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Preclinical and Clinical Experience with Dasatinib in Philadelphia Chromosome-Negative Leukemias and Myeloid Disorders

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Abstract

Recent advances in the molecular characterization of Philadelphia chromosome-negative (Ph⁻) leukemias and related myeloid disorders have provided a clear rationale for investigating novel targeted therapies. Dasatinib is a tyrosine kinase inhibitor with activity against BCR-ABL, platelet-derived growth factor receptors (PDGFRs), c- KIT, fibroblast growth factor receptors (FGFRs), SRC family kinases (SFKs), and EPHA receptors, all of which have been implicated in the pathogenesis of Ph⁻ leukemias and myeloid disorders. This review presents emerging data on the preclinical and clinical activity of dasatinib in these diseases, which suggest that larger clinical studies are warranted.

Keywords

Leukemia; myeloid; Philadelphia-negative; dasatinib; myelodysplastic syndrome; myeloproliferative neoplasm

1. Introduction

The discovery that chronic myeloid leukemia (CML) results from the presence of the Philadelphia chromosome (Ph) and resultant expression of the BCR-ABL fusion gene led to the development of targeted tyrosine kinase inhibitors (TKIs), which have significantly improved clinical management and patient outcomes. Subsequent progress in elucidating the molecular pathogenesis of related Ph-negative (Ph⁻) diseases has suggested that therapies targeted at specific tyrosine kinases may provide a new approach to treatment. As a result, several new TKIs and other therapeutic agents are currently under investigation for use in these diseases.

By targeting the tyrosine kinase activity of BCR-ABL, TKIs such as imatinib produce high rates of treatment responses in CML [1]. In vitro studies have shown that in addition to Abl, imatinib potently inhibits platelet-derived growth factor receptor (PDGFR) and the class III tyrosine kinase KIT [2], leading to clinical studies of imatinib in other hematologic diseases where these molecular targets may be important [3].

Dasatinib is a BCR-ABL inhibitor approved for CML and Ph-positive acute lymphoblastic leukemia (ALL) following imatinib failure. Importantly, as dasatinib also inhibits several other oncogenic tyrosine kinases, it may have clinical potential in other hematologic diseases. The aim of this review is to explore the pathogenesis of Ph– leukemias and myeloid diseases to determine if there is a rationale for performing clinical studies with dasatinib outside of its current indications and to discuss preliminary clinical data.

2. Dasatinib

Dasatinib is the most potent BCR-ABL inhibitor, inhibiting unmutated BCR-ABL 325-fold more potently than imatinib [4]. Other tyrosine kinases potently inhibited by dasatinib include those inhibited by imatinib, i.e., PDGFR and KIT, and additional targets, including SRC family kinases (SFKs) such as LYN, EPHA receptors, fibroblast growth factor receptor 1 (FGFR1), and epidermal growth factor receptor (EGFR) [5]. SFKs modulate signal transduction through multiple oncogenic pathways, particularly growth factor receptors including EGFR, PDGFR, FGFR, and vascular epidermal growth factor receptor (VEGFR). Because SFKs have key roles in cell adhesion, migration, and cell-cycle progression, and have subsequently been implicated in the development of cancer, they are attractive targets for novel anticancer therapy [6].

Dasatinib has broad efficacy against all-but-one BCR-ABL mutations that cause imatinib resistance in patients with CML [4,7]. Gain-of-function mutations of KIT play an important role in the oncogenesis of certain human malignancies, including a subset of hematologic neoplasms [8, 9]. Although imatinib is a potent inhibitor of wild-type KIT [10], many activating KIT mutations confer imatinib resistance [11]. Dasatinib potently inhibits wild-type KIT and imatinib-resistant KIT activation loop mutants, suggesting dasatinib may have clinical efficacy against hematologic neoplasms associated with these gain-of-function KIT mutations [12,13].

3. Philadelphia-negative leukemias and myeloid disorders

The hematologic disorders discussed in this review are categorized within the World Health Organization (WHO) classification of hematopoietic and lymphoid neoplasms [14]. Five main categories of myeloid diseases are recognized: acute myeloid leukemia (AML); myelodysplastic syndromes (MDS); myeloproliferative neoplasm (MPN); myelodysplastic/myeloproliferative neoplasms (MDS/MPN); and MPN associated with eosinophilia and abnormalities of PDGFR α , PDGFR β , or FGFR1. Other Ph– leukemias discussed include chronic lymphocytic leukemia (CLL) and ALL, both of which are categorized as lymphoid neoplasms [15]. There is preclinical evidence to indicate that molecular targets of dasatinib may be involved in the pathogenesis of several of these diseases.

