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MONOGRAPH

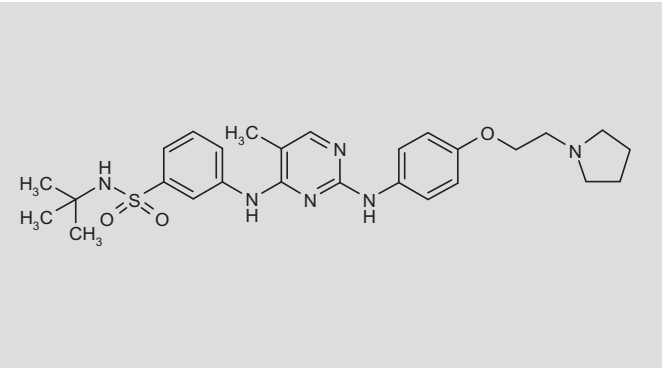
TG-101348

Tyrosine-Protein Kinase JAK2 Inhibitor
Receptor-Type Tyrosine-Protein Kinase FLT3 Inhibitor
Treatment of Myeloproliferative Diseases

SAR-302503

N-tert-Butyl-3-[5-methyl-2-[4-[2-(1-pyrrolidinyl)ethoxy]phenylamino]pyrimidin-4-ylamino]benzenesulfonamide

InChI: 1S/C27H36N6O3S/c1-20-19-28-26(30-21-10-12-23(13-11-21)36-17-16-33-14-5-6-15-33)31-25(20)29-22-8-7-9-24(18-22)37(34,35)32-27(2,3)4/h7-13,18-19,32H,5-6,14-17H2,1-4H3,(H2,28,29,30,31)



$C_{27}H_{36}N_6O_3S$
Mol wt: 524.678
CAS: 936091-26-8
EN: 441519

SUMMARY

TG-101348 is an orally available, small-molecule inhibitor of the tyrosine-protein kinase JAK2 (Janus kinase 2, JAK-2) that is under investigation for the treatment of myelofibrosis. Myelofibrosis and the related myeloproliferative neoplasms polycythemia vera and essential thrombocythemia are associated with the JAK2 V617F mutation, which causes hyperactivation of cytokine signaling pathways that control myeloid hematopoiesis. Several preclinical studies have demonstrated a pathogenic role for JAK2 V617F and the therapeutic potential of TG-101348 in polycythemia vera. In a phase I trial, TG-101348 reduced constitutional symptoms and splenomegaly in a JAK2 V617F-enriched population of intermediate- and high-risk myelofibrosis patients. Adverse events included nausea, vomiting, diarrhea, anemia and thrombocytopenia. A phase I/II extension study in the patients who experienced benefit from TG-101348 is in progress. Here we discuss the therapeutic rationale, pharmacokinetics, safety, efficacy and future potential of TG-101348.

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SYNTHESIS*

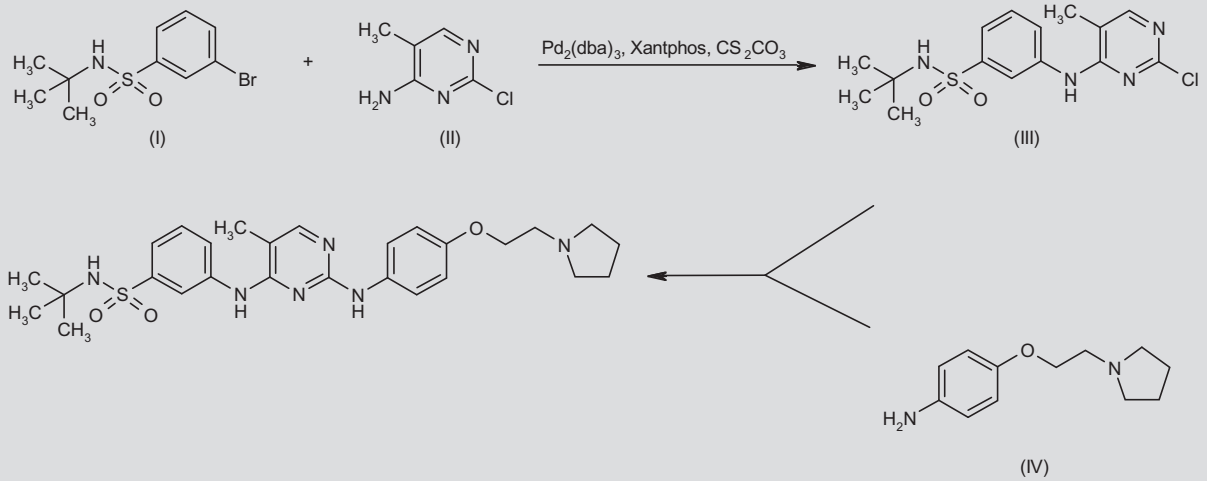
TG-101348 is prepared by condensation of 3-bromo-*N-tert*-butylbenzenesulfonamide (I) with 2-chloro-5-methylpyrimidin-4-ylamine (II) in the presence of $Pd_2(dba)_3$, Xantphos and Cs_2CO_3 in refluxing dioxane to give the sulfonamide derivative (III), which is finally coupled with 4-[2-(pyrrolidin-1-yl)ethoxy]phenylamine (IV) in AcOH at 150 °C (1, 2). Scheme 1.

