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Myeloproliferative Neoplasms Associated with Mutation in JAK2V617F and Tyrosine Kinase Inhibitors as Therapeutic Strategy

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Abstract

MPNs include a heterogeneous group of clonal or oligoclonal hemtopathies characterized by proliferation and accumulation of mature myeloid cells involving CML, PV, ET, PMF, SM, CEL, CNL, UMPN. While PV, ET, and PMF classified as a negative Philadelphia chromosome, JAK2V617F have shown in about 90% of PV patient and over 50% of ET and PMF patients. JAK2 is involved in EPO signaling pathway, and its mutations lead to EPO-independent spontaneous phosphorylation. Most tyrosine kinase inhibitors (TKI) are small molecules that compete with ATP for binding the ATP-binding site in tyrosine kinase domains, since ATP is a source of phosphate groups used by Tks to phosphorylate the target protein. There are many TKI agents that are studying for treatment of the MPNs with JAK2 tyrosine kinase mutations. The most important TKI drugs including CEP701, CYT387, LY2784544, SB1518, TG101348, XL019, and INCB18424. The main mechanisms of drug actions are to reduce the splenomegaly, improvement of constitutional symptoms (improvement of bone marrow fibrosis and anemia). Although this drugs are useful, they have some side affects that gastrointestinal diseases (GI), diarrhea, nausea and vomiting, anemia, thrombocytopenia, thrombosis, leukocytosis, thrombocytosis, peripheral neuropathy, transient loss of blood pressure and lightheadedness are the most common side effects.

Keywords: MPN; JAK2; TKI

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Introduction

MPNs form a heterogeneous group of clonal or oligoclonal hamtopathies characterized by proliferation and accumulation of mature myeloid cells. In 2008, WHO revised the classification of myeloid and lymphoid malignancies, which was an improvement of 2001 classification. Myeloid malignancies include five disorders: AML, MDS, MPN, MPN/MDS as well as myeloid and lymphoid malignancies with eosinophilia and rearrangement in PDGFR or FGFR1. MPN is divided into 8 separate subclasses, including CML, PV, ET, PMF, SM, CEL, CNL, and UMPN. MPN is a clonal disorder in HSCs usually occurring in fifth to seventh decades of life; however CML and ET are observed in children. MPNhas an annual incidence of 1:6110 per 100000 Population (1, 2). Although MPN represents a change in multipotent stem cell, molecular its pathology is not completely clear yet (3). Clinical signs include erythrocytosis, thrombocytosis, leukocytosis, pancytopenia, extramedullary hematopoiesis, increased risk of thrombosis and progression to acute myeloid leukemia (AML) (4-6). ET, PV, and PMF are the most common MPN subtypes showing JAK2 mutation. Polycythemia Vera (PV) is a clonal myeloproliferative disorder characterized by increased production of red blood cells, granulocytes, and platelets. It usually affects people aged 40-60 years with an annual incidence of 14 per million populations. JAK2 tyrosine kinase mutation is the most common molecular lesion identified in 90% of

cases. JAK2 is involved in EPO signaling pathway, and its mutations lead to EPO-independent spontaneous phosphorylation (7, 8). PMF is a Ph-negative myeloproliferative neoplasm due to clonal stem cell disorder. It is also associated with bone marrow fibrosis, extramedullary hematopoiesis with anemia, splenomegaly, dacryocytes and leukoerythroblastosis in PB, showing JAK2 V617F mutation in over 50% of cases (9).

Janus kinase and JAk2

Janus kinases are 120 to 140 kDa tyrosine kinases. All JAK family members share a similar sequence containing 7 domains known as JAK Homology (jH). When a cytokine is not bound to its receptor, Janus kinase is strongly associated with intracellular parts of cytokine receptor via FERM and SH2 domains and remains in an inactive state. Cytokine binding to its receptor leads to structural changes in the receptor, which is transmitted to JAK associated cytoplasmic domain, leading to their phosphorylation and activation (10).