3.1 AML and MDS

AML is a heterogeneous clonal disorder of hematopoietic progenitor cells and is the most common myeloid leukemia, with a prevalence of 3.8 cases per 100,000, rising to 17.9 cases per 100,000 adults aged 65 years and older [16]. In adults, AML is characterized by nonrandom clonal chromosome aberrations and pathogenically relevant genetic lesions

occurring in leukemic blasts [17]. The first stage of treatment, using a combination of cytarabine and an anthracycline, aims to achieve complete hematologic remission (CHR; marrow with less than 5% blasts, a neutrophil count greater than 1,000, and a platelet count greater than 100,000), with a secondary treatment phase to prolong remission. The long-term survival rate in adults with AML is approximately 30%. Most patients require salvage therapy because of resistance to treatment or relapse after remission [16].

MDS refers to a group of clonal disorders characterized by excessive apoptosis in the erythrocyte, granulocyte, and megakaryocyte hematopoietic pathways. According to French-American-British (FAB) classification, there are five types of MDS: refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-T), and chronic myelomonocytic leukemia (CMML) [18]. Although clonal, the acquisition of additional genetic abnormalities, including abnormalities in tyrosine kinases, means that MDS often progresses to AML [19,20]. To improve overall survival and slow the evolution to AML, treatment options range from high-intensity chemotherapy requiring hospitalization, to low-intensity treatment in an outpatient setting (e.g., differentiation-inducing agents or immunosuppressive agents), or supportive care only, depending on prognostic factors such as age and pretreatment genetic findings [21].

The c-KIT proto-oncogene, inhibited by imatinib and dasatinib in vitro, is expressed in more than two-thirds of AML and MDS cases [22]. However, in a study of 18 patients with AML or MDS treated with a single daily oral dose of imatinib 400 mg, imatinib did not show any significant activity, suggesting that inhibition of c-KIT alone is not sufficient to support a response to imatinib [23]. Mutated KIT has been associated with inferior outcomes and a higher cumulative incidence of relapse in AML [24,25]. Among patients with core-binding-factor acute myeloid leukemia (CBF-AML), which accounts for approximately 10%–15% of cases of AML, KIT mutations in the extracellular portion of the receptor (exon 8) or in the activation loop at codon 816 (exon 17) are detectable in approximately 30% of patients [26].

Different KIT mutations have varying sensitivity to specific TKIs. In vitro, cells carrying exon 17 mutations involving the N822 are sensitive to imatinib, whereas cells that harbor a D816 mutation in the A-loop region are resistant [11]. By contrast, dasatinib inhibits the D816 mutated form of KIT [12,13]. In a study of patients with de novo CBF-AML, exon 17 KIT mutations occurred exclusively at codon D816 in patients with inv(16) ($n = 61$), whereas in patients with t(8;21) ($n = 49$), they occurred at codons D816 or N822 [24]. In a study of 75 patients with MDS or MDS-derived acute myeloid leukemia (MDS-AML), a KIT mutation was detected in patients with RAEB-T, CMML, and MDS-AML. While some mutations (~7%) were at codon 816, the remainder were unique [27]. These data provide a rationale for using dasatinib in patients with AML or MDS who harbor imatinib-resistant mutations.

Recent in vitro studies suggest that SFKs are constitutively active in AML cells. In one study, the SFK family member LYN was found to be expressed in an active form in AML cells, and silencing LYN expression using small interfering RNA strongly inhibited proliferation [28]. A further study investigated the role of SFKs in the regulation of Signal

Transducer and Activator of Transcription (STAT) activation in myeloid leukemia cells. Two of six AML cell lines displayed constitutive STAT5 activation, whereas four cell lines had constitutive SFK activity. Treatment with SFK inhibitors suppressed STAT5 activation, decreased viability, and suppressed cell proliferation [29]. In addition, the internal tandem duplication of FMS-like tyrosine kinase 3 (FLT3/ITD) is a frequent mutation in AML that activates FLT3 and its downstream signal components, including LYN [30]. All of these studies indicate that LYN inhibition by dasatinib could be an additional benefit for patients with AML.