BACKGROUND

TG-101348 is a selective inhibitor of the tyrosine-protein kinase JAK2 (Janus kinase 2, JAK-2) that is currently under investigation for the treatment of myelofibrosis (MF). MF and the related conditions polycythemia vera (PV) and essential thrombocythemia (ET) are Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) that result from clonal expansion of a hematopoietic progenitor cell. MF can arise de novo (primary MF) or develop from PV or ET (post-PV or post-ET MF). The incidence of MF in the U.S. is estimated to be 0.2 in 100,000. Risk factors include advanced age, radiation and chemical exposure, and blood cell disorders. Patients with MF present with marrow fibrosis, myeloid metaplasia, extramedullary hematopoiesis causing splenomegaly, constitutional symptoms (weight loss, fever, fatigue and night sweats), anemia, and abnormal numbers of platelets and white blood cells. In some cases, MF undergoes aggressive leukemic transformation. Life expectancy is 5-7 years for patients with MF compared to more than 15 years for patients with PV and ET. Diagnosis generally occurs in the sixth to eighth decades of life (3, 4).

Currently available therapies for MF are primarily supportive and do not prolong survival. For example, cytoreductive agents, such as hydroxyurea, are used to manage organomegaly, blood cell counts and the risk of thrombosis. In some cases, splenectomy may be indi-

Scheme 1. Synthesis of TG-101348



cated for pain control, cachexia or transfusion-dependent anemia. Although constitutional symptoms have been correlated with long-term survival in primary MF, they are not well managed with current therapies (5). Allogeneic stem cell transplant is the only potentially curative treatment strategy, but is associated with limited donor availability and high rates of morbidity and mortality (6).

Treatment of Philadelphia-negative MPNs has lagged behind that of chronic myelogenous leukemia (CML), which is associated with the constitutively active tyrosine kinase BCR-ABL encoded by the Philadelphia chromosome. The development of the small-molecule inhibitor imatinib, which competes for the ATP binding pocket of BCR-ABL, revolutionized the treatment of CML and was a groundbreaking advancement in molecular medicine, since it represented the first therapy targeted against a signaling pathway that is aberrantly active in neoplastic cells. On the other hand, the molecular basis for the Philadelphia-negative MPNs had long remained enigmatic until 2005, when a gain-of-function mutation in *JAK2* was described in half of the patients with ET and MF and nearly all patients with PV (7-10). Genome-wide association studies have demonstrated that a genetic haplotype predisposes to acquisition of this mutation through somatic mutagenesis (11-13).

Named for the two-faced Roman god Janus, JAK2 contains a functional tyrosine kinase domain and an autoinhibitory pseudokinase domain that does not have enzymatic activity. The *JAK2* gain-of-function mutation prevalent in Philadelphia-negative MPNs is a valine-to-phenylalanine single amino acid substitution (V617F) that results in impairment of the autoinhibitory pseudokinase domain and constitutive JAK2 kinase activity. JAK2 functions downstream of cytokine receptors that lack intrinsic tyrosine kinase activity, including type I cytokine receptors with the common glycoprotein 130 subunit (e.g., interleukin-6 [IL-6] receptor) or with the common β-chain (e.g., IL-3 receptor) and type II cytokine receptors (e.g., interferon [IFN] receptors) (14). Importantly, JAK2 is the sole tyrosine kinase that signals in response to IL-3, IL-5, growth hormone (GH), prolactin (PRL), erythropoietin (Epo), thrombopoietin, granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF). Upon ligand binding, JAK2-associated receptors undergo oligomerization and JAK2-dependent phosphorylation, which is necessary for the recruitment of Src homology 2 (SH2) domain-containing proteins (15). In particular, the signal transducers and activators of transcription (STATs), such as STAT3 and STAT5, are important JAK2 targets in the myeloid compartment. STAT family members homo- or heterodimerize following JAK2 activation and translocate to the nucleus, where they activate transcription of genes that control cell proliferation, differentiation, angiogenesis and inhibition of apoptosis (14). The MAPK/ERK and PI3K/Akt/mTOR pathways have also been implicated in JAK2 signaling. JAK2 is negatively regulated by phosphatases, such as SHP-1 and CD45, and suppressor of cytokine signaling (SOCS) proteins.

