressors of cytokine signalling (SOCS) and protein tyrosine phosphatases (PTPs), ultimately downregulate the active STAT signalling.

Correspondence to J.T. jturkson@cc.hawaii.edu.

*These authors contributed equally to this work.

Competing interests statement

The authors declare no competing financial interests.

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STAT proteins promote fundamental cellular processes, including cell growth and differentiation, development, apoptosis, immune responses and inflammation^{1,2} (FIG. 2). STAT1 signalling is activated in response function partly by controlling the growth and apoptosis of immune cells⁴. STAT1 signalling regulates T helper type 1 (T_H1) cell-specific cytokine production that alters both immune function and inflammatory responses by shifting the balance between T_H1 and T_H2 cells⁵. Indeed, STAT1 deficiency abrogates IFN responsiveness, leading mice to succumb to bacterial and viral infections⁶. Furthermore, the loss of responsiveness to IFN γ due to STAT1 deficiency provides malignant cells with a growth advantage and leads to increased tumour formation⁴. This outcome suggests that STAT1 has a tumour-suppressive function; although recent data indicate that the protein has a more complex role in carcinogenesis⁴. Moreover, in STAT1-null mouse models of atherosclerosis-susceptible bone-marrow transplantation, these mice have reduced foam cell formation and atherosclerosis, which suggests that STAT1 has a pro-atherogenic function⁷. By contrast, gain-of-function mutations in the *STAT1* gene — which lead to STAT1 hyperactivation and defective nuclear dephosphorylation — affect T_H1 and T_H17 cell responses and cause chronic mucocutaneous candidiasis^{8,9}.

STAT2 signalling is important for the induction of antiviral effects. STAT2-null mice and STAT2-null cell lines have defective antiviral responses to IFN α and IFN β , as well as blunted apoptotic effects to IFN α and IFN β ¹⁰. Evidence further suggests that altered STAT2 signalling may partly contribute to carcinogenesis through the upregulation of interleukin-6 (IL-6) production, which promotes STAT3 activation¹⁰. Notably, the colon of STAT2-deficient mice showed markedly lower levels of IL-6, which was associated with diminished tumour progression¹⁰. By contrast, the reconstitution of STAT2 in the null background upregulated IL-6 production and increased the levels of pSTAT3.

STAT3 function is essential for early embryonic development, which is clearly demonstrated by the death on day 8.5 of STAT3-deficient mouse embryos¹¹. The biological importance of STAT3 was further studied using tissue-specific STAT3-deficient mice¹². *In vitro* cultured STAT3-deficient T cells did not respond to IL-6 stimulation and could not be rescued by IL-6 from apoptotic cell death, indicating that STAT3 functions are essential for IL-6-mediated anti-apoptotic responses¹³. Furthermore, STAT3-deficient keratinocytes showed a poor wound-healing response *in vitro* and *in vivo*, which was due to the limited migration of the cells¹⁴. In addition, the dys-regulation of the role of STAT3 in keratinocyte physiology is thought to contribute to the induction of skin carcinogenesis¹⁵. STAT3 function is often aberrant in the context of cancer, and this abnormality represents an underlying mechanism of STAT3 for promoting malignant transformation and progression. Of clinical significance, constitutively active STAT3 is detected in numerous malignancies, including breast, melanoma, prostate, head and neck squamous cell carcinoma (HNSCC), multiple myeloma, pancreatic, ovarian, and brain tumours^{16–23}. Aberrant STAT3 signalling promotes tumorigenesis and tumour progression partly through dysregulating the expression of critical genes that control cell growth and survival, angiogenesis, migration, invasion or metastasis^{16–19,21–23}. These genes include those that encode p21^{WAF1/CIP2}, cyclin D1, MYC, BCL-X, BCL-2, vascular endothelial growth factor (VEGF), matrix metalloproteinase 1 (MMP1), MMP7 and MMP9, and survivin^{16–19,21–23}. Evidence also

supports a role of STAT3 in the suppression of tumour immune surveillance^{24,25}. Consequently, the genetic and pharmacological modulation of persistently active STAT3 was shown to control the tumour phenotype and to lead to tumour regression *in vivo*^{18–23,26–30}. Of further clinical significance, mutations within the SH2 domain-coding sequence in *STAT3* occur in patients with a rare primary immunodeficiency, hyper-immunoglobulin E syndrome³¹, and in large granular lymphocytic leukaemia³². These mutations reportedly increase the stability of the functional STAT3–STAT3 dimers and are associated with the disease pathogenesis^{32,33}.

STAT4 is a crucial mediator of IL-12 function that regulates the differentiation of T_H1 cells and their inflammatory responses⁵. Accordingly, STAT4 signalling is associated with autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) induction, an animal model of multiple sclerosis³⁴. Indeed, mice deficient in the *Stat4* gene were protected from developing EAE³⁵. Additionally, STAT4-null lymphocytes showed decreased proliferation and had an impaired response to IL-12 (REF. 36). Treatment with a STAT4-specific antisense oligonucleotide (ASO) led to an improvement in the systemic lupus erythematosus (SLE) phenotype, severe lupus nephritis in mouse models, which suggests that STAT4 also has an important role in SLE, even though STAT4 knockout had no effect on the clinical presentation of the disease in mouse models³⁶.

STAT5 has two isoforms, STAT5A and STAT5B, which are encoded by distinct genes and share 96% sequence homology, with notable differences occurring in the transactivation domain³⁷. Normal STAT5 signalling is important in mammary gland development and milk production, and in haematopoiesis³⁸. Mice deficient in both STAT5A and STAT5B show defects in IL-2 receptor- α expression in T lymphocytes¹². By contrast, mice that are deficient in STAT5B only showed loss of sexual dimorphism of body growth rate, which was accompanied by a decreased expression in male-specific liver genes³⁹ and the attenuation of the cytolytic activity of natural killer cells⁴⁰. Constitutive STAT5 activation is also implicated in the pathogenesis of HNSCC, chronic myelogenous leukaemia (CML)⁴¹, breast⁴², prostate and uterine cancers¹⁹. The aberrant STAT5 activation by BCR–ABL in CML⁴³ is particularly noteworthy. It is widely recognized that STAT5 and STAT3 share similar functions in promoting cancer, including the induction of pro-proliferative and anti-apoptotic genes^{44,45}.

STAT6 signalling is induced by IL-4 and IL-13 and supports immune function, notably regulating the balance between inflammatory and allergic immune responses^{46–49}. Beyond the immune system, STAT6 signalling promotes luminal mammary epithelium development and is implicated in the pathology of lung and airway disease, including the promotion of airway hyperactivity and mucus production in the lung epithelium, and the regulation of allergic skin inflammation^{46,50–52}.

Mutations in JAKs, as observed in certain haematological disorders, immunodeficiencies and cancers, lead to dysregulation of STAT signalling that contributes to these disease phenotypes^{53,54}. The clinical significance of the JAK–STAT pathway in diseases is exemplified by an ongoing clinical study of patients with primary immunodeficiency

disorders to identify mutations in the *JAK3*, *STAT1* and *STAT4* genes, among others (ClinicalTrials.gov identifier: NCT00001788).

Therapeutic approaches that target STAT proteins

It has been more than a decade since the first peptide inhibitor of a STAT protein was reported³⁰ and efforts to target STAT signalling for therapeutic purposes continue. Extensive drug discovery activities targeting STAT3 are well underway, as evident by the numerous publications related to this protein. This is in contrast to the moderate research efforts for inhibitory modulators of STAT1, STAT2, STAT4, STAT5 and STAT6, as demonstrated by the few published reports. Various inhibitory strategies against STAT signalling and function are being pursued (FIG. 3). Most of these focus on inhibiting STAT dimerization using peptides or peptidomimetics generated through structure-based design, small molecules identified by molecular modelling, virtual or library screening, or natural products. STAT dimerization and signalling can also be blocked by inhibiting tyrosine kinases that phosphorylate STAT proteins, or by inducing phosphatases that dephosphorylate STAT proteins. Other approaches, discussed in BOX 1 and BOX 2, include the use of oligodeoxynucleotide (ODN) decoys as specific STAT DNA-binding domain inhibitors and ASOs that interfere with *STAT* mRNA.

Box 1

Oligodeoxynucleotide decoy approach to inhibit STAT functions

One approach to modulate STAT signalling is to use oligodeoxynucleotide (ODN) decoys as specific DNA-binding domain inhibitors^{28,197} (FIG. 3c; TABLE 1). ODN decoys compete with endogenous promoter sequences for binding to the target transcription factor, thereby preventing gene expression. In a rat cardiac allograft model of atherosclerosis, a 25-bp STAT1 ODN decoy based on the γ -activated sequence (GAS) element¹⁹⁸ specifically downregulated the protein and mRNA levels of CD40. *CD40* is a STAT1 target gene that is involved in T-cell activation, and suppression of its expression by the STAT1 ODN may account for the protective effects of the STAT1 ODN against atherosclerosis development¹⁹⁹. Additionally, in a myelogenous leukaemia cell line (K562), treatment with a 21-bp ODN element containing a modified version of the GAS element that binds STAT5 suppressed STAT5 transcriptional activity, which led to cell cycle arrest and apoptosis²⁰⁰. This result suggests that blocking STAT5, which is downstream of the constitutively activated BCR–ABL, with an ODN decoy can be a promising therapeutic approach to overcome imatinib resistance in chronic myelogenous leukaemia²⁰⁰.