JAK2 is a member of JAK Tyrosine kinase family and mediates the function of several cytokines as well as EPO, including growth hormone, prolactin, TPo, GM-CSF, IL-3, and IL-5. The unique relationship between EPO and JAK2 indicates that JAK2 is the only kinase associated with EPOR, and the only transmitter of EPO signal. The importance of this phenomenon was shown by studying the three-dimensional structure of kinase domains, false kinases and regulatory domains of Janus kinase2. This three-dimensional structure indicates the precise regulation of kinase activity associated with EPOR for its phosphorylation and activation (11).

JAK/STAT signaling pathway is among a handful of signaling cascades for transduction of multiple signals for development and homeostasis in animals, humans and insects. In mammals, JAK/STAT is a major signaling pathway for a wide range of cytokines and growth factors. JAK activation stimulates cell proliferation, differentiation, migration and apoptosis. These cellular events are essential for hematopoiesis, immune development, adipogenesis, dual sexual development, and other cellular processes. Mutations which reducing the activity of JAK/STAT pathway are deemed to affect these processes (12).

Several mutations (about 70) have been described in the structural domains of JAK since the discovery of JAK2 V617F mutation. Many of them (about 30) have been biochemically proven to lead to constitutively active proteins (13). JAK2 activates STAT, MAPK, and PI3K signaling pathways, which can cause transformation of hematopoietic progenitors (14).

Detrimental consequences have been resulted from the impairment of these regulatory mechanisms. Several studies have reported an active mutation in pseudo-kinase domain of JAK2 in more than 90% of PV as well as 50% of ET and IMF cases. In all these studies, it was clear that JAK2 overactivity contributes in increased erythropoietic activity and erythron mass with splenomegaly, with a similar phenotype that in thalassemia (3, 7, 15-17).

Mutations in kinase domains can have a direct impact upon activation, but the molecular consequence of mutations in other domains of JAK are not easily understood. Mutations in Pseudokinase (V617F) appear to reduce the negative regulatory role of reactions between pseudokinase and kinase domains, resulting in constitutive kinase activation. It has recently been described that pseudokinase has residual kinase activity and phosphorylates inhibitory amino acid positions in JAK2, including serine 523 and Tyr570 (18).

JAK2V617F mutation in exon 14 of JAK2 gene (on chromosome 9p24) is the most common mutation in MPN patients with rate of 96% in PV, 65% in ET and PMF (11, 13). This mutation affects the autoinhibitory domain in JH2 in JAK2, leading to constitutive activation of JAK2 and JAK/STAT signaling pathway (19).

Discovery of JAK2 V617F mutation would define JAK2 as a therapeutic target in Philadelphia chromosome-negative MPN patients. Although JAK2 V617F is not present in all ET and PMF patients, partial activation of JAK/STAT plays an important role in pathogenesis of MPN patients. It not only causes proliferation but also mediates the function of inflammatory cytokines, the levels of which are usually increased in myelofibrosis. Since 2005, several JAK2 inhibitors have been developed, and a number of them are in clinical trials. Pre-clinical studies have confirmed the activity of these agents through induction of apoptosis in both in vivo and in vitro models (20, 21).

Recently, several somatic mutations have been reported in MPN patients as well as JAK2V617F. These mutations included IDH1, ASXL1, TET 2, MPL, IDH2, CBL, LNK, IKZF, and EZH2, originate from the stem cell progenitors, and their role in disease pathogenesis is not fully understood (22-24).

The prevalence of these mutations except for JAK2 is less than 20% of MPN patients. These include: MPL (Myeloproliferative Leukemia virus oncogene), LNK (a membrane-bound adaptor protein), TET2 (TET oncogene family member2), ASXL1 (Additional sex combs-like l), IDH1/IDH2 (isocitrate dehydro-genase), EZH2 (enhancer of zeste homology2), DNMT3A (DNA cytosine methyltransferase 3 a), CBL (casitas B-lineage lymphoma Proto-oncogene),

IKZF1 (Ikaros family zinc finger), TP53 (Tumor Protein p53), and SF3B1 (Splicing factor 3B subunit1) (2, 25).