MPNs may represent a spectrum of disorders whose phenotypes depend on the ratio of mutant to wild-type *JAK2* alleles. In transgenic mice expressing human *JAK2* V617F in the hematopoietic compartment, high *JAK2* mutant allele burden correlates with a PV phenotype, whereas low mutant allele burden correlates with an ET phenotype (16). *JAK2* V617F is not the only relevant allele, since mutations in exon 12 of *JAK2*, which affect the region immediately upstream of its autoinhibitory kinase domain, have been observed in *JAK2* V617F-negative MPNs (17-19). Despite the genetic complexity of MPNs, JAK2 inhibition offers a promising therapeutic approach for disease management and symptomatic relief.

PRECLINICAL PHARMACOLOGY

TG-101348 was developed by TargeGen (now Sanofi) using structure-based design methods to target JAK2. This small molecule competes for the ATP binding pocket of JAK2, which is responsible for ATP hydrolysis and the formation of inorganic phosphate that is used for phosphorylation of JAK2 substrates. The concentration of TG-101348 that reduces enzymatic activity of both wild-type and mutant JAK2 by 50% is 3 nM (IC_{50}). Although TG-101348 can also inhibit receptor-type tyrosine-protein kinase FLT3 and proto-oncogene tyrosine-protein kinase receptor Ret (IC_{50} = 15 and 48 nM, respectively), its potency against more than 30 other kinases, including the Janus kinase family members JAK1, JAK3 and non-receptor tyrosine-protein kinase TYK2, is significantly lower (20, 21). In addition, TG-101348 limits the proliferation of JAK2-dependent cell lines (IC_{50} = 300 nM) and induces apoptosis preferentially in JAK2-dependent cell lines (1-3 μM) compared to JAK2-independent cell lines (5-10 μM) (20), suggesting that the drug may represent a targeted treatment strategy for patients with *JAK2* V617F$^+$ MPNs. Several preclinical studies discussed below support the rationale for testing the efficacy of this agent in patients with MPNs.

A few seminal studies have demonstrated the importance of the *JAK2* V617F mutation in PV pathogenesis and biological response to TG-101348. In a murine PV model in which *JAK2* V617F-transduced bone marrow was injected into lethally irradiated mice (22), TG-101348 treatment was associated with dose-dependent improvement in survival, hematocrit, splenomegaly, extramedullary hematopoiesis, splenic architecture and marrow fibrosis (20). Compared to vehicle control-treated mice, bone marrow and spleen from TG-101348-treated mice (120 mg/kg) produced fewer erythroid blood-forming units (BFU-E) and fewer colony-forming units of granulocyte–macrophage (CFU-GM) and megakaryocyte (CFU-M) lineages. TG-101348 also caused a reduction in *JAK2* V617F allele burden, suggesting that quantification of the disease-associated allele may provide a marker of biological response. Additionally, immature erythroblasts and myeloid cells harvested from *JAK2* V617F$^+$ mice and subsequently stimulated with Epo and IL-3 demonstrated reduced phosphorylation of the JAK2 effectors STAT5, ERK and ribosomal protein S6 kinase (S6K) in response to TG-101348, indicating a reduction in JAK2 signaling, although the degree of phosphorylation was similar to that of baseline levels in serum-starved cells. A similar trend in STAT5 and S6K phosphorylation was observed in immature erythroblasts from bone marrow of mice treated with oral TG-101348 (120 mg/kg). The off-target effect of TG-101348 on various hematopoietic lineages is unclear from this study due to fulminant myeloproliferative disease in mice following irradiation and bone marrow transplant, but TG-101348 appears to target *JAK2* V617F$^+$ cells preferentially (20). Therefore, TG-101348 can inhibit JAK2 signaling, limit expansion of *JAK2* V617F-dependent cell populations, and improve parameters associated with the development and progression of PV in murine models.