Clinically, the efficacy of dasatinib in nine patients with Ph⁺ AML and six patients with MDS/CMML was investigated as part of a phase II open-label study of 67 patients with various Ph⁺ myeloid diseases. A durable complete response (15 months) was observed in a male aged 80 years with AML, who was previously treated with intensive chemotherapy and expressed KIT in 66% of his bone marrow (BM) blasts. However, no KIT mutational analysis was performed. No objective clinical responses to dasatinib were observed in patients with MDS [31]. In a study evaluating dasatinib in 41 children and adolescents with various leukemia subtypes, including 14 patients with Ph⁺ AML, preliminary results demonstrated that one patient with Ph⁺ AML achieved no evidence of leukemia with dasatinib although BM regeneration was not observed [32]. A pilot study of dasatinib in patients with MDS is currently recruiting (NCT00624585).

3.2. MPN

The MPN category includes three classic Ph⁺ neoplasms: polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic or primary myelofibrosis (PMF), and other rare MPNs: chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), hypereosinophilic syndrome (HES), and mast cell (MC) diseases, including systemic mastocytosis (SM) [14].

Primary clinical features of classic MPNs are increased red-cell mass (PV), high platelet count (ET), and BM fibrosis (PMF). These disorders share many characteristics, including the risk of leukemic transformation. The annual incidences of PV and ET are both 2.2 cases per 100,000, and PMF is less common [33,34]. Phlebotomy and low-dose aspirin are the cornerstones of PV therapy, and aspirin of ET therapy, with the goal to diminish the high risk of thrombotic complications. PMF, which has a significantly worse prognosis than PV or ET, is managed with supportive care measurements, allogeneic stem cell transplant (allo-SCT), or experimental drug therapy [35].

Elevated plasma levels of PDGF found in patients with ET, PV, and PMF is thought to stimulate, along with several other cytokines, bone marrow fibrosis, a process that should be reversible with appropriate treatment (e.g., PDGFR inhibition) [3,36]. The clinical benefits of imatinib in PV have been reported in two studies where treatment with imatinib resulted in the reduction of phlebotomy requirements in some patients [37,38], thought to be the result of its non specific myelosuppressive effect. A high proportion of patients with PV, ET, or PMF have a somatic point mutation (V617F) in the gene coding for JAK2, a nonreceptor tyrosine kinase that regulates the phosphorylation of several pathways (e.g. JAK/STAT). Indeed, the JAK2-V617F mutation is a diagnostic criterion in the revised

classification of PV, ET, and PMF [14,39]. In a mouse model of PV, constitutive activation of STAT5 and detectable expression of the STAT5 target gene BCL-XL was observed in BM of both normal and polycythemic mice, with increased STAT5 phosphorylation and BCL-XL observed in spleens from JAK2-V617F recipients [40]. An in vitro study of the effect of imatinib on reporter cells expressing JAK2-V617F showed imatinib specifically inhibited cells expressing mutant JAK2 but not wild-type JAK2, albeit at concentrations too high to achieve in vivo and significantly higher than imatinib's effective therapeutic concentration in CML. Imatinib decreased the phosphorylation of mutant JAK2 and STAT5 [41]. In vitro, dasatinib inhibits STAT5 signaling and down-regulates the expression of STAT5 target genes, including BCL-X, MCL-1, and cyclin D1, resulting in the inhibition of cell proliferation and induction of apoptosis [42]. Dasatinib also suppresses the proliferative capacity of progenitor cells from PV in vitro. As JAK2-V617F transcript levels did not decrease, however, the molecular target of inhibition was undefined [43]. Eleven patients with PMF were included in the phase II trial of dasatinib in Ph⁻ myeloid diseases, and no objective clinical responses were observed, suggesting that dasatinib effects on STAT5 are insufficient in this disease [31].