TKI and JAK2 inhibitor

Most tyrosine kinase inhibitors (TKI) are small molecules that compete with ATP for binding the ATP-binding site in tyrosine kinase domains, since ATP is a source of phosphate groups used by Tks to phosphorylate the target protein (26). JAK2 V617f mutation site is located out of TK domain in JAK, and common JAK2 inhibitors can target both wild type and mutated JAK2. Wild Type (wT) JAK2 targeting leads to myelosuppresion and probably accounts for anemia and thrombocytopenia in clinical trials with these agents. So far, most clinical studies with JAK2 inhibitors have focused on MF patients, with clinical response usually involving a reduction in spleen size, improved systematic symptoms and exercise ability, etc. (27). Beneficial effects of several kinase inhibitors in inhibition of cells expressing JAK2 V617F has been indicated. It has recently been shown that combination of Aurora kinase inhibitor with JAK2 inhibitor synergistically reduces the proliferation of JAK2V617F expressing cells (28). Moreover, combined use of JAK2 inhibitors and suppressors of PI3k/Akt/MTOR signaling pathway synergistically decreases the proliferation of JAK2V617F cells (29). In addition, a combined inhibitory program with dual feature of Mitogen-activated Protein kinase (MEK) -selumetinib (AZD6244) and JAK2 inhibitor has been shown to synergistically cause proliferation of JAK2 V617F cells (30). Furthermore, epigenome modifier agents have been tested for potential therapeutic activity in MPN patients. However, therapeutic indications of DNA demethylation in MPN are unclear, since there are disputed reports on the pattern of DNA methylation changes. Demethylation agents such as azacitidine and decitabine have been tested as single agents in combination with JAK2 inhibitors in MPN (31). It was also noted that Histone deacetylase (HDAC) causes epigenetic regulation of gene expression by removing the acetyl group from lysine site in histone and non-histone transcription factor like proteins. The level and function of HDACs is increased in PMF patients, so the therapeutic effects of a potent Pan-HDAC inhibitor known as Panobinostat (LBH589) has been evaluated in vitro for the cells expressing JAK2V617F (10).

Treatment with Panobinostat reduces the expression levels of JAK2 V617F as well as downstream signaling pathway likely due to hyperacetylation of Heat shock Protein 90 (HSP-90), disrupting the association between JAK2 and chaperones, after which proteasome damage follows. Patients with myelofibrosis are treated with Panobinostat as an agent improving fixed symptoms and reducing the size of spleen. In addition, when combined JAK2 inhibitor and Panobinostat are used, proliferation of JAK2 V617F cells is synergistically reduced, and increased therapeutic effect of this agent has been demonstrated compared with single-agent use in MPN mouse models (32-35).

In general, the majority of studies have not been observed significant changes in allelic burden of JAK 2V617F and BM fibrosis due to several reasons. First, compared with chronic phase of CML as a disease with a single affected gene (BCR/ABL), MF has a more complicated molecular biology characterized by mutation in several genes (JAK2, MPL, TET2), cytogenetic abnormalities in more than 40% of cases as well as multiple clones and SuBCRones, which may not be sufficient for targeting a pathway by tyrosine kinase inhibitors (TKI). This wide array of mutations complicates the therapeutic approach (36, 37).

The second hypothesis is mechanism of action of JAK2 inhibitors is related to functional inhibition of proinflammatory cytokines in normal and neoplastic cells (37). JAK2 inhibitors sometimes affect JAK1 in addition to inhibiting the JAK2 signaling pathway, and inhibit cytokine receptors leading to significant improvement in spleen rehabilitation and improve-ment of systematic symptoms in MF patients (38).