Due to uncertainties regarding retroviral expression of PV, an improved murine model has also been developed in which physiological *JAK2* V617F expression driven by the *Jak2* promoter causes a lethal PV-like myeloproliferative neoplasm (23). TG-101348 treatment of these mice by oral gavage (120 mg/kg) resulted in markedly reduced spleen size, restoration of the splenic architecture and fewer erythroid cells. However, serial transplant experiments demonstrated that bone marrow from mice inoculated with disease-transmitting hematopoietic stem cells and treated with TG-101348 still caused a dramatic increase in hematocrit in recipient mice, indicating that while TG-101348 can control growth in the myeloid compartment, it cannot eliminate the myeloid progenitor cell population that initiates myeloproliferative neoplastic transformation in vivo.

Taken together, these studies provide proof of principle that PV secondary to the *JAK2* V617F mutation and enhanced JAK2 signaling can be effectively targeted by TG-101348 using endpoints such as survival, splenomegaly, hematocrit and *JAK2* V617F allele burden. Furthermore, they posit that TG-101348 may provide clinical benefit for patients with myeloproliferative disease.

TG-101348 has also been studied using PV progenitor cells from patients. In a xenogeneic transplant model, human PV progenitor cells transplanted intrahepatically into immunocompromised mice demonstrated increased erythroid differentiation and engraftment in hematopoietic organs compared to normal human hematopoietic stem cells. Engraftment and allele burden were attenuated by a 12-day course of oral TG-101348 (120 mg/kg/day). In a similar experiment, engraftment by *JAK2* V617F-transduced normal cord blood progenitor cells was reduced by 65% in the spleen and 95% in the liver following TG-101348 treatment, whereas engraftment by cells transduced with wild-type *JAK2* was reduced by 40% in the spleen and 89% in the liver. Therefore, cell populations expressing the mutant allele of *JAK2* may be more sensitive to TG-101348 than those expressing the wild-type allele, which could have positive implications for the toxicity profile of TG-101348 in clinical studies (21).

Since Epo-independent erythroid colony formation is a hallmark of MPNs (24), bone marrow and peripheral blood from PV patients were plated in the absence of Epo and the growth-inhibitory effect of TG-101348 on colony formation was assessed. Colony formation of all hematopoietic lineages derived from bone marrow and spleen of PV patients was completely abolished by TG-101348 (300 nM) (20). STAT5 phosphorylation was also reduced in BFU-E derived from PV patient bone marrow following ex vivo TG-101348 treatment (600 nM), demonstrating functional inhibition of JAK2 signaling. Furthermore, BFU-E growth from cultured bone marrow was eliminated by mouse serum 2 hours after administration of TG-101348 (60 mg/kg), suggesting a pharmacokinetic profile for TG-101348 that is suitable for *JAK2* V617F inhibition.

In another study, hematopoietic stem cells and common myeloid progenitor cells from *JAK2* V617F$^+$ PV patients produced fewer BFU-E and mixed-lineage colonies when treated with TG-101348 (300 nM). In addition, TG-101348 (300 nM) reduced *JAK2* V617F allele burden in PV hematopoietic stem cells. To demonstrate that this effect was due to *JAK2* V617F targeting, *JAK2* V617F-transduced cord blood progenitor cells were also treated with TG-101348 (300 nM), resulting in reduced erythroid colony generation compared to those transfected with wild-type *JAK2* (21). Finally, TG-101348 (300 nM) inhibited JAK2-dependent activation of the erythroid transcriptional program, as evidenced by decreased phosphorylation of Akt, erythroid transcription factor (GATA-binding factor 1, GATA-1) and STAT5 in an Epo-responsive cell line. Interestingly, TG-101348 (300 nM) inhibited STAT5 and Akt phosphorylation more effectively than AG-490, a JAK2/JAK3 inhibitor. It also inhibited phosphorylation of

GATA-1, an erythroid transcription factor downstream of Akt, to a greater extent than the PI3K inhibitor LY-294002, despite similar decreases in Akt phosphorylation. Therefore, TG-101348 can decrease proliferation of human *JAK2* V617F$^+$ cells by preventing activation of downstream effectors of JAK2.

Limited data from experiments testing the effect of TG-101348 on peripheral mononuclear cells from MF and PV patients with *JAK2* V617F, *JAK2* exon 12 or thrombopoietin receptor (*MPL*) mutations suggest that TG-101348 can effectively attenuate the growth of mutation-harboring clones in both the presence and absence of Epo and that the therapeutic potential of TG-101348 is not limited to patients with the *JAK2* V617F mutation (25). Despite the heterogeneity of mutations associated with MPNs, JAK2 may represent a common node in disease pathogenesis that can be specifically targeted with TG-101348.