Nonclassic MPNs are rare disorders characterized by chronically increased levels of peripheral blood neutrophils (CNL) or eosinophils (CEL/HES) [44,45]. Current management includes observation only for asymptomatic patients with no evidence of organ damage, systemic corticosteroid therapy for control of symptoms, and interferon alpha or hydroxyurea as steroid-sparing agents. In patients refractory to standard therapy that have life-threatening disease complications, the use of investigational drugs such as alemtuzumab or mepolizumab might be considered, but data on long-term efficacy and safety are limited [46]. The JAK2 mutation has been described in a few cases of CNL, but because it is rare disorder, determination of the true incidence is difficult [44]. FIP1L1-PDGFR α fusion is the most frequent genetic aberration in CEL/HES. An interstitial deletion on chromosome 4q12 results in the fusion of two genes, FIP1L1 and PDGFR α , with the resultant protein having constitutive tyrosine kinase activity [45]. It should be noted, however, that in strict terms HES patients do not present detectable molecular lesions and diagnosis is based in part on excluding other causes of eosinophilia. Patients with an HES phenotype who display a clonal cytogenetic or molecular abnormality are reclassified as having CEL [47]. The role of FIP1L1-PDGFR α in disease induction has been confirmed by its disappearance in patients successfully treated with imatinib [46,48].

In a study of 63 patients treated with imatinib 100 to 400 mg daily, 27 patients were identified as carrying the FIP1L1-PDGFR rearrangement. All 27 achieved CHR and became negative for fusion transcripts. After a median follow-up of 25 months, all 27 patients remained in CHR and continued imatinib treatment. Of the 36 patients who did not carry the rearrangement; only five achieved a CHR, which was lost in all cases after 1 to 15 months [49]. Relapses with imatinib have been attributed to mutations in PDGFR that confer resistance; however, this is very rare occurrence. In one of only two cases reported, sequencing of the PDGFR kinase domain at the time of relapse showed the fusion protein had acquired a T674I mutation in the ATP-binding region of PDGFR [50].

In vitro, dasatinib causes dose-dependent inhibition of proliferation and apoptosis in an eosinophil leukemia cell line expressing FIP1L1-PDGFR (EOL-1) [51], thereby providing a rationale for its use in these patients. In addition, a novel fusion of TEL (an ETS family transcription factor) with LYN was identified in a patient with CEL and myelofibrosis who had a chromosomal abnormality (12;8)(p13;q11q21). This patient was refractory to both imatinib therapy and allo-SCT, and subsequently died of blastic transformation. In vitro, the proliferation of TEL-LYN-transfected Ba/F3 cells and the formation of colonies by hematopoietic stem cells (HSCs) expressing TEL-LYN were suppressed by dasatinib but not imatinib [52], providing a further example of a molecular target in HES/CEL that may be of clinical relevance for dasatinib treatment.

In the phase II study of Ph⁺ myeloid diseases, which included eight patients with HES/CEL, one woman aged 48 years with HES previously treated with imatinib but without a response, achieved a complete response with dasatinib. Tests for KIT-D816V and PDGFR α were negative. The response was durable and the patient relapsed after 58 weeks while off therapy because of toxicity [31].

3.2.1. MC Diseases—MC diseases are defined by an abnormal accumulation of tissue MCs in one or more organ systems. SM is a persistent disease that can be categorized as indolent SM (ISM), aggressive SM (ASM), SM with associated clonal hematologic non MC-lineage disease (AHNMD), and MC leukemia (MCL) [53]. Although standard cytoreductive therapies for SM may provide symptomatic improvements, responses are transient and the overall prognosis, which is poor for patients with ASM, SM-AHNMD, or MCL, remains unchanged [54]. In a study of ten patients with symptomatic systemic MC disease who were treated with imatinib at a dose of either 100 mg or 400 mg per day, five had a measurable response to the drug, with two experiencing complete clinical and histologic remission. In five patients with eosinophilia, three had complete clinical and hematologic remission. The other two patients, who did not respond to imatinib, had a KIT-D816V mutation [55]. The clonal nature of SM is evidenced by detection of the imatinib-resistant D816V mutation of KIT in more than 80% of SM cases [11,13,55].