In a number of studies, it has been shown that the use of JAK2 inhibitor and persistent treatment with it leads to decreased serum levels of proinflammatory cytokines (26). In vitro studies showed that increased concentration of cytokines leads to growth reduction induced by JAK2 inhibitors in JAK2V617F positive cells (39). In 1995, the first JAK2 inhibitor, tyrophostin B42 (AG 490), was discovered by Meydon et al. This agent had a significant effect on leukemic cells and induced cell apoptosis with no harmful impact upon normal hematopoiesis. However, it was subsequently reported that although AG 490 was a potent JAK2 inhibitor, it was not specific (29). To solve this problem, researchers used different methods to identify the new selective JAK2 inhibitor. Flowers et al showed that a small JAK2 inhibitory peptide called Tkip, the inhibitory SOCS1 mimetic peptide, specifically inhibited JAK2 phosphorylation in tyrosine 1007, preventing IFNγ signaling (40). Another agent named C7 was reported in 2005 that could directly inhibited JAK2 autophosphorylation in a dose and time-dependent manner (41). The majority of JAK2 inhibitors are classified in two groups. Class I inhibitors are specific against JAK2 structure to compete with ATP. Class II inhibitors were first developed as inhibitors against other kinase targets, and later their

likely JAK2 inhibitor activity was addressed. Moreover, they affect constitutive hemostasis through inhibition of non-JAK2 tyrosine kinases signaling, and may be associated with a separate toxicity profile (22, 42).

JAK protein kinase inhibitors

1. INCB 018424

This is a selective oral inhibitor for JAK1 (IC50 = 3.3 nM) and JAK2 (IC50 = 2.8 nM) unlike JAK3 (IC50 = 428 nM) and Tyk2 (IC50 = 19nM) (43). A pre-clinical study showed that the activation and phosphorylation of JAK2V617F and downstream targets of ERK, STAT5, and STAT3 in HEL and Baf/3 cells that express JAK2V617F is inhibited by INCB 018424. Inhibition of JAK2V617F JAK2 induces apoptosis in JAK2V617F positive cells. INCB 018424 inhibits the clonogenic growth of erythroid progenitor cells in PV patients with JAK2V617F mutation. In murine cells with MPN, INCB 018424 treatment reduced the level of proinflammatory cytokines (IL-6 and TNFα), improved spleen size and increased the survival of treated mice (44).

Phase I/II clinical trial with INCB 018424 in primary or Post-PV/Post ET MF was conducted independent of JAK2 mutation features (96). Patients with neutropenia (ANC ≤ 1.5×109/L) or thrombocytopenia (Plt count ≤ 100 × 109/L) were excluded from the trial. Out of 153 patients in this study, 115 were still being treated (73%) with a mean treatment follow-up period of 7 and 14 months. Their mean age was 65 years, 82% had JAK2V617F mutation, and 92% had splenomegaly at the start of treatment with medium spleen size of 19cm (below left costal margin). During Phase I trial, groups receiving 25 mg dose twice daily and 100 mg dose once per day were defined. Thrombocytopenia was observed during Phase II during the trial, 25 mg groups expanded twice daily and 50 mg once per day, and additional therapeutic dose was evaluated in diet to indicate the most effective therapeutic regimens to reduce thrombocytopenia. Based on drug response and toxicity, an optimized Table of 15mg twice daily (10 mg twice a day if platelet count was 100 - 200 × 109) and monthly dose escalation to 25 mg twice a day if there was toxicity without response was considered (44).

Santos and Verstovsek reported INCB 018424 as an effective and well-tolerated drug to control splenomegaly and systemic symptoms in PMF patients (5). In 39 patients with PV, who were JAK2 V617F positive and 76% were phlebotomy dependent, HCT was normalized in the absence of phlebotomy (mean HCT was 39% after 6 months). The continuous improvement in platelet and WBC count, spleen size, pruritus, a bone pain and night sweat was observed. Complete response was observed in 45% of patients, 52% had a partial response and 68% showed complete hematologic response. In 39 ET patients, 67% of whom were JAK2 mutation positive and 87% had experienced no clinical response to hydroxyurea, INCB 018424 decreased platelets from a mean baseline of 884000 to 558000 for over 6 months, 13% of them had complete response and 77% partial response to treatment with INCB 018424. INCB 018424 is well tolerated with general adverse effects of anemia (12% PV, 18% ET), low platelet count (PV 3%) and neutropenia (ET = 5%) (4). Ongoing trial was conducted for 73 PV/ET patients who could not tolerate and respond to hydroxyurea. Santos and Verstovsek stated that INCB 018424 had a significant effect in PV/ET treatment in those who are resistant or do not tolerate hydroxyurea (45).