The use of ODN decoys to target STAT3 is further advanced. A 15-bp STAT3 ODN decoy (5'-CATTTCCCGTTAATC-3') was initially designed on the basis of the sequence of the high-affinity *cis*-inducible element of the *FOS* promoter²⁸. A modified version of this STAT3 ODN decoy (5'-CATTTCCCGTAAATC-3') was evaluated in mice bearing glioblastoma xenografts²⁰¹. Intratumoural injection of this modified version downregulated STAT3 target genes and decreased tumour growth²⁰¹. Furthermore, the inhibition of STAT3 function by the same ODN decoy sensitized resistant head and neck

squamous cell carcinoma (HNSCC) and bladder cancer cells to the epidermal growth factor receptor (EGFR) inhibitors cetuximab and erlotinib²⁰². Although this STAT3 ODN decoy disrupted STAT1 signalling *in vitro*, its inhibitory effect on tumour cell growth is reportedly independent of the STAT1 activation status²⁰³.

To evaluate the potential clinical application of ODN decoys, a single-dose intramuscular injection of the STAT3 ODN decoy with the sequence 5'-CATTTCCTCGTTAATC-3' was evaluated for toxicities in a non-human primate model²⁸. Encouragingly, this ODN decoy showed no systemic or organ-specific toxicity when it was given over a 2-week period. There was no observable dose–response effect on STAT1 activation in peripheral blood cells, whereas pSTAT3 levels decreased²⁸. A Phase 0 clinical trial of the STAT3 ODN decoy 5'-CATTTCCTCGTTAATC-3' (ClinicalTrials.gov identifier: NCT00696176) assessed the safety of a single dose intratumoural injection in patients with HNSCC¹⁹⁶. This decoy showed a reasonable safety profile and suppressive effects on the expression of STAT3 target genes¹⁹⁶.

More stable cyclic versions of the STAT3 ODN decoy 5'-CATTTCCTCGTTAATC-3', which are resistant to serum nucleases, also decreased the expression of STAT3-regulated genes and suppressed the viability of HNSCC and bladder cancer cells¹⁹⁶. A hairpin ODN version with a modified consensus sequence containing two STAT3-binding sites that achieves a STAT3/STAT1 discriminating property was efficacious in SW480 colon cancer cells¹⁹⁷. Together, these studies suggest that the clinical development of STAT3 ODN decoys has great promise¹⁹⁶.

Box 2

Antisense oligonucleotide approach to inhibit STAT functions

The use of antisense oligonucleotides (ASOs) to target aberrant signal transducer and activator of transcription 3 (STAT3) signalling in cancer has yielded promising results. Preclinical studies have shown that STAT3-specific ASOs inhibited STAT3 expression and downregulated the STAT3 target gene vascular endothelial growth factor (*VEGF*) in melanoma and mammary carcinoma models *in vitro*²⁰⁴. Additionally, they suppressed the malignant phenotype in prostate cancer²⁰⁵ and hepatocellular carcinoma cells²⁰⁶.

Moving towards a therapeutic application of ASOs to inhibit STAT functions, a phosphorothioate-modified chimeric ASO sequence that targets human *STAT3* mRNA has been designed by ISIS Pharmaceuticals (ISIS 481464). Studies in cynomolgus monkeys showed that ISIS 481464 given at a dose of 10 mg per kg per week for 6 weeks was sufficient to decrease STAT3 protein levels by up to 90%²⁰⁷. Importantly, the agent was well tolerated when given at doses of up to 30 mg per kg per week for 6 weeks, with no treatment-related changes in clinical signs or treatment-related deaths²⁰⁷.

In the following section, we discuss direct inhibitors reported for each member of the STAT protein family. There are no significant discoveries of STAT2 inhibitors, and so these are not discussed here, while inhibitors of STAT1 and STAT4 are discussed together owing to the low volume of reports and their relatively similar functional roles. Tyrosine kinase

inhibitors (TKIs), which broadly affect the induction of multiple STAT proteins by inhibiting the kinases that phosphorylate and thereby activate the STAT proteins, are discussed at the end of the section.

Inhibitors of STAT3

There are several human cancers that could benefit from therapeutics that target aberrantly active STAT3. Owing to its therapeutic significance, STAT3 is the target in many drug discovery research efforts (TABLE 1). Approaches to target STAT3 also include TKIs, ODN decoys (BOX 1) and ASOs (BOX 2), which are discussed separately. STAT3 drug discovery research has mostly focused on targeting the pTyr-SH2 domain interaction^{21–23} given its importance in promoting STAT3 dimerization and function, and these efforts have generated various inhibitory agents. However, there are many reported STAT3 inhibitors that may induce their effects through multiple mechanisms.

Peptides and peptidomimetics

The STAT3 SH2 domain (FIG. 1) is required for promoting dimerization (FIG. 2). One of the limitations of targeting protein dimerization is the practicality of targeting the dimer interface, which is challenging owing to the planarity of the large surface area. Nevertheless, novel peptide inhibitors that target the pTyr-SH2 domain interaction were the first STAT3 inhibitors to be established^{23,30,55–57} (FIG. 3a; TABLE 1).

A semi-rational, structure-based design approach identified the first SH2 domain-binding peptides and peptidomimetics that disrupt the STAT3 pTyr-SH2 domain interactions and STAT3–STAT3 dimerization^{21,30,55,58}. The native, parent pTyr peptide, PY*LKTK (where Y* stands for pTyr) and its modified forms blocked the DNA-binding and transcriptional activities of STAT3 at high micromolar concentrations³⁰. Peptidomimetic and non-peptide analogues, including ISS-610 and S3I-M2001, exhibited improved potency against STAT3 activity *in vitro* and against diverse malignant cells harbouring aberrantly active STAT3 (REFS 27,30,55,58). S3I-M2001 inhibited the growth of human breast tumour xenografts, while ISS-610 inhibited cell growth and induced apoptosis *in vitro*^{27,55}.

Furthermore, phosphopeptide binding sequences with the primary structure pTyr-Xxx-Xxx-Gln (where Xxx represents any amino acid) derived from leukaemia inhibitory factor (LIF), IL-10 receptor, epidermal growth factor receptor (EGFR), granulocyte colony-stimulating factor (GCSF) receptor or glycoprotein 130 (gp130), similarly inhibited STAT3 activation^{57,59}. In these studies, the peptidomimetic Ac-pTyr-Leu-Pro-Gln-Thr-Val-NH₂ was derived, which inhibited STAT3 activity (IC₅₀ values of 150 nM)^{56,59}. Moreover, a 28-mer native peptide identified as SPI, derived from the STAT3 SH2 domain, inhibited the STAT3 pTyr-SH2 domain interaction and signalling. The activity of SPI was moderate, but it did suppress cell viability and induced apoptosis of human breast, pancreatic, prostate and non-small cell lung cancer (NSCLC) cells *in vitro*⁶⁰.

Other studies identified CJ-1383 as a SH2 domain-binding peptidomimetic inhibitor that induced anti-tumour cell effects against human breast cancer cells⁶¹, as well as a phosphatase-stable, cell-permeable SH2 domain-targeting phosphopeptide mimetic prodrug,

PM-73G⁶³. PM-73G potently inhibited both constitutive and IL-6-stimulated STAT3 phosphorylation *in vitro* in breast cancer cells⁶², and in *in vivo* tumour xenografts further inhibited VEGF expression, angiogenesis and tumour growth⁶³. However, at low concentrations PM-73G did not inhibit the STAT3 target genes that encode cyclin D1, BCL-2 or survivin, or promote apoptosis^{62,63}. When the concentration of PM-73G was increased 50-fold cell proliferation inhibition was observed⁶². The lack of effects on cell growth and survival and on the related target genes at the lowest concentration of PM-73G tested in these studies are in contrast to the large body of data on the inhibition of STAT3 activity by other modalities that resulted in tumour cell growth blockade and apoptosis. Together, these data suggest that the role of STAT3 and the impact of STAT3 inhibitors in tumour cell biology are more complex than currently known. Despite the prolific research activity into peptide inhibitors, metabolic instability, poor cell permeability and other peptide-associated liabilities have precluded their clinical development as therapeutics.

Small molecules

Many small-molecule inhibitors of STAT3 activity have been identified through computational modelling, docking studies and virtual screening of chemical libraries (TABLE 1). In most cases, these agents function as disruptors of STAT3–STAT3 dimerization (FIG. 3a). STA-21 (also known as NSC628869) was identified from the screening of the National Cancer Institute (NCI) chemical library as an inhibitor of STAT3 dimerization, DNA-binding activity and transcriptional function in breast cancer cells, with a potency of 20 M⁶⁴. Its structural analogue, LLL-3 (which showed improved membrane permeability), decreased cell viability *in vitro* and intracranial tumour size *in vivo* in glioblastoma animal models⁶⁵.

A catechol (1,2-dihydroxybenzene) compound was identified from Wyeth's proprietary small-molecule collection as a STAT3 SH2 domain inhibitor, and this agent was active at 106 µM against a multiple myeloma cell line⁶⁶. Furthermore, a structure-based, virtual screen of the NCI chemical library targeting the STAT3 SH2 domain discovered S3I-201 (also known as NSC74859) as a STAT3–STAT3 dimerization disruptor, with a potency of 60–110 µM²⁶. S3I-201 inhibited STAT3 DNA-binding and transcriptional activities, induced growth inhibition and apoptosis of tumour cells harbouring constitutively active STAT3, and suppressed the growth of human breast cancer xenografts²⁶. Several of its derivatives, including S3I-201.1066, BP-1-102 and S3I-1757, showed improved potencies, with IC₅₀ values of 35 µM (S3I-201.1066), 6.8 µM (BP-1-102) and 13.5 µM (S3I-1757), and inhibited cell growth, malignant transformation, survival, migration and invasiveness *in vitro* of malignant cells harbouring aberrantly active STAT3 (REFS 67–69). In particular, S3I-201.1066 and BP-1-102 inhibited growth of human breast or NSCLC xenografts in mice, and notably BP-1-102 is orally bioavailable.