Because dasatinib inhibits various KIT mutants, wild-type KIT, and FIP1L1-PDGFR α in vitro, it is likely to be useful in patients who have SM, particularly those with an associated CEL (SM-CEL) [51]. In the phase II study of Ph⁺ myeloid diseases, the overall response rate to dasatinib in patients with SM was 11/33 (33%). Only two patients, however, one with SM-PMF (KIT-D816V negative, FIP1L1-PDGFR α negative, JAK2-V617F positive) and one with SM-CEL (KIT-D816V negative, FIP1L1-PDGFR α negative, JAK2 V617F negative), achieved complete responses (elimination of mastocytosis) lasting 5 and 16 months, respectively. The patient with SM-CEL had a cytogenetic abnormality involving the gene for PDGFR β that was sensitive to dasatinib and was the probable reason for response. Nine patients (six with ISM and three with ASM) had symptomatic improvement. One responding patient with ISM was KIT-D816V negative, whereas others were positive; none had cytogenetic abnormality [31].

In preliminary studies and case reports, clinical responses to dasatinib have been reported among patients with various forms of SM who have the KIT-D816V mutation. Of six

patients with ASM and a detectable D816V mutation, major responses were observed in three patients, and a measurable decrease in the burden of neoplastic MCs was achieved in five patients. All patients, however, had to suspend therapy because of toxicity [56]. A case study of one patient with SM-AHNMD harbouring the c-KIT mutation reported symptomatic improvements after dasatinib treatment for 13 weeks [57]. The responses of two patients with SM and a D816V mutation treated with dasatinib were also recently reported. Dasatinib therapy was well tolerated by both patients, and clinical features rapidly improved. A BM aspirate performed on the first patient after 18 months demonstrated residual involvement with the disease, with the disappearance of D816V point mutation, mild leukocytosis, and resolution of anemia and thrombocytopenia. Both patients remained on dasatinib at the time of reporting [58]. In a case study of four patients with SM, early adverse effects were reported in two patients, likely to have been mediated by mast cell mediator release. However, major symptomatic improvement was observed with the resolution of diarrhoea and pruritus. Two further cases showed no response to dasatinib therapy [59]. This small series of studies suggests that dasatinib has clinical activity in some patients with SM, and can reduce symptoms.

The KIT-D816V mutant is not considered to represent a fully transforming oncoprotein in SM and it has been hypothesized that other signaling pathways may play a role in malignant transformation/progression. One possible novel pathway involving LYN and Bruton's tyrosine kinase (BTK) has been described. In primary neoplastic MCs and in the human MC leukemia cell line HMC-1, LYN and BTK were constitutively phosphorylated. Dasatinib inhibited LYN/BTK activation in both cell types [60], which may also explain the synergistic growth-inhibitory effects of dasatinib and a second TKI (PKC412) on neoplastic MCs expressing KIT-D816V in vitro [61].

3.3. MDS/MPN

The WHO MDS/MPN category, signified by increased cell numbers in peripheral blood accompanied by defective hematopoiesis, includes chronic and juvenile myelomonocytic leukemias (CMML and JMML) and atypical (BCR-ABL-negative) CML. No consistently effective therapy is available for MDS/MPN. Various chemotherapy regimens have been tried, but with modest success. Because only allo-SCT appears to be curative, targeted therapies are currently under evaluation, including farnesyltransferase inhibitors that target RAS protein maturation [62]. Although mutations involving the RAS signal transduction pathway are the most prevalent in JMML and CMML, other mutations identified in CMML include an activating G1849T mutation of JAK2 [63] and FIP1L1-PDGFR α [64], suggesting a potential use for dasatinib in a subset of patients.

EPHA receptors may also have an oncogenic role in MPN. Among 280 patients with MPN (73 PV, 65 ET, 24 ISM, 24 CMML, 8 HES, 50 CML, and 36 Ph- CML) and 38 healthy controls, normal blood cells were negative for EPHA3 expression but EPHA3 was significantly elevated in 45% of PV, 55% of ET, 90% of CMML, 100% of ISM, 30% of CML, 15% of HES, and 80% of Ph-CML patients. Incubation with dasatinib resulted in significant inhibition of EPHA3 phosphorylation, apoptosis, colony growth reduction, and proliferation [65].

To date, there are no clinical data for dasatinib treatment in patients with MDS/MPN.