In summary, preclinical studies using murine models, human cell lines, *JAK2* V617F-transduced human cord progenitor cells and PV patient-derived cells provide an abundance of data regarding the therapeutic potential for TG-101348 in MPNs. TG-101348 preferentially limits differentiation along the erythroid lineage by disrupting the activation of downstream effectors such as STAT5, ERK, S6K, Akt and GATA-1. In murine models of PV that demonstrate extramedullary hematopoiesis and splenomegaly, TG-101348 can increase survival and reduce ectopic erythroid differentiation, while reducing splenomegaly and restoring architectural integrity to the spleen. Despite some discrepancy in various experimental models of PV, TG-101348 appears to function specifically on JAK2-dependent cells of the erythroid lineage, while having only modest effects on other hematopoietic compartments. Finally, quantification of the *JAK2* V617F allele burden may provide a reliable marker for response to TG-101348.

PHARMACOKINETICS AND METABOLISM

The pharmacokinetic profile of TG-101348 has been studied in mice and humans. In C57BL/6 mice, total plasma exposure increased linearly according to dose following oral administration of TG-101348, with peak levels occurring 2-4 hours after dosing. In addition, the pharmacokinetic profile of orally administered TG-101348 (100 mg/kg) suggested that concentrations above both the enzymatic and cellular IC_{50} could be achieved with twice-daily administration (20). Similarly, in a phase I clinical trial of TG-101348, peak plasma concentrations in MF patients were achieved 1-4 hours after dosing, although increases in maximum plasma concentration and area under the concentration–time curve were greater than expected based on dose escalation, suggesting increased bioavailability at higher doses. The terminal phase half-life of TG-101348 was 16-34 hours across all doses, consistent with first-order kinetics and linear drug elimination (26).

CLINICAL STUDIES

Due to the low incidence of MF, TG-101348 was awarded orphan drug status by the U.S. Food and Drug Administration to promote therapeutic development for this rare disease. The safety and toxicity of TG-101348 have been investigated in a phase I trial in patients with MF (MF-TG101348-001; NCT00631462) (26). Fifty-nine patients enrolled in this study who had received a diagnosis of primary, post-PV or post-ET MF were considered high- or intermediate-risk based on the presence of at least one of the following: age > 65 years, constitutional symptoms, hemoglobin < 10 g/dL, white blood cell count > 25 x 10^9/L and/or blood blasts > 1% (27). In addition, 86% of patients carried the *JAK2* V617F allele. The primary endpoints were safety, tolerability, dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and pharmacokinetic behavior. Clinical response according to spleen size, constitutional symptoms, body weight, leukocytosis, thrombocythemia and *JAK2* V617F allele burden was followed as a secondary endpoint. Patients who benefitted from TG-101348 were treated beyond six cycles on an extension study (MF-TG101348-002; NCT00724334).

Overall, TG-101348 had an acceptable safety profile. The MTD was 680 mg/day and the DLT in two of six patients treated at 800 mg/day was asymptomatic and reversible hyperamylasemia (MF-TG101348-001; NCT00631462) (26). Patients from all cohorts (30-800 mg/day) developed grade 3-4 nausea (3%), vomiting (3%), diarrhea (10%), thrombocytopenia (24%) and anemia (35%). The hematological adverse events generally developed in the first three cycles of treatment and occurred mostly in patients who enrolled in the study with grade 1-2 anemia or thrombocytopenia.

The clinical responses of this study are promising for the development of TG-101348 as a targeted treatment strategy for MF. At the time of enrollment, 83% of patients had spleens palpable ≥ 10 cm below the left costal margin (median 18 cm). A 25% reduction in spleen size was observed by clinical examination in greater than half of the patients, both overall and in the MTD cohort, after 12 cycles of TG-101348 according to guidelines set by the International Working Group for MPN Research and Treatment (IWG-MRT). Patients who were *JAK2* V617F$^-$ also demonstrated a reduction in splenomegaly. Responses were also observed in constitutional symptoms, including early satiety, night sweats, cough and pruritus. Dramatic improvement or complete resolution of these symptoms was observed by one to six cycles in more than half of the patients who reported them at the time of enrollment. Like the improvement in splenomegaly, which lasted an average of 315 and 288 days, respectively, for the overall and MTD cohorts, responses in constitutional symptoms were also prolonged. Considering the relationship between constitutional symptoms and long-term survival in primary MF (5), this is a particularly important endpoint that needs further evaluation. Body weight did not differ from baseline for any cohort. In both the overall cohort and the MTD cohort, by six cycles more than half of the patients with leukocytosis and nearly all patients with thrombocythemia achieved normal white blood cell and platelet numbers, respectively. Fifty-one patients (86%) were positive for the *JAK2* V617F mutation (median allele burden of 20%) and most experienced a reduction in mutant allele burden. This response seemed to be greater for individuals with significant allele burden at baseline (> 20%), although correlation of clinical response to reduction in allele burden could not be performed due to the limited size of the study.