2. SB 1518

SB1518 is a kinase inhibitor drug acting as a competitive inhibitor of ATP in JAK2 and mutated JAK, and is more specific for JAK2 compared with JAK1 and JAK3. It can also inhibit FLT3 and its mutation of D835Y, the proliferation of leukemia and lymphoma cells in humans depending on the activation of FLT3 or JAK2 (46-48). Pre-clinical assessments confirmed proliferation inhibition of EPOR and JAK2V617F expressing Ba/F3 cells by SB1518, which is associated with reduced JAK2 and STAT5 phosphorylation (4).

There are two clinical trials for patients with MF. First, 43 patients (MF= 36, AML = 7), 78% of whom were JAK2 mutation-positive and 65% had enlarged spleen (splenomegaly) (median size of 13cm), were studied. Out of 25 patients with an enlarged spleen, 7 had 50% or higher reduction in size and 7 others showed a reduction in size of 35-50%. Side effects include diarrhea, vomiting and low PLT count (34). In the second trial, 20 patients were investigated. 85% had an enlarged spleen (median 17cm) and 85% were JAK2 mutation positive. This experiment was carried out only for a few months but reduced size of spleen was observed in some cases, and 2 out of 9 transfusion-dependent patients no longer needed blood transfusion. Side effects included nausea, fatigue, diarrhea and dehydration (49, 50).

3. TG 101348

TG 101348 is a selective JAK inhibitor with selective inhibition capacity of JAK2, inhibiting the growth of hematopoietic colonies with MPLW515K and JAK2V617F mutations or mutations in exon 12 of JAK2. Studies showed that the drug is well tolerated in MF patients, and reduced the size of spleen and

improved symptoms in high doses (51). JAK2 inhibitory capacity in moderate or high-risk PV, POST PV and POST ET MF patients has been indicated (52).

Table 1. JAK inhibitors in clinical trials (6, 10, 78, 79).

Drug name	Target	Clinical trial	Improvemwnt	Side effects
CEP701	JAK2	Phase II in MPN	Splenomegaly ↓	GI, diarrhea nausea and vomiting, anemia, thrombocytopenia, throm bosis, leukocytosis, thrombocytosis
CYT387	JAK1/2, Tyk2168	Phase I/II in MPN	Splenomegaly ↓ Improvement of constitutional symptoms Improvement of anemia	Thrombocytopenia, increased transaminases, peripheral neuro-pathy, transient loss of blood pressure and light -headedness as first-dose effect
LY2784544	JAK2222	Phase I ongoing in MPN	Splenomegaly ↓ Improvement of constitutional symptoms (Improvement of bone marrow fibrosis)	N/A
SB1518	JAK2, Tyk2225	Phase II in MPN	Splenomegaly ↓ Improvement of constitutional symptoms	GI symptoms, diarrhea, nausea, thrombocytopenia
TG101348	JAK2227	Phase III in MPN	Splenomegaly ↓ Improvement of constitutional symptoms Normalization of leukocytosis and thrombocytosis (JAK2V617f allele burden	N/A
XL019	JAK2228	I/terminated	Neuronal toxicity	N/A
INCB18424	JAK1, JAK2	phase I/II MPN	Splenomegaly ↓ Improvement of constitutional symptoms	N/A

The trial was conducted on 59 moderate and high-risk MF patients with splenomegaly. 98% had enlarged spleen, 86% were JAK2 mutation positive and 37% were blood transfusion-dependent. 49% experienced significant reduction in spleen size. In case of treatment discontinuation, spleen size reduction was reversed within a few weeks. High WBC count was normalized in 73% of patients, and the majority of patients with high platelet counts were normalized. Improvement in fixed symptoms such as fatigue, itching and night sweats was observed. Allele burden was reduced in 59% of evaluated patients (mean reduction of 60%), and reduced BM fibrosis was seen in some patients. Side effects included diarrhea (76%), nausea (70%), vomiting (69%), neutropenia (15%), thrombocytopenia (33%) and transfusion-dependent anemia start (67%) (53-55).