3.4. MPN associated with eosinophilia and abnormalities of PDGFR α , PDGFR β , or FGFR1

Currently, imatinib is the first-line treatment option for patients with MPN associated with abnormalities of PDGFR α or PDGFR β . However, imatinib is ineffective in MPN associated with FGFR1 translocations and only allo-SCT appears to eradicate or suppress the malignant clone [66]. A fusion mutation involving the FGFR1 gene on chromosome 8p11 is the characteristic feature of stem cell leukemic/lymphoma syndrome (SCLL), a condition with features of both lymphoma and eosinophilic MPD [67]. PDGFR α activation is associated with CEL and results from fusion mutations that include KIF5B-PDGFR α , BCR-PDGFR α and CDK5RAP2-PDGFR α , all of which are inhibited by imatinib [68]. Fusions of the tyrosine kinase-encoding region of PDGFR β to a variety of other genes have also been described and are most commonly associated with CMML with eosinophilia (ETV6-PDGFR β , KIAA1509-PDGFR β , HIP1-PDGFR β) and unclassified MPD with eosinophilia (NIN-PDGFR β , TP53BP1-PDGFR β , PDE4DIP-PDGFR β) [68]. A case report of two patients with PDGFR β fusions to the genes PRKG2 and SPTBN1 demonstrated that both patients achieved complete molecular remission after introduction of imatinib therapy [69]. Responses with imatinib have also been reported in cases of MPN associated with eosinophilia [3]. Four patients who had MPN, eosinophilia, and either the ETV6-PDGFR β fusion gene (three patients) or a t(5;12) translocation involving PDGFR β and an unknown partner gene, were treated with imatinib 400 mg once daily. In all four patients, a normal blood count was achieved within 4 weeks of treatment. The t(5;12) translocation was undetectable by 12 weeks in three patients and by 36 weeks in the fourth patient. In the three patients with ETV6-PDGFR β , the transcript level decreased, and in one patient, it became undetectable by 36 weeks. All responses were durable at 9 to 12 months of follow-up [70]. Because dasatinib has been shown to inhibit PDGFR and FGFR in vitro, it may also be expected to show activity against these mutations [5], although no case reports have been published.

3.5. ALL

ALL can originate from several genetic lesions in lymphoid progenitor cells, and chromosomal translocations are a defining characteristic. ALL affects both children and adults. In B-cell ALL, the most frequent form of ALL in children, approximately 25% of cases originate from a translocation between chromosomes 12 and 21 [71]. In adults, the most frequent chromosomal translocation is the Ph chromosome, which has an overall incidence of approximately 25%, rising to more than 50% among patients older than 55 years [71,72]. The efficacy of dasatinib in patients with Ph+ ALL following imatinib failure is well established [73], and is outside the scope of this review.

With the exception of patients with mature B-cell ALL, who are treated only with relatively short-term intensive chemotherapy, treatment for ALL typically includes long-term maintenance therapy to prevent relapse. During maintenance, patients receive chemotherapy (mercaptopurine and methotrexate) for 2–2.5 years. The most intensive form of treatment for ALL is allo-SCT, which can be curative, but is associated with substantial morbidity and mortality [71].

Translocations resulting in oncogenic fusion transcription factors occur frequently in ALL, with amplification of the NUP214-ABL1 oncogene detected in patients with T-cell ALL [74]. In one study, the activity of dasatinib, alongside the dual ABL/SRC TKIs bosutinib and INNO-406, was assessed against a human NUP214-ABL1-expressing cell line. Each compound potently inhibited proliferation and induced apoptosis, suggesting these agents represent promising therapeutic approaches for NUP214-ABL1-positive leukemia [75]. Preliminary data suggest that dasatinib has superior activity against NUP214-ABL1-expressing cell lines compared with imatinib and nilotinib. The inhibition of cellular proliferation was associated with time-dependent induction of apoptosis and inhibition of ABL, CRKL, and STAT5 phosphorylation. Moreover, dasatinib was active in a NUP214-ABL1-positive leukemia xenograft murine model and in marrow lymphoblasts from a patient with NUP214-ABL1-positive T-ALL [74]. In the study evaluating dasatinib in pediatric leukemia, seven patients had Ph- ALL. One patient had a temporary significant decrease in peripheral blood blast count with no decrease in BM blasts and without achieving remission [32].