Virtual ligand screening identified Cpd30 (4-(5-((3-ethyl-4-oxo-2-thioxo-1,3-thiazolidin-5-ylidene) methyl)-2-furyl)benzoic acid), which selectively inhibited STAT3 with moderate potency, blocked STAT3 nuclear translocation upon IL-6 stimulation, and induced apoptosis in breast cancer cells harbouring constitutively active STAT3 (REF. 70). The related agent, Cpd188 (4-((3-((carboxymethyl)thio)-4-hydroxy-1-naphthyl)amino)sulphonyl)benzoic acid),

in combination with docetaxel, decreased tumour growth in chemotherapy-resistant human breast cancer xenograft models⁷¹.

Stattic, a non-peptide small molecule discovered through virtual screening of chemical libraries, targets the STAT3 SH2 domain and inhibits STAT3 signalling at 10 μM ⁷². It induced apoptosis of STAT3-dependent breast cancer and HNSCC cells and inhibited growth of orthotopic HNSCC tumour xenografts⁷². STX-0119, another STAT3 SH2 domain antagonist, induced antitumour cell effects *in vitro* and antitumour effects *in vivo* in a human lymphoma model⁷³, possibly by disrupting STAT3–STAT3 dimerization⁷⁴, with little effect on STAT3 phosphorylation⁷³. Fragments of STX-0119 and stattic were chemically fused to generate HJC0123 (REF. 75), which suppressed STAT3 phosphorylation and transcriptional activity in breast cancer cells and induced antitumour cell effects against breast and pancreatic cancer cells *in vitro* at IC_{50} values of 0.1–1.25 μM . The oral administration of HJC0123 led to inhibition of growth of human breast cancer xenografts⁷⁵.

Compound 6, which was identified through structure-based virtual screening, inhibited breast cancer cell viability *in vitro*, with an IC_{50} value of 20 μM , and decreased STAT3 phosphorylation and transcriptional activity in hepatocellular carcinoma (HCC) cells⁷⁶. Furthermore, an agent identified as OBP-31121 was reported to inhibit STAT1, STAT3 and STAT5 phosphorylation. OBP-31121 induced loss of viability and apoptosis *in vitro* and inhibited tumour growth *in vivo* in gastric cancer models, and it further sensitized gastric cancer cells to cisplatin and 5-fluorouracil⁷⁷. OBP-31121 has progressed to a Phase I clinical trial to determine the maximum tolerated dose (ClinicalTrials.gov identifier: NCT00955812).

Natural product inhibitors and their derivatives

Natural products have been an important resource in STAT3 inhibitor discovery and these efforts have yielded several lead candidates (TABLE 1). In many of these cases, however, the mechanism of action of these candidates with regard to STAT3 activity are unclear. It is possible that they inhibit STAT3 indirectly and are likely to block several targets.

Curcumin, a phenolic compound derived from the perennial herb *Curcuma longa* was shown to suppress JAK–STAT signalling at 15 μM , induce cell cycle arrest and inhibit cell invasion *in vitro* in a small cell lung cancer model⁷⁸. In mice bearing gastric cancer xenografts, treatment with curcumin inhibited IL-6 production by IL-1 β -stimulated myeloid-derived suppressor cells, which was associated with decreased activation of STAT3 and nuclear factor- κB (NF- κB)⁷⁹.

Curcumin analogues with improved bioavailability and stability, such as FLLL32, had enhanced potency (IC_{50} values of 0.75–1.45 μM) in suppressing both pSTAT3 and total STAT3, and they also induced STAT3 ubiquitylation and possible proteasomal degradation in canine and human osteosarcoma cells *in vitro*⁸⁰. Another curcumin analogue, HO-3867, similarly downregulated STAT3 signalling in cisplatin-resistant human ovarian cancer cells, thereby promoting enhanced sensitivity to cisplatin⁸¹. HO-3867 also induced apoptosis in BRCA1-mutated human ovarian cancer cells that harbour aberrantly active STAT3 (REF. 82).

Studies suggest that LLL12 (TABLE 1), another small-molecule inhibitor of STAT3 signalling that is based on curcumin^{83,84}, might suppress STAT3 activation by blocking its recruitment to the receptor and thereby preventing phosphorylation by tyrosine kinases, and by interfering with dimerization⁸⁵. LLL12 suppressed cell viability, induced apoptosis, and repressed colony formation and migration *in vitro* in studies of glioblastoma, osteosarcoma and breast cancer cells^{84,86}. It also inhibited angiogenesis, tumour vasculature development, and tumour growth *in vivo* in osteosarcoma xenograft models^{84,85}. Except for the finding that the analogues FL32 and FL62 may interact with the STAT3 SH2 domain⁸⁷, questions remain regarding how curcumin or its analogues inhibit STAT3 signalling.

Melanoma cells harbouring aberrantly active STAT3 were shown to be responsive to BBMD3, a derivative of the natural bis-benzylisoquinoline alkaloid berbamine from *Berberis amurensis*, with an IC₅₀ value of 2.9 µM. Melanoma cells treated with BBMD3 had decreased levels of pJAK2, pSRC and pSTAT3, and reduced cell viability⁸⁸. *In vitro* kinase assays showed that BBMD3 directly inhibited the autophosphorylation of the mutant JAK2^{V617F} (REF. 88).

The bis-indole alkaloid indirubin, which is derived from a mixture of plants used in the traditional Chinese medicine called Danggui Longhui Wan, was reported to inhibit VEGF receptor-mediated JAK–STAT3 signalling⁸⁹. Indirubin regulated angiogenesis in both chick embryo chorioallantoic membrane and mice corneal micropocket assays⁸⁹. Indirubin derivatives, including IRD E804 and MLS-2488, directly inhibited SRC activity *in vitro* (IC₅₀ value of 0.43 µM), suppressed pJAK, pSTAT3 and pAKT activity, suppressed STAT3 DNA-binding activity, repressed myeloid cell leukaemia sequence 1 (*MCL1*) and survivin expression, and induced apoptosis in human breast cancer cells^{90,91}. Recently, a more water-soluble indirubin derivative, E738, was identified that inhibits pJAKs (IC₅₀ values of 10.4 nM (JAK1), 74.1 nM (JAK2) and 0.7 nM (TYK2)) and SRC kinases (IC₅₀ value of 10.7 nM). Studies of E738 showed that it downregulated pJAK2, pSRC and pSTAT3 activity, and STAT3 transcriptional activity in pancreatic cancer cells at 1–2 µM⁹².

Resveratrol (3,5,4'-trihydroxystilbene), a polyphenolic compound found in red grapes and several other plants, was reported to inhibit STAT3 signalling at high micromolar concentrations, and it is likely that this effect contributes to the antitumour cell responses to this agent. Constitutive and IL-6-induced STAT3 activation in multiple myeloma, leukaemia and other tumour cell types were inhibited on resveratrol treatment, leading to a decreased expression of BCL-2 and other anti-apoptotic proteins, and apoptosis induction *in vitro*⁹³. In leukaemia-bearing mice, treatment with resveratrol prolonged their life span and this was associated with decreased levels of pSTAT3 in liver tissue lysates⁹⁴. The resveratrol derivative LYR71 suppressed breast cancer cell viability (IC₅₀ value of 20 µM), and inhibited STAT3-mediated MMP9 expression⁹⁵. Reports also showed that the inhibition of the JAK–STAT3 pathway by resveratrol or its analogue, piceatannol (3,3',4,4'-trihydroxystilbene), decreased BCL-X_L and BCL-2 expression and sensitized lung carcinoma, multiple myeloma, prostate and pancreatic cancer and the glioblastoma multiforme patient-derived CD133-positive cells to radiation or chemotherapy *in vitro*^{96–98}. A combination of resveratrol and 5-fluorouracil induced synergistic cytotoxicity in human colon cancer cells, which was accompanied by decreased STAT3 activity⁹⁹. Despite these

observations, the mechanisms for the inhibition of STAT3 signalling by resveratrol or its analogues remain poorly understood.

Caffeic acid, a natural phenolic acid compound present in fruits, wine and coffee¹⁰⁰, and its synthetic derivative, CAPDE, were reported to inhibit tumour growth and angiogenesis in a renal carcinoma mouse xenograft model. Its effects are induced partly by suppressing active STAT3 and the expression of hypoxia-inducible factor 1 α (HIF1 α) and VEGF¹⁰¹. Another caffeic acid analogue, WP1193, inhibited STAT3 phosphorylation and blocked the growth of murine melanoma¹⁰² and human glioma xenograft tumours¹⁰³. However, the mechanistic details of the inhibition of STAT3 phosphorylation remain undefined.

Treatment with 100 μ M capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide), which is present in chilli peppers, inhibited cell proliferation and induced apoptosis of multiple myeloma cells by inhibiting both constitutively active STAT3 and inducible STAT3 expression¹⁰⁴. Apart from promoting gp130 depletion, the molecular mechanisms of how capsaicin modulates IL-6, gp130 and STAT3 signalling are not fully understood.

Studies also indicate that cryptotanshinone, the active component in the herbal plant *Salvia miltiorrhiza* Bunge (danshen), has a moderate inhibitory effect on STAT3 phosphorylation and its functions, possibly by binding to the STAT3 SH2 domain and interfering with dimerization, although this remains to be confirmed¹⁰⁵. Prostate cancer cells treated with cryptotanshinone showed decreased expression of known STAT3-regulated genes, including those that encode cyclin D1, BCL-X_L and survivin¹⁰⁵.