4. XL 019

XL019 can reversibly inhibit JAKs, and is more specific for JAK2 compared with JAK1, 3. This drug can inhibit the growth of erythroid cells via inhibition the phosphorylation of EPO-stimulated STAT5. It also inhibits the growth of cell lines with active or overexpressed JAK2, including cell lines derived from patients with Hodgkin lymphoma, AML, ET and erythroleukemia (56-58).

In a study, 30 primary or Post/PV/ET patients were studied. A high dose leads to peripheral neuropathy. Consequently, low dose in 21 JAK2 mutation positive patients showed a reduction in spleen size in 12 patients. In 7 out of 9 patients, decreased WBC count was observed. Four patients had initially a pre-leukemic myelofibrosis with 10-19% blast count. Three of them showed reduction in blasts and two showed normalization of BM blasts. Adverse side effects including peripheral neuropathy, fatigue, and unsteady gait were reported (57, 58).

5. CYT 387

This is a pyrimidine derivative that effectively inhibiting JAK1 and JAK2 signals and to a lesser extent JAK3 signals. It also affects hematopoietic cells and causes their apoptosis, but has no effect on non-hematopoietic cells (59). In mouse models; it normalized WBC count and hematocrit, spleen size

and returned the level of inflammatory cytokines back to normal condition (59). It can also inhibit the growth of human erythroleukemia cells with jak2v617f and mplw515l but has little activity against bcr-abl positive cells (60). Phase I/II clinical trial was conducted on MF patients in 2009. 36 patients were recruited, 23 PMF, 8 Post-PV MF and 5 Post-PV MF patients, 81% of whom were JAK2 V617F mutation positive. Initial reports indicated a reduction in spleen size, control of some persistent symptoms of MF and improvement of anemia (61).

6. R723

This is a small bioavailable and selective JAK2 inhibitor with little impact on jak3, jak1, and Tyk (62).

In experiments on mouse models, in both low and high dose groups, a significant decrease in cell count occurred. The decrease in platelet count was observed in high dose group, with some reduction in spleen size, but anemia was not improved. Little effect was observed on the progression of BM fibrosis (62). No test has been conducted on humans up to now.

7. Ly2784544

This is a histone deacylase inhibitor with anti-inflammatory effect on infections. It is safe in healthy subjects but can reduce the proinflammatory cytokines without any effect on inflammatory cytokine production. Studies have also indicated the apoptosis induction capacity of this selective inhibitor in malignant jak2v617f clones while it has minimum impact on normal hematopoietic cells (63).

The main symptoms are diarrhea, nausea, vomiting, and gastrointestinal pains (64). The study that started in 2010 and is currently ongoing in PV, ET and MF patients, has been shown that this drug inhibits the spontaneous proliferation of hematopoietic PV and ET cells with jak2v617f mutation (65).

8. CEP-701

This is an oral tyrosine kinase inhibitor. It does not specifically target JAK2 but simultaneously inhibits mutated JAK2 WT and JAK2. In fact, it is a pseudo-alkali indocarbazole acting like an inhibitor of FLT3 and JAK2. In a preclinical study, CEP-701 inhibited mutant JAK2 and JAK2 WT signaling with IC50 of 1 nM (20).

Recently, two studies have been conducted. In the first study, 22 patients with JAK2+ primary or Post-PV myelofibrosis were recruited. 6 patients (27%) showed clinical improvement, reduced spleen size was observed in 3 patients, 2 patients became transfusion independent, and 1 patient showed reduced spleen size and improved cytopenia. Decrease in JAK2 allele burden or fibrosis was not observed in patients. Significant side effects, including anemia (14%), low platelet count (23%) and digestive problems (72%) were reported. In the second study, 26 patients (16 primary PMF, 3-Post-ET, 7 Post-PV) received a high dose of the drug. 6 patients showed spleen size reduction (median of 5.8 cm) but no changes in transfusion or decreased WBC count was observed. In patients receiving liquid compounds of this inhibitor, gastric complications are the major problem, with 71% grade (1-2) diarrhea and 29% grade (1-2) nausea. Capsule combination is better tolerated with 37% diarrhea and 37% nausea (66).