3.6. CLL

CLL is the most common leukemia in Europe and North America, although it is rarely diagnosed in people younger than 50 years. CLL is characterized by the accumulation of mature B-cells, which is due to defective apoptosis rather than increased proliferation. In contrast to other B-cell malignancies, the most frequent genetic abnormalities in CLL result from mutations, deletions, or trisomies rather than translocations [76]. Management of CLL involves chemotherapy using purine analogues such as fludarabine, monoclonal antibodies (e.g. rituximab or alemtuzumab), or in selected cases, high-dose chemotherapy, or allo-SCT. Because the B-cell receptor is devoid of intrinsic kinase activity, nonreceptor kinases (e.g. SFKs) are thought to play crucial roles in the intracellular transduction of survival signals [77]. Indeed, LYN is aberrantly expressed in CLL cells, correlating with defective apoptosis [78].

The effects of dasatinib on the intracellular signaling and survival of CLL cells has been investigated in vitro. Dasatinib 100 nM treatment decreased levels of the activated, phosphorylated forms of SFKs, AKT, ERK1/2 and p38, inhibited proliferation, and induced apoptosis [77]. Because kinase inhibition and the DNA damage response induce apoptosis by different mechanisms, sensitization of CLL cell lines to the cytotoxic drug fludarabine with dasatinib was investigated. A combination of dasatinib 5 μ M and fludarabine increased the apoptosis induction of each by approximately 50% [77]. Dasatinib also sensitized CLL cells to the chemotherapeutic agents chlorambucil [79] and methylprednisolone [80]. Also, in primary CLL samples, cells with nonmutated immunoglobulin variable heavy chain (IgVH) genes were more sensitive to dasatinib than those with mutated IgVH genes [77]. In a second in vitro study, dasatinib induced apoptosis in primary leukemic cells from 40 patients with CLL. The pro-apoptotic activity was not observed in normal B- or T-cells or blood mononuclear cells from healthy donors, suggesting that dasatinib has a specific effect on CLL cells. Leukemic cells that expressed ZAP-70 were significantly more sensitive to dasatinib-induced apoptosis than CLL cells lacking expression of ZAP-70. The process was associated with impairment of B-cell receptor signaling, decreased tyrosine kinase activity

and regulation of genes and proteins related to apoptosis [81]. The efficacy of dasatinib in patients with previously treated CLL/SLL is currently being investigated in a phase II clinical study (NCT00438854) [82].

Summaries of preclinical and clinical experience with dasatinib in Ph⁺ leukemias and myeloid disorders are provided in Table 1.

4. Conclusions

Increased understanding of the molecular pathogenesis of Ph⁺ hematologic disorders and the pathogenic role of PDGFRs, KIT mutations, SFKs, and constitutively active PDGFR α and PDGFR β fusion proteins supports the therapeutic use of dasatinib in a range of conditions outside of its current indications. The inhibitory activity of dasatinib, as demonstrated in vitro, suggests dasatinib represents a potential new treatment option. Preliminary results have been obtained from the clinic across several different diseases and larger clinical studies are warranted.

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Table 1

Preclinical and clinical studies with dasatinib in Philadelphia chromosome-negative leukemias and myeloid disorders

Indication	Preclinical		Clinical	
	Dasatinib molecular target	Patients, n	Response	
Acute myeloid leukemia/myelodysplastic syndromes	D816 Mutated Form of KIT; LYN; STAT5 [12,13,28,29]	15	One patient with complete response [31]	
		14	One patient with no evidence of leukemia [32]	
Myeloid neoplasms	EPHA [65]			
Polycythemia vera, essential thrombocythemia, primary myelofibrosis	STAT5 [42]	11	No objective clinical responses [31]	
Chronic eosinophilic leukemia/hypereosinophilic syndrome	FIP-IL1-PDGFR α TEL-LYN [51,52]	8	One patient with complete response [31]	
Systemic mastocytosis	D816 Mutated Form of KIT LYN/BTK [12,13,61]	33	Two patients with complete response Nine patients with symptomatic improvement [31]	
		6	Three patients with major response Five patients with decrease in neoplastic mast cells [56]	
		1	Symptomatic improvement [57]	
		2	Symptomatic improvement [58]	
		4	Two patients had major symptomatic improvement [59]	
MPN associated with eosinophilia and abnormalities of PDGFR α , PDGFR β , or FGFR1	PDGFR, FGFR [5]			
Acute lymphoid leukemia (excluding Ph+ ALL)	NUP214-ABL1 [74,75]	7	One patient with decrease in peripheral blood blast count [32]	
Chronic lymphocytic leukemia	SFKs [77,81]			