Two agents that target microtubules have been reported to inhibit STAT3 phosphorylation in breast cancer cells: vinorelbine and paclitaxel¹⁰⁶. Vinorelbine is a microtubule destabilizer and a semi-synthetic analogue of the vinca alkaloids isolated from the leaves of *Catharanthus roseus*. Paclitaxel is a microtubule stabilizer and was originally isolated from the bark of the yew tree. Both agents were reported to inhibit STAT3 phosphorylation in breast cancer cells; paclitaxel was shown to disrupt STAT3 interaction with tubulin¹⁰⁶. Although these findings are interesting, the mechanistic basis for the inhibition of STAT3 phosphorylation by these two agents is unclear.

Drug-sensitive and drug-resistant multiple myeloma cells treated with 2.5 μ M celastrol, a triterpene derived from the Chinese medicinal plant *Tripterygium wilfordii*, showed decreased expression of STAT3-regulated genes, diminished cell proliferation and enhanced sensitivity to chemotherapy¹⁰⁷. Celastrol also inhibited growth of human HCC tumour xenografts in mice¹⁰⁸. Although this agent blocked constitutive and inducible STAT3 activation and STAT3 nuclear translocation, the precise mechanism of action remains unknown.

Avicin D, a triterpenoid saponin that is present in the cactus plant *Acacia victoriae*, also decreased pSTAT3 activity and BCL-2 expression and induced apoptosis in both cutaneous T-cell lymphoma lines and CD4⁺ T cells isolated from patients with Sézary syndrome¹⁰⁹. However, very little is known about how avicin D inhibits STAT3 phosphorylation.

Studies further show that withaferin A, a triterpenoid derived from *Withania somnifera*, inhibits constitutive and IL-6-induced STAT3 activation in human breast cancer cell lines at low micromolar concentration¹¹⁰. Withaferin A also decreased STAT3 and JAK2 protein levels and JAK2 activity in renal carcinoma Caki cells¹¹¹. However, not much is understood of how withaferin suppresses the JAK–STAT3 signalling pathway.

The pentacyclic triterpene betulinic acid, which is isolated from the bark of the plant *Ziziphus mauritiana*, moderately inhibited both constitutive and inducible STAT3 activation, nuclear translocation and DNA-binding activity in multiple myeloma cells. It blocks JAK1, JAK2 and SRC activities and blocks the induction of the protein tyrosine phosphatase SHP1 (also known as PTPN6)¹¹². The inhibition of STAT3 activity by betulinic acid also sensitized multiple myeloma cells to the apoptotic effects of bortezomib and thalidomide¹¹².

Another pentacyclic triterpenoid, ursolic acid (3 β -hydroxyurs-12-en-28-oic-acid), a natural dietary component found in many fruits, moderately suppressed both constitutive and inducible STAT3 activation in prostate cancer and other tumour cells by inhibiting SRC, JAK1 and JAK2 activities^{113,114}. These effects led to decreased cyclin D1, BCL-2, survivin, MCL1 and VEGF expression¹¹³.

Oleanolic acid, found in *Ganoderma lucidum* and other plants, and its more potent derivative, CDDO-Me (active at 0.1 nM)¹¹⁵, inhibited the JAK–STAT3 pathway in multidrug-resistant ovarian cancer¹¹⁶ and in osteo-sarcoma cells¹¹⁷. CDDO-Me also induced antitumour responses *in vivo* in an aggressive oestrogen receptor-negative breast cancer model by modulating EGFR and STAT3 activities¹¹⁸. Details of the mechanism of inhibition of the EGFR–STAT3 or JAK–STAT3 signalling by CDDO-Me are limited.

Cucurbitacin agents isolated from Cucurbitaceae, Cruciferae (Brassicaceae) and other plant families have been identified as modulators of the JAK–STAT3 pathway. Cucurbitacin I (JSI-124) inhibited JAK–STAT3 signalling (IC₅₀ value of 500 nM) and suppressed the growth *in vivo* of human and mouse tumours harbouring aberrantly active STAT3 (REF. 119). Treatment with cucurbitacin I blocked cell proliferation and enhanced the radiation sensitivity of CD133⁺ cancer stem cells isolated from patients with NSCLC¹²⁰. Cucurbitacin I also suppressed growth of medulloblastoma¹²¹ and growth of thyroid cancer cells *in vitro* and *in vivo* in xenograft models¹²². Low micro-molar concentrations of cucurbitacin B in combination with cisplatin induced strong apoptotic effects in laryngeal squamous carcinoma cells by inhibiting the STAT3 pathway¹²³. Reports also show that cucurbitacin E blocked VEGFR2-mediated JAK2–STAT3 activation in human umbilical vein endothelial cells and suppressed angiogenesis in human prostate tumour xenograft models¹²⁴. This compound also induced viability loss and apoptosis of pancreatic¹²⁵ and bladder cancer cells *in vitro*¹²⁶ partly through inhibition of STAT3 phosphorylation.

Diosgenin, a steroidal saponin present in a number of plants, inhibited both constitutive and inducible STAT3 signalling in HCC cells, decreased cell proliferation, and induced chemosensitization by mechanisms that are unclear, but are likely to involve the induction of the protein tyrosine phosphatase SHPTP2, suppression of tyrosine kinases and/or blockade of STAT3 nuclear translocation¹²⁷.

Studies in multiple myeloma cells show that emodin, which is derived from the root and rhizome of *Rheum palmatum*, moderately blocked JAK2 and STAT3 activation and inhibited cell viability¹²⁸. Thymoquinone, the active ingredient from the volatile oil of black seed (*Nigella sativa*), blocked SRC and JAK2 activation, inhibited IL-6-induced and constitutive STAT3 activity, downregulated the expression of STAT3 target genes, and suppressed the viability of multiple myeloma cells with IC₅₀ values of 8.5–10 μ M^{129,130}.

Furthermore, reports show that honokiol, isolated from the bark of *Magnolia officinalis*, moderately or weakly inhibited STAT3 activation in HNSCC, HCC and gastric cancer cells by undefined mechanisms, but probably involve the reduction of EGFR levels and the elevation of SHP1 expression^{131–133}.

Similarly, evodiamine, an alkaloid isolated from *Evodia rutaecarpa*, inhibited STAT3 signalling in HCC by blocking JAK activity and inducing SHP1 phosphatase¹³⁴, and it induced antitumour responses *in vivo* in a HCC xenograft model¹³⁴.

Carbazole, the active compound of coal tar and its *N*-alkyl derivatives, decreased IL-6-stimulated STAT3 activation and DNA-binding activity in embryonic kidney or human monocytic leukaemia cells through undefined mechanisms, without affecting phosphorylation of STAT3 (REFS 135,136).

A clinically used drug, sanguarine, a benzophenanthridine alkaloid extracted primarily from the bloodroot plant, was recently shown to inhibit constitutive STAT3 activation through mechanisms that are currently unclear, but might contribute to its suppressive action on cell proliferation, migration and invasion of prostate tumour cells¹³⁷.

A member of the vitamin E superfamily, γ -tocotrienol and acetyl-11-keto- β -boswellic acid, the active compound isolated from the Indian *Boswellia serrate* plant, both reportedly inhibited constitutive or inducible STAT3 signalling in HCC and multiple myeloma cells through the blockade of tyrosine kinases and the induction of SHP1 phosphatase activity^{138,139}. These effects led to a decreased expression in cyclin D1, BCL-2, MCL1 and VEGF, inhibition of cell proliferation, and induction of apoptosis^{138–140}. Furthermore, 3,3'-diindolylmethane, an indole compound found in cruciferous vegetables, inhibited the JAK–STAT pathway through a yet undefined mechanism, which was partly responsible for the antitumour effects and the enhanced cisplatin sensitivity in an ovarian cancer model¹⁴¹. Brevilin A, a novel natural product isolated from *Litsea glutinosa*, inhibited the JAK JH1 (Janus homology 1) domain and attenuated STAT3 activation in prostate and breast cancer cells¹⁴².

Inhibitors of STAT1 and STAT4

Few inhibitors of the STAT1 or STAT4 signalling pathway have been reported in the literature (TABLE 1). Most STAT1 and STAT4 inhibitors are natural products except for pravastatin, a synthetic small-molecule inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which reportedly modulated IFN γ activity and IFN γ -mediated STAT1-dependent events^{143,144}. In studies of apolipoprotein E-deficient mice fed on a cholesterol-rich diet, pravastatin treatment suppressed IFN γ levels in both serum and

atherosclerotic lesions, which was associated with decreased STAT1 activation, reduced IFN regulating factor 1 (IRF1) expression, and induced activity of SOCS1 in aorta tissue¹⁴⁴. Although the mechanistic details are limited, these findings suggest a potential application of pravastatin in diseases in which STAT1 signalling is implicated¹⁴⁴.

Studies also identified a series of phosphopeptide mimetics of STAT1, including ISS-840, which directly inhibited STAT1 signalling with moderate potency (IC₅₀ value of 31 μ M) through disruption of dimerization⁵⁸. Furthermore, studies showed that an alkaloid isolated from *Berberis vulgaris*, berbamine, could modulate STAT1 signalling. In the EAE multiple sclerosis model, treatment with berbamine decreased the severity of the disease partly by suppressing IFN γ production and activity¹⁴⁵.

Given the key role of STAT1 in the development of T_H1 cells, which are implicated in the onset of EAE, compared with T_H2 cells that promote recovery from the disease, the inhibition of IFN γ -dependent STAT1 activation could be used as a means to normalize the T_H1–T_H2 balance and promote recovery from the EAE phenotype^{145,146}.