Santos et al in December 2009 reported that CEP 701 lead to medium effectiveness, but gastrointestinal toxicity is common in MF patients (13). In a study, 39 PV/ET patients with JAK2 mutation were subject to CEP 701. In 15 out of 18 cases with splenomegaly, decreased spleen size was seen, and 3 out of 5 phlebotomy dependent patients required less phlebotomy after 6 months of treatment. Plt and WBC counts did not show improvement and in some cases were aggravated. Gastric side effects are usually present. In 5 patients, new thrombotic events were observed, which indicated that CEP 701 could not inhibit thrombosis in high-risk PV/ET patients (67).

9. ITF 2357 (Givinostat)

This is an oral histone deacetylase (HDAC) inhibitor that appears to reduce spleen size and improve fixed signs in MPN patients. It is safe in healthy subjects but can reduce proinflammatory cytokines without affecting the production of inflammatory cytokines (68). HDAC inhibitor is not specific for JAK2 but affects mutant JAK2 gene as part of its extensive impact upon cancer cells (68, 69). HDAC inhibitors caused growth cessation and lead to cancer cells death. A small study was conducted on safety and efficacy of Givinostat in a number of JAK2 positive PV, ET, and MF patients. In 13 PV/ET patients, 1 showed complete response to treatment, 6 had partial response, and 4 patients had no response in the end of study, while 2 patients discontinued the study. Among 16 MF patients, major response was achieved in 3 patients. Reduction in spleen size was seen in 75% of PV/ET and 38% of MF patients, and most patients reduced pruritus, and in some cases reduced JAK2 V617F allele burden was observed (62, 70, 71).

10. MK-0683(Vorinostat)

This is an inhibitor of histone deacetylase used in solid tumors, lymphoma, and myeloma, regulating cell proliferation and apoptosis pathways in various types of tumors, the impact of which on MF has been demonstrated (72, 73). This drug inhibits spontaneous

proliferation of hematopoietic cells in PV and ET patients with JAK2 V617F mutation. It also normalizes peripheral blood cell counts and reduces splenomegaly in mice with JAK2V617F mutation as well as normalizing platelet count in mice. Renal function impairment together with increase in creatinine level, hair loss, fatigue, and diarrhea are among its complications (74).

11. LBH589 (Panobinostat)

This is an HDACi, that reduces the levels of JAK2 V617F in MF patients. In a preliminary study of LBH589 (Panobinostat) in patients with progressive hematologic malignancies, 2 to 4 MF patients showed clinically tolerable recovery (75).

12. RAD 001

This is an oral inhibitor of MTOR kinase, part of a signaling systemin blood cells.. MTOR is often aberrantly activated in cancer cells (76). Initially successful investigations have been conducted on the effects of MTOR inhibitors on solid cancers and lymphoma. Inhibitory effects of RAD 001 in proliferation of JAK2 mutant cells and reduction of basic symptoms in MPN patients were reviewed, and it was concluded that the use of RAD 001 in 26 MF patients leads to acceptable reduction in spleen size, reduces underlying disease symptoms and itching, and is well tolerated. However, no significant changes in JAK2 V617F allelic burden was observed in studied subjects (77).

Conclusion

In this review article we express some TKI which are used or using in different clinical trial phases to treatment the negative Philadelphia chromosome such as PV, ET, and PMF. Although tyrosine kinase inhibitors, have side effects, they are useful rather than common methods to treatment the negative Philadelphia patient.

Authors' contributions

Tari: writing and rivising the paper. Yarhmadi, Kaviani, Abroun and Soleimani: writing the paper. Hajifahali: final review the paper. Atashi: Correspondence

Conflicts of Interest

The authors declare that they have no conflict of interest.

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