TG-101348 has not been tested in combination with other drugs and there are no publicly available data on the interactions between TG-101348 and other agents. However, pharmacokinetic data from ongoing studies will provide pertinent information regarding shared metabolic pathways for drug elimination and altered bioavailability.

CONCLUSIONS

The orphan status approval of TG-101348 for MF serves as a solid proof of concept demonstrating the relevance of a novel target, JAK2, in human disease. Animal models and a phase I trial investigating the toxicity profile of TG-101348 in patients with MF offer promising evidence that TG-101348 may be an effective palliative agent for splenomegaly and constitutional symptoms associated with MF. TG-101348 also corrected leukocytosis and thrombocythemia for a large portion of phase I patients, suggesting that in the future this drug could supplant current cytoreductive therapies, such as hydroxyurea, whose mechanisms of action are less specific in the context of MF. Additional studies are needed to determine the effect of TG-101348 on survival, to compare secondary endpoints in MF patients treated with TG-101348 and standard therapies, and to investigate the potential for TG-101348 in the other Philadelphia chromosome-negative MPNs, PV and ET.

Although the *JAK2* V617F mutation and equivalent mutations in the autoinhibitory domains of other Janus kinases have not been detected in nonhematological malignancies (28-30), aberrant JAK/STAT signaling has been implicated in the pathogenesis of solid tumors. For example, mutations in *JAK1* and *JAK3* have been described in breast, gastric and lung carcinomas (31), and amplification of the *JAK2* locus has been detected in lung sarcomatoid carcinoma (32). The tumor suppressor breast cancer type 1 susceptibility protein (*BRCA1*) can interact with JAK2 and cause activation of STAT3, although this interaction in breast and ovarian cancers has not been confirmed (33). Therefore, in some instances, JAK2 may be directly involved in tumorigenesis.

The putative role for TG-101348 and other JAK2 inhibitors in the treatment of solid tumors is most strongly buttressed by the involvement of activated STAT proteins in various cancers. In particular, activation of STAT3 and STAT5 has been described in cancers of the breast, head and neck, lung, ovary, pancreas, prostate and skin (34-36). Activated STAT3 is required for cancer cell survival in some cases due to its role in regulating cell cycle, apoptosis and angiogenesis (37-39). However, the involvement of JAK2 upstream of aberrantly activated STATs is unclear, since STATs can also be activated by Src family kinases and growth factor receptors with intrinsic tyrosine kinase activity, such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGF-R) (34, 40-42).

Direct evidence supporting the use of JAK2 inhibitors in the treatment of solid tumors comes from studies investigating AZD-1480, another small-molecule inhibitor of JAK2 similar to TG-101348. AZD-1480 blocks STAT3 signaling and oncogenesis in multiple solid tumor cell lines in vitro and in xenograft models (43). STAT3 phosphorylation and nuclear translocation were reduced by AZD-1480, but not inhibitors of Src or EGFR, suggesting that STAT3 activation is primarily JAK2-dependent. Importantly, AZD-1480 also blocks IL-6-driven STAT3 activation (43), which proceeds through JAK2 and supports autocrine and/or paracrine cell growth in several cancers (44, 45). AZD-1480 is currently under phase I investigation for MPNs (NCT00910728) and solid tumors (NCT01112397, NCT01219543). One likely challenge will be dosing of JAK2 inhibitors to inhibit solid tumor growth without causing hematological adverse events. These studies will guide expectations for the therapeutic potential of TG-101348 in nonhematological malignancies.

The acceptable toxicity profile of this agent and the growing body of evidence suggesting the relevance of JAK2 in other diseases such as solid tumors has enhanced our interest in future applications of TG-101348. Indeed, this and other JAK2 inhibitors are now being developed in phase I for solid tumors, and the results of those trials are eagerly awaited to better position this agent and the class in the oncology drug development landscape.

SOURCE

Sanofi SA (FR).

DISCLOSURES

The authors state no conflicts of interest.

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