STAT4 protein levels also decreased in response to berbamine treatment, with little change in tyrosine (Y693) phosphorylation¹⁴⁵. Studies also showed that the oral administration of water extract of cinnamon bark decreased IFN γ expression and inhibited STAT4 activation in activated murine T cells¹⁴⁷.

The potential to modulate STAT1 or STAT4 and therefore modulation of T-cell functions suggests that berbamine and water extract of cinnamon bark could be used as therapeutics in inflammatory disorders¹⁴⁷. Details of the mechanisms by which these two agents modulate IFN γ function and STAT1 or STAT4 signalling and their overall effects on the immune system, however, are unclear.

It is also reported that treatment with resveratrol (40–60 μ M) inhibited both JAK1 phosphorylation and STAT1 activation in human epidermoid carcinoma cells¹⁴⁸; the mechanism of action of resveratrol in this scenario is currently undefined.

Inhibitors of STAT5

Few STAT5 inhibitors have been reported. Pimozide, a diphenylbutylpiperidine derivative that is approved by the US Food and Drug Administration (FDA) as a neuroleptic drug, was shown to inhibit STAT5 activation with moderate activity⁴¹. In CML cells harbouring constitutively active STAT5, treatment with 5 μ M pimozide suppressed pSTAT5 activity and induced apoptosis⁴¹. The mechanism of inhibition of STAT5 phosphorylation, however, is unclear but is suggested to be independent of dopamine D₂ receptor activity⁴¹, and proceeds through the modulation of the negative regulators of STAT5 signalling⁴⁵. Owing to its perceived inhibitory effect on STAT5 activity, it has been suggested that pimozide could be combined with TKIs, such as imatinib or dasatinib, as an approach to reduce the incidence of drug resistance in patients with CML⁴¹.

A non-peptidic chromone-based nicotinyl hydrazine, discovered through a screen of chemical libraries, was shown to weakly inhibit STAT5 activity (IC₅₀ of 47 ± 17 μ M)¹⁴⁹.

This agent selectively inhibited the phosphorylation of STAT5 in lymphoma cell lines by an undefined mechanism¹⁴⁹. Inhibition of STAT5 activity is also reported for indirubin derivatives, including E804, which blocked pTyr-STAT5 and STAT5 DNA-binding activity in BCR-ABL-positive CML cells at 5 μ M¹⁵⁰. This effect was associated with the downregulation of MCL1 and BCL-X_L expression¹⁵⁰. The mechanism for the inhibition of STAT5 signalling is likely to be suppression of tyrosine kinase activities.

Inhibitors of STAT6

Reports of inhibitors of STAT6 signalling are minimal (TABLE 1). The membrane-permeable version of the peptide STAT6BP, which was derived from the STAT6-binding region of IL-4R α , inhibited IL-4-dependent STAT6 phosphorylation and transcriptional activity¹⁵¹. A recent report also showed that a chimeric STAT6-inhibitory peptide, STAT6-IP, blocked the T_H2 cytokines IL-4 and IL-13, and chemokine production. The intranasal delivery of STAT6-IP inhibited the ovalbumin-induced lung inflammation, mucus production and eosinophil accumulation in the lungs¹⁵², while its topical application in an *in vivo* model of chronic ragweed-induced asthma suppressed the production of T_H2 cytokines¹⁵³.

Treatment of the human B lymphoma cell line DND39 with TMC-264, an agent identified from the fermentation broth of *Phoma* spp., inhibited IL-4-dependent tyrosine phosphorylation and activation of STAT6 (REF. 154). At low micromolar concentrations (1.6 μ M), TMC-264 selectively inhibited the phosphorylation of STAT6 over that of STAT5 or STAT1, and it also blocked STAT6 DNA-binding activity in a dose-dependent manner¹⁵⁴. Its derivative, AS1517499, showed a significantly improved potency against STAT6 activity (IC₅₀ of 21 nM) and inhibited IL-13-induced STAT6 phosphorylation⁵¹.

Other notable inhibitors of STAT6 signalling include YM-341619, a derivative of 4-aminopyrimidine-5-carboxamide (IC₅₀ value of 0.70 nM)¹⁵⁵, and AS1810722, a fused bicyclic pyrimidine derivative (IC₅₀ value of 2.4 nM)¹⁵⁶. Both AS1810722 and YM-341619 are potent, orally active STAT6 inhibitors that could be developed for the treatment of STAT6-dependent allergic diseases, such as asthma.

Leflunomide, an isoxazol derivative, *N*-(4-trifluoro-methylphenyl)-5-methylisoxazol-4-carboxamide, was reported to inhibit IL-13-induced STAT6 phosphorylation in human bronchial smooth muscle cells, but with a relatively weak potency (250 μ M)⁵⁰. The mechanisms are currently unknown and may include the inhibition of protein tyrosine kinases⁵¹.

Studies also indicate that the calcium-activated chloride channel blocker, niflumic acid, at 100 μ M, inhibited JAK2 and STAT6 activation in the transformed human airway epithelial cell line BEAS-2B, and suppressed the pulmonary effects of IL-13 (REF. 157). While these findings suggest that niflumic acid could have potential in controlling IL-13-induced asthma, details of the mechanisms of action remain to be defined¹⁵⁷.

Furthermore, berbamine reportedly inhibited STAT6 activation, as well as STAT1 and STAT4, implicating STAT6 in its pharmacological effect on T_H2 cell development¹⁴⁵.

Finally, (*R*)-76 and its synthetic derivative, (*R*)-84, were identified through a chemical library screen and shown to bind and block STAT6 tyrosine phosphorylation, without competing at the phosphopeptide binding site of the SH2 domain¹⁵⁸. These two small molecules also inhibited eotaxin-3 secretion in the bronchial epithelial cell line BEAS-2B¹⁵⁸.

TKIs as modulators of STAT proteins

Tyrosine kinases have long been attractive targets for therapeutic development owing to their importance in promoting many cellular processes and hence, their involvement in human diseases. Occasions in which the abnormal activation of tyrosine kinases has been implicated in human diseases include the gain-of-function dominant mutation JAK2^{V617F} identified in patients with myeloproliferative neoplasia^{159,160} and the BCR–ABL mutation that drives CML⁴³. It is important to recognize that STAT activities could be involved in tyrosine kinase-associated diseases. Thus, the modulation of STAT function could be part of the underlying mechanisms for the therapeutic responses to TKIs, as long as dysregulated STAT signalling is evident downstream of the tyrosine kinase (FIG. 3b; TABLE 1). Several TKIs have been developed and approved for the treatment of various cancers, and these are predominantly small molecules that directly target the kinases or antibody-based therapeutics that compete for binding to the respective growth factor receptors on the cell surface. A few of these TKIs are presented here as examples.

Imatinib and dasatinib are small molecules that inhibit BCR–ABL, SRC and other tyrosine kinases and are approved for the treatment of CML¹⁶¹, gastrointestinal stromal tumour¹⁶², and other leukaemias including Philadelphia chromosome-positive acute lymphoblastic leukaemia, and myelodysplastic and myeloproliferative disorders¹⁶³. Both imatinib and dasatinib inhibit STAT5 signalling^{164,165}.

Similarly, gefitinib and erlotinib are small-molecule EGFR inhibitors approved for treating patients with advanced NSCLC with EGFR overexpression¹⁶⁶ or for treating patients with metastatic NSCLC and pancreatic cancer. Both agents inhibit STAT signalling. Erlotinib moderately suppresses STAT3 expression levels and the formation of malignant lesions in a 4-nitroquinoline-1-oxide-induced mouse model of oral cancer¹⁶⁷. Furthermore, γ -tocotrienol, in combination with erlotinib or gefitinib, reportedly inhibited STAT5 signalling in mouse mammary tumour cells¹⁶⁸.

The modulation of STAT3 signalling may be part of the underlying mechanisms for the survival benefits induced by the multiple kinase inhibitor sorafenib in patients with late-stage hepatocellular carcinoma (HCC) in Phase III clinical trials^{169,170}. This conclusion is supported by the observation that HCC cells overexpressing mutant EGFR variant III (EGFRvIII) had a diminished response to sorafenib, whereas the combination of sorafenib and the EGFRvIII-specific monoclonal antibody CH12 enhanced the antitumour effect. The effect of sorafenib occurred partly by inhibiting multiple factors including STAT3, extracellular signal-regulated kinase (ERK), and phosphoinositide 3-kinase–AKT pathways¹⁷¹. Furthermore, sorafenib inhibited proliferation and induced apoptosis in glioblastoma cell lines and in primary cultures of cells from patients with grade IV

glioblastoma, which were associated with the suppression of STAT3 signalling and the downregulation in expression of cyclin D, cyclin E and MCL1 proteins¹⁷². Moreover, treatment with sorafenib inhibited both JAK2 and STAT3 phosphorylation and the growth of human neuroblastoma tumours in a mouse xenograft model¹⁷³. Studies of sorafenib analogues, including SC-1 and SC-49, further confirmed the inhibitory effects on STAT3 phosphorylation in HCC and liver adenocarcinoma *in vitro* and in tumour models *in vivo*^{174,175}. Notably, the inhibitory action of the analogues against STAT3 phosphorylation was observed to be independent of their effects on kinases, and instead mediated through the induction of SHP1 phosphatase^{174,175}. Specifically, SC-1 and SC-1 analogues that are structurally designed to preclude the binding to and the inhibition of kinases, were able to block STAT3 phosphorylation through the induction of SHP1 (REFS 174–176).

Tetracyclic pyridone 2-*tert*-butyl-9-fluoro-3,6-dihydro-7*H*-benz[*h*]-imidaz[4,5-*f*]isoquinoline-7-one (P6), a reversible ATP inhibitor, decreased JAK2 phosphorylation and selectively inhibited the viability of murine and human myeloma cell lines harbouring activated STAT3 and JAKs¹⁷⁷. Nifuroxazide, an oral nitrofurantoin antibiotic commonly used as an anti-diarrhoeal agent, was identified from the proprietary Prestwick chemical library, which was screened for agents that inhibit the activation of JAK2, TYK2 and STAT3 and decrease cell viability in multiple myeloma cells¹⁷⁸.

Antibody-based tyrosine kinase-inhibiting therapeutics include cetuximab, which binds to the extracellular EGFR and is approved for certain types of HNSCC or colorectal cancers¹⁷⁹. Cetuximab inhibited EGFR phosphorylation and the downstream signalling events, including STAT3 activation, in gastric and NSCLC cells¹⁸⁰. Trastuzumab, which binds to EGFR2 (also known as HER2) and is approved to treat HER2-positive cancers, such as breast, gastric and gastroesophageal junction cancers¹⁸¹, inhibited the growth of gastric and breast cancer cell lines, decreased HER2 and STAT3 phosphorylation, and induced a synergistic response in combination with cisplatin¹⁸².

Several JAK inhibitors are also in clinical trials against various diseases, some of which are discussed here. Notable ones include the JAK inhibitor tofacitinib, which showed significant efficacy in rheumatoid arthritis and is FDA-approved for this indication¹⁸³. Through the inhibition of JAKs, tofacitinib abrogated anti-CD3-induced IFN γ , IL-4 and IL-17 production in CD4⁺ T cells isolated from the peripheral blood of healthy volunteers¹⁸³. Tofacitinib was also shown to inhibit STAT1, STAT3, STAT4, STAT5 and STAT6 activation in cultured anti-CD3-stimulated T cells, which suggests that targeting the cytokine signalling pathway with TKIs may be a consideration for immunosuppression¹⁸³. Furthermore, the FDA-approved drug ruxolitinib, a potent inhibitor of JAK1 (IC₅₀ value of 3.3 nM) and JAK2 (IC₅₀ value 2.8 nM), blocked STAT3 and STAT5 activation in a human erythroleukemia cell line (HEL) expressing JAK2^{V617F} (REF. 184). In a NSCLC model, this agent inhibited STAT3 activity without affecting cell proliferation and viability, and inhibited growth in soft agar and xenografts *in vivo*¹⁸⁵. Adult patients with T-cell leukaemia are currently being recruited into a Phase II clinical trial (Clinical trials.gov identifier: NCT01712659) to examine the safety and effectiveness of this agent.

Additionally, the pyrazolyl pyrimidine AZD1480, which potently inhibits JAK1 (IC₅₀ value of 1.3 nM) and JAK2 (IC₅₀ value of 0.4 nM)¹⁸⁶, differentially suppressed STAT1, STAT3, STAT5 and STAT6 activation at 0.1–5 µM concentration in prostate, ovarian, glioma, and breast cancer cells *in vitro*^{187,188}. AZD1480 also inhibited prostate and ovarian tumour growth *in vivo*¹⁸⁷, and, at the highest concentration (5 µM), inhibited the proliferation *in vitro* of Hodgkin's lymphoma cells harbouring activated JAK by mechanisms that include effects on other targets¹⁸⁶. Other studies showed that AZD1480 suppressed STAT3 activation in human and murine kidney carcinoma cell lines¹⁸⁹ and in myeloid-derived suppressor cells in a murine renal carcinoma model, supporting the notion that targeting STAT3 could be used to control the tumour microenvironment¹⁸⁹.

The cationic amphiphilic compound atiprimod (SK&F 106615) decreased JAK2 and pJAK2 levels in K562 cells¹⁹⁰, inhibited STAT3 and STAT5 phosphorylation, and induced antitumour cell effects in multiple myeloma cell models¹⁹¹. In a model of essential thrombocythaemia harbouring an active JAK2 mutation, atiprimod induced potent antiproliferative and pro-apoptotic effects, which were associated with decreased STAT3 and STAT5 phosphorylation¹⁶⁰. Moreover, the gold complex auranofin, which is currently undergoing Phase II clinical trials in chronic lymphocytic leukaemia (CLL) and ovarian and lung cancers (Clinical trials.gov identifiers: NCT01419691, NCT01747798 and NCT01737502, respectively), is reported to inhibit IL-6-induced phosphorylation of JAK1 and STAT3, leading to decreased MCL1 expression, caspase 3 activation, and the induction of apoptosis in multiple myeloma cells^{192,193}. Although it inhibited JAK1 activity in *in vitro* kinase assays, the exact mechanism of auranofin-induced modulation of JAK–STAT3 signalling remains unclear¹⁹².

Notably, treatment with TKIs does not always lead to a suppressive response on STAT signalling. This may be partly due to the presence of compensatory mechanisms, whereby non-targeted tyrosine kinases can induce activation of STAT proteins. This is the case for lung cancer cells treated with the SRC family kinase inhibitor dasatinib, which showed no observable effect on STAT3 activation¹⁹⁴, possibly due to the induction by JAKs and other tyrosine kinases¹⁹⁵. Overall, STAT signalling modulation could be incorporated into studies that evaluate the underlying events for the responses to TKIs.

Conclusion and clinical perspectives

Although there is strong evidence supporting the role of STAT proteins in human diseases to justify the discovery of novel therapeutics against this family, there are no STAT-inhibiting modalities in clinical application, with the exception of TKIs. Even with the TKIs, their clinical applications are not specifically directed towards providing insights into the potential therapeutic benefits of targeting STAT proteins. It is only upon the availability of suitable small-molecule STAT inhibitors or other effective modalities as drugs that the clinical benefits of inhibiting STAT signalling can be realized.

With regard to STAT3, the public literature is full of reports of agents that modulate this pathway, although many of them have suboptimum potency and pharmacokinetic parameters, and/or poorly defined mechanisms. More recently reported small-molecule

inhibitors of STAT3, including S3I-201.1066 and BP-1-102, have shown good *in vitro* activities and *in vivo* efficacies in preclinical models^{67,68}, with the expectation that clinical candidates of direct STAT3 inhibitors could be forthcoming. Furthermore, it is particularly noteworthy that many of the reported inhibitors of STAT3 signalling have been identified from natural product sources, a pattern that parallels the trend that many of the existing drugs in clinical application trace certain aspects of their origin to natural products. Indeed, the large chemical diversity presented by natural products favours the chances of discovering active leads targeting the JAK–STAT signalling pathway. Yet, there are only isolated cases of research activity that currently tap into natural products as potential sources for discovering JAK–STAT inhibitors and these studies largely focus on targeting STAT3 signalling. These research efforts will need to be intensified to take full advantage of the potential that natural products offer in representing a promising source of drug leads.

So far, the only reported clinical trial of direct modulators of STAT signalling is the recent Phase 0 study of STAT3 ODN decoys for safety and pharmacodynamic monitoring²⁸, the results of which are encouraging and indicate minimal toxicity in patients with HNSCC¹⁹⁶. There is also a Phase I clinical trial that is presently recruiting patients with advanced cancers which are linked to STAT3, to evaluate the safety and clinical activity of the ISIS-STAT3Rx antisense oligonucleotide as a STAT3 inhibitor (ClinicalTrials.gov identifier: NCT01563302). Data from this trial should shed more light on the clinical and therapeutic significance of the inhibition of STAT3 function in cancer patients.

In the absence of clinical trials of direct small-molecule STAT inhibitors, the potential to safely modulate aberrant STAT signalling in human cancers can be evaluated based on the therapeutic use of TKIs. There are many TKIs that are in clinical application against human cancers, a few of which have already been discussed above. Other cases include an ongoing Phase II study in patients with CLL of dasatinib based on the strong *in vitro* cytotoxic effects observed following the pretreatment of primary CLL cells with this agent (ClinicalTrials.gov identifier: NCT01441882). Young patients with newly diagnosed glioma are also being recruited into a Phase I/II trial to determine the dose-limiting toxicity of erlotinib administered together with radiation therapy (ClinicalTrials.gov identifier: NCT00124657). The direct effect of the above-mentioned drugs on the activity of STAT3 or other STAT proteins is not one of the intended goals of these trials. However, the antitumour responses are likely to be a cumulative effect on their respective targets and against STAT (or STAT3) signalling, assuming the latter is aberrant as a consequence of the hyperactive tyrosine kinase that represents the target for the drug under evaluation.

Furthermore, a few natural products are presently undergoing clinical trials or have been approved for clinical applications that also modulate STAT signalling among others. The effect of curcumin on pancreatic cancer is being evaluated in a Phase II trial (ClinicalTrials.gov identifier: NCT00094445). The tolerability and pharmacodynamic properties of resveratrol are also being studied in patients with colorectal cancer (ClinicalTrials.gov identifier: NCT00433576). The effectiveness of 3,3'-diindolylmethane in the treatment of breast cancer is being studied in a Phase II/III study (ClinicalTrials.gov identifier: NCT01391689).

An ongoing pilot clinical study is also evaluating the tumour sensitivity of patients with melanoma to recombinant IFN α 2b by determining the cellular levels and the activation status of STAT1 (ClinicalTrials.gov identifier: NCT01460875). There is also a Phase II study on the pharmacodynamic effects of the TKI AZD0530 on SRC and downstream STAT3 and STAT5 activation in patients with metastatic HNSCC (ClinicalTrials.gov identifier: NCT00513435), while a Phase I clinical trial will determine the maximum-tolerated dose and the pharmacokinetic profile of the multi-kinase inhibitor AT9283 in young patients with relapsed or refractory acute leukaemia, together with evaluating phosphorylated STAT5 as a means for monitoring the kinase inhibitory effects *ex vivo* and *in vivo* (ClinicalTrials.gov identifier: NCT01431664). Furthermore, an observational clinical study is ongoing to assess the activation status of STAT1, STAT3, STAT5A, STAT5B and STAT6 in leukocytes isolated from patients with rheumatoid arthritis treated with tocilizumab, a humanized IL-6R-specific monoclonal antibody (ClinicalTrials.gov identifier: NCT01633346).

The clinical benefits of exploiting dysregulated STAT signalling in human diseases remain to be harnessed, and the expectation is that clinical candidates of direct STAT-inhibitory modalities are forthcoming in the near future.

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Biographies

Gabriella Miklossy

Gabriella Miklossy obtained her Ph.D. degree in biomedical sciences at the University of Debrecen, Hungary. She was interested in designing dominant negative HIV-1 protease inhibitors and testing their activity using a novel high-throughput microtitre-plate fluorescent assay. She pursued her postdoctoral fellowship at the State University of New York, Upstate Medical University, Syracuse, New York, USA, where she studied the biochemistry of transaldolase and HRES1/RAB4 in the pathophysiology of systemic lupus erythematosus. She subsequently joined the laboratory of James Turkson at the University of Hawaii Cancer Center, Honolulu, USA, to pursue research on discovering natural product-based novel anticancer drugs targeting STAT3 signalling.

Tyvette S. Hilliard

Tyvette Hilliard received her Ph.D. degree in medicinal chemistry from the University of Illinois at Chicago, USA. During her pre-doctoral studies, she assisted in the development of a three-dimensional (3D) alginate organ culture system to study ovarian cancer. She investigated the role of the gonadotropins on normal ovarian surface epithelium (OSE) proliferation using the 3D system. This novel system allowed her to identify several oncogenic pathways activated in the OSE by the gonadotropins. She is presently a postdoctoral fellow in the Natural Products and Experimental Therapeutics Program in the

laboratory of James Turkson pursuing the evaluation of marine natural products for novel therapeutics for cancers harbouring aberrant STAT3 signalling.

James Turkson

James Turkson obtained his Ph.D. degree in pharmacology from the University of Alberta, Edmonton, Alberta, Canada, where he studied the therapeutic significance of targeting protein kinase C to platelet and neutrophil functions and to nucleoside transport mechanisms in leukaemia. He pursued his postdoctoral fellowship training in molecular oncology with Richard Jove at the Moffitt Cancer Center (MCC), University of South Florida, Tampa, USA, where he studied the mechanisms of STAT3-mediated oncogenesis. He transitioned to join the faculty at MCC and pursued drug discovery research in collaboration with Richard Jove, Said Sebt and Andrew Hamilton focusing on targeting STAT3 for the discovery of small-molecule inhibitors as novel anti-cancer drugs. This research led to the identification of the first direct inhibitor of a STAT protein, which was a small peptide that disrupted STAT3 dimerization, signalling and functions. He then moved to the Burnett School of Biomedical Sciences at the University of Central Florida in Orlando, USA, and maintained a rigorous drug discovery research programme targeting STAT3. He subsequently relocated to the University of Hawaii Cancer Center, University of Hawaii, Honolulu, USA, where he is currently Professor and the Program Director of the Natural Products and Experimental Therapeutics Program. His current research interests include targeting STAT3 and other cancer-relevant signalling molecules for anticancer drug discovery, natural products, and drug resistance in ovarian and pancreatic cancers.

Key points

- Considerable evidence supports the crucial roles of the signal transducer and activator of transcription (STAT) family of proteins in human diseases, particularly in immune and inflammatory disorders, infection and cancer
- Increasing emphasis is being placed on developing direct STAT inhibitors for clinical application, mainly through the discovery of small molecules, oligonucleotides and natural product derivatives.
- A large part of the ongoing STATs drug discovery research for therapeutics is focused on targeting STAT3, of which the efforts to develop small-molecule STAT3 inhibitors is extensive. While research into oligodeoxynucleotide (ODN) decoys and antisense oligonucleotides (ASOs) as STAT-inhibitory approaches is not as widespread, these efforts appear to be advancing as a STAT3 ODN has progressed to clinical trials (Phase 0).
- Tyrosine kinase inhibitors (TKIs) as therapeutic modalities are widely explored, and this approach is highly established. TKI agents may be therapeutic considerations in STAT-associated diseases in so far as a causal link could be established between the target tyrosine kinase and dysregulated STAT signalling that is prevalent in the disease.
- The precedence of natural product-based therapeutics for many diseases and the number of reports on natural product inhibitors of STAT3 signalling together highlight the potential of this resource as an important source of leads for developing STAT inhibitors.
- There is a clinical trial to evaluate ISIS-STAT3Rx ASO against advanced cancers. Other clinical trials are focusing on therapeutic modalities that can affect STAT function and STAT-associated diseases, including the evaluation of curcumin for pancreatic cancer, resveratrol for colorectal cancer and 3,3'-diindolylmethane for breast cancer, and TKIs against many cancer types.

Figure 1. A schematic representation of the structures of the STAT proteins

Linear representations of the domain structures of the seven members of the signal transducer and activator of transcription (STAT) protein family: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6. The transactivation domain contains a crucial tyrosine (Y) residue, the phosphorylation of which initiates STAT activation and dimerization between two monomers through a reciprocal phospho-Tyr-SH2 domain interaction. The serine (S) residue present in the transactivation domain of certain STAT proteins is thought to enhance transcriptional activity when it is phosphorylated.

Figure 2. STAT signalling pathway, functions and associated diseases
Activation of signal transducer and activator of transcription (STAT) is promoted when a ligand (L) binds its receptor (R). The ligand–receptor interaction induces receptor phosphorylation (P), which recruits the STAT proteins to the phospho-motifs of the receptor. Phosphorylation of the critical tyrosine residue in the STAT protein is then initiated by tyrosine kinases, such as growth factor receptors, Janus kinases (JAKs) and SRC family kinases. Two phosphorylated STAT monomers dimerize through reciprocal pTyr-SH2 domain interactions, and the STAT–STAT dimers translocate to the nucleus where they bind to specific STAT-response elements in the target gene promoters and regulate transcription. The STAT-dependent induction of genes is essential for many physiological functions. The activation of normal STAT signalling is controlled by physiological negative modulators, such as suppressors of cytokine signalling (SOCS) and protein tyrosine phosphatases (PTPs), in accordance with normal cellular functions. Although the STAT proteins (STAT1 to STAT6) are differentially activated and promote varying cellular processes depending on the ligand and the context, collectively, their normal induction regulates cell growth, differentiation, survival and apoptosis. Their normal induction also regulates inflammatory and immune responses, embryonic development and mammary gland development. By contrast, defective or abnormal STAT signalling is associated with various human diseases, including susceptibility to infection, immune disorders, many types

of cancer, asthma and allergic diseases. IFN, interferon; IL-2R α , interleukin-2 receptor- α ; T_H, T helper.

Figure 3. Models of inhibition of STAT signalling

a | Small-molecule dimerization disruptors (SMDDs) or phospho-peptidomimetic inhibitors (PPMIs) target the phospho-Tyr-SH2 domain interaction at the interface of dimers of signal transducer and activator of transcription (STAT) proteins. This leads to the disruption of STAT-STAT dimers and the formation of STAT-SMDD or STAT-PPMI heterocomplexes, leading to a suppression of STAT signalling and function. **b** | The binding of ligands, such as growth factors and cytokines, to their cognate receptors on the cell surface induces STAT tyrosine phosphorylation and activation, and this process is competitively inhibited by antibody-based therapeutics. Tyrosine kinases that mediate STAT phosphorylation, leading to STAT-STAT dimer formation, are the targets of small-molecule tyrosine kinase inhibitors. The overall effect of these modulators is to block the induction of STAT phosphorylation and signalling. **c** | Decoy oligodeoxynucleotides (ODNs) compete against the DNA-response elements in the target gene promoters for the binding of the active STAT-STAT dimers. The interaction of ODNs with STAT-STAT dimers precludes the binding of the dimers to target gene promoters, thereby preventing the induction of STAT-responsive genes. **d** | The induction of protein tyrosine phosphatases has been noted to occur in response to certain STAT inhibitors, including sorafenib analogues and natural products.

This promotes the dephosphorylation of the phospho-STAT proteins leading to the suppression of their signalling and function.

Table 1

STAT signalling modulators, their targets and effects, and clinical indications

Molecule	Target	Effect	Indication (clinical trial phase)	Refs
<i>Peptide and peptidomimetics</i>				
PY*LKTK	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	30
Ac-pTyr-Leu-Pro-Gln-Thr-Val-NH ₂	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	56,59
ISS-610	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	7,30,55,58
PM-73G	STAT3 SH2 domain	Inhibits phosphorylation of STAT3	Not applicable	62,63
CJ-1383	STAT3 SH2 domain	Inhibits phosphorylation of STAT3	Not applicable	61
ISS-840	STAT1 SH2 domain	Inhibits STAT1–STAT1 dimerization	Not applicable	58
<i>Non-peptide small molecules</i>				
S3I-M2001	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	27,55
STA-21	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Psoriasis (Phase I/II)	64
Catechol moiety	STAT3 SH2 domain	Inhibits STAT3 DNA binding	Not applicable	66
Stattic	STAT3 SH2 domain	Inhibits phosphorylation of STAT3	Not applicable	72
LLL12	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	83,84
FLLL32	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	80
S3I-201	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	26
BP-1-102	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	68
S3I-201.1066	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Not applicable	67
Pravastatin	STAT1	Inhibits phosphorylation of STAT1, decreases levels of IFN γ	Schizophrenia (Phase IV), pre-eclampsia (Phase I), hyperlipidaemia (Phase I/II/III/IV), cirrhosis (Phase II/III), gastroesophageal cancer (Phase IV), myeloid leukaemia (Phase I/II), pneumonia (Phase 0)	143,144
Pimozide	STAT5	Inhibits phosphorylation of STAT5	Schizophrenia (Phase II/IV), Tourette's syndrome (Phase II), psychosis (Phase III), post-operative nausea (phase not provided)	41
Chromone nicotinyI hydrazone	STAT5 DNA-binding domain	Inhibits phosphorylation of STAT5	Not applicable	149
AS1517499 ⁵¹	STAT6	Inhibits phosphorylation of STAT6	Not applicable	51
Leflunomide	Various protein kinases	Inhibits phosphorylation of STAT6	Rheumatoid arthritis (Phase I/II/III/IV), lupus nephritis (Phase II/III), uveitis (Phase II), melanoma (Phase I/II), prostate cancer (Phase II/III), glioblastoma multiforme (Phase III)	50
Niflumic acid	JAK2, STAT6	Inhibits phosphorylation of STAT6	Not applicable	157
YM-341619	STAT6, IL-4	Inhibits phosphorylation of STAT6	Not applicable	155

Molecule	Target	Effect	Indication (clinical trial phase)	Refs
AS1810722	STAT6, IL-4	Inhibits phosphorylation of STAT6	Not applicable	156
<i>Natural products</i>				
Capsaicin	STAT3, gp130	Inhibits STAT3 expression, depletes gp130	Chronic obstructive pulmonary disease (Phase 0/I/II), psoriasis (Phase IV), chronic neck pain (Phase II), rhinitis (Phase I/II/IV), pulmonary hypertension (Phase II), HIV infections (Phase II/III), peripheral nervous system diseases (Phase II/III), migraine (Phase I), burning mouth syndrome (Phase 0)	104
Curcumin	JAK1, JAK2, JAK3, STAT3	Inhibits phosphorylation of STAT3	Pancreatic cancer (Phase II/III), colon cancer (Phase I/ II/ III), breast cancer (Phase II), head and neck cancer (Phase 0), osteosarcoma (Phase I/II), multiple myeloma (Phase II), atopic asthma (phase not provided), dermatitis (Phase II/ III), type 2 diabetes (Phase IV), schizophrenia (Phase I/II), Alzheimer's disease (Phase I/II), multiple sclerosis (Phase II), rheumatoid arthritis (Phase 0)	78,79
Avicin D	JAK1, JAK2, STAT3	Inhibits phosphorylation of STAT3	Not applicable	109
Resveratrol	JAK1, STAT3	Inhibits phosphorylation of STAT3	Colorectal cancer (Phase I), follicular lymphoma (Phase II), cardiovascular diseases (Phase I/II), type 2 diabetes (Phase I/II/III), obesity (Phase II), Alzheimer's disease (Phase II/III), memory impairment (phase not provided)	93
Piceatannol	JAK1, STAT3	Inhibits phosphorylation of STAT3	Not applicable	96–98
Sanguarine	JAK2, SRC, STAT3	Inhibits phosphorylation of STAT3	Not applicable	137
Celastrol	JAK2, SRC, STAT3	Inhibits phosphorylation of STAT3	Not applicable	107,108
Withaferin A	JAK2, STAT3	Inhibits phosphorylation of STAT3	Schizophrenia (phase not provided)	110,111
Cucurbitacin I	JAK2, STAT3	Inhibits phosphorylation of STAT3	Not applicable	119–122
Cucurbitacin B	JAK2, STAT3	Inhibits phosphorylation of STAT3	Not applicable	123
3,3'-diindolyl-methane	JAK2, STAT3	Inhibits phosphorylation of STAT3	Breast cancer (Phase I/II/III), prostate cancer (Phase I/II),	141

Molecule	Target	Effect	Indication (clinical trial phase)	Refs
			cervical dysplasia (Phase III), laryngeal papilloma (Phase II), thyroid disease (Phase 0)	
Emodin	JAK2, STAT3	Inhibits phosphorylation of STAT3	Polycystic kidney disease (phase not provided)	128
Cucurbitacin E	JAK2, VEGFR2, STAT3	Inhibits phosphorylation of STAT3	Not applicable	124–126
Oleanolic acid/CDDO-Me	JAK2, SRC, EGFR, STAT3	Inhibits phosphorylation of STAT3	Solid tumours and lymphomas (Phase I), chronic kidney disease and type 2 diabetes (Phase I/II/III), diabetic nephropathy (Phase II), hepatic dysfunction (Phase I/II)	116–118
Caffeic acid	JAK2, SRC, STAT3	Inhibits phosphorylation of STAT3	Not applicable	100
Vinorelbine	STAT3	Inhibits STAT3 phosphorylation, disrupts STAT3–tubulin interaction	Non-small cell lung cancer (Phase I/II/III/IV), breast cancer (Phase I/II/III/IV), prostate cancer (Phase I/II), rhabdomyosarcoma (Phase I/II/III), gastric cancer (Phase II), melanoma (Phase II), low-grade gliomas (Phase II), Hodgkin's lymphoma (Phase I/II/III)	106
Paclitaxel	STAT3	Inhibits STAT3 phosphorylation, disrupts STAT3–tubulin interaction	Peritoneal cavity carcinoma (Phase I/II/III), breast cancer (Phase I/II/III/IV), ovarian cancer (Phase I/II/III/IV), cervical cancer (Phase I/II/III), non-small cell lung cancer (Phase I/II/III/IV), bladder cancer (Phase I/II/III), melanoma (Phase I/II/III), oesophageal cancer (Phase I/II/III), thyroid cancer (Phase I/II/III), gastric cancer (Phase I/II/III)	106
Evodiamine	JAK2, SHP1, STAT3	Decreases JAK2 phosphorylation, increases SHP1 expression leading to inhibition of activated STAT3	Not applicable	134
Cryptotanshinone	STAT3 SH2 domain	Inhibits STAT3–STAT3 dimerization	Polycystic ovary syndrome (phase not provided)	105
Honokiol	EGFR, SHP1, STAT3	Decreases EGFR expression, increases SHP1 expression leading to inhibition of activated STAT3	Not applicable	131–133
Berberamine	JAK2, SRC, STAT3	Inhibits phosphorylation of STAT3, decreases level of STAT4, induces STAT6 activation	Not applicable	145,146

Molecule	Target	Effect	Indication (clinical trial phase)	Refs
Cinnamon bark	STAT4	Inhibits phosphorylation of STAT4, decreases levels of IFN γ	Polycystic ovary syndrome (Phase I), hypercholesterolaemia and type 2 diabetes (Phase II)	147
Indirubin	JAK, STAT3 DNA-binding domain, STAT5 DNA-binding domain	Inhibits phosphorylation of STAT3, inhibits phosphorylation of STAT5	Not applicable	89
TMC-264	STAT6 DNA-binding domain,	Inhibits phosphorylation of STAT6, decreases IL-4 signal transduction	Not applicable	154
<i>Tyrosine kinase inhibitors</i>				
Tofacitinib	JAK3	Inhibits phosphorylation of STAT1, STAT3, STAT4, STAT5 and STAT6	Rheumatoid arthritis (Phase I/II/III), juvenile idiopathic arthritis (Phase I/II/III), psoriasis (Phase I/II/III), ankylosing spondylitis (Phase II), keratoconjunctivitis sicca (Phase II), ulcerative colitis (Phase III)	183
Sorafenib	JAK2, STAT3	Inhibits phosphorylation of STAT3	Hepatocellular carcinoma (Phase I/II/III/IV), head and neck squamous cell carcinoma (Phase I/II), gastric cancer (Phase I/II), breast cancer (Phase I/II/III), prostate cancer (Phase I/II), thyroid cancer (Phase II/III), non-small cell lung cancer (Phase I/II/III), pancreatic cancer (Phase I/II/III), bladder cancer (Phase I/II), colorectal cancer (Phase I/II), kidney cancer (Phase I/II/III/IV), liver cancer (Phase I/II/III), glioblastoma multiforme (Phase I/II), leukaemia (Phase I/II/III), melanoma (Phase I/II/III)	169–173
SC-1, SC-49	STAT3, SHP1	Increases SHP1 activity leading to inhibition of activated STAT3	Not applicable	174,175
AZD1480	JAK1, JAK2	Inhibits phosphorylation of STAT1, STAT3, STAT5 and STAT6	Hepatocellular carcinoma, lung carcinoma and gastric cancer (Phase I), essential thrombocythaemia myelofibrosis and post-polycythaemia vera (Phase I)	186–189
Atiprimod	JAK2	Inhibits phosphorylation of STAT3, inhibits phosphorylation of STAT5	Neuroendocrine carcinoma (Phase II), multiple myeloma (Phase I/II)	160,190,191
Auranofin	JAK1	Inhibits phosphorylation of STAT3	Chronic lymphocytic leukaemia (Phase II),	192,193

Molecule	Target	Effect	Indication (clinical trial phase)	Refs
			squamous cell lung cancer (Phase II), ovarian cancer (phase not provided)	
<i>DNA-binding modifier</i>				
Oligodeoxy-nucleotide decoy	DNA-binding domain of STAT1, STAT3 and STAT5	Inhibits transcription activity of STAT1, STAT3 and STAT5	Head and neck cancer (Phase 0)	28,196–203

EGFR, epidermal growth factor; gp130, glycoprotein 130; IFN, interferon; IL, interleukin; JAK, Janus kinase; pTyr, phosphotyrosine; STAT, signal transducer and activator of transcription; VEGFR2, vascular endothelial growth factor receptor 2.