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Novel strategies for patients with chronic myeloproliferative disorders

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Abstract

Purpose of review—This review focuses on new strategies for unmet clinical needs and on new targeted therapies in classical Philadelphia-negative myeloproliferative neoplasms (MPNs).

Recent findings—Meta-analyses in essential thrombocythemia (ET) documented *JAK2* V617F being associated with increased risk of thrombosis. New studies reinforced the evidence of leukocytosis as an independent risk factor for thrombosis in polycythemia vera (PV) and ET. In a phase II trial of Peg-IFN-α2a in PV patients, a decrease of *JAK2* mutant expression up to undetectable levels was demonstrated. New trials documented that 5-azacytidine and bortezomib have negligible effect in primary myelofibrosis (PMF), while thalidomide and tipifarnib produce 22% and 44% of response, respectively. In PMF, the JAK2 inhibitor INCB018424 resulted in a rapid and marked reduction in splenomegaly and a clinical improvement, with a modest effect on *JAK2* V617F burden.

Summary—Treating low-risk ET and PV patients presenting leukocytosis or *JAK2* V617F mutation in order to prevent thrombosis deserves a prospective validation. Pursuing clonal remission in PV by IFN needs new evidence. Tipifarnib may be added to conventional therapeutic instruments for symptomatic PMF. The results of anti-JAK2 targeted therapies are encouraging as regards symptoms reduction but not clonal remission.

Keywords

chronic myeloproliferative neoplasm; essential thrombocythemia; polycythemia vera; primary myelofibrosis; *JAK2* V617F

Introduction

The classical Philadelphia-negative myeloproliferative neoplasms (MPNs) [1], namely essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF), lack a therapeutic strategy aimed at efficiently target the molecular hallmark leading to malignancy. Thus, therapies are addressed to prevent the inherent propensity to incur clinically relevant events, like thrombosis and hemorrhage in PV and ET, or to alleviate the

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symptoms that mostly influence the patient's quality of life, like microvascular disturbances in PV and ET, or anemia and splenomegaly in PMF. Resolutive treatments, like stem cell transplantation, are limited to a minority of patients, and the risk of transplant-related mortality renders the appropriate selection of patients problematic, considering the chronic and often indolent nature of the diseases.

This review highlights recent developments in therapeutic strategies for patients with MPNs and critically evaluates the chance they have in resolving the unmet clinical needs and in contrasting the newly discovered molecular abnormalities, first of all the *JAK2* V617F gain of function mutation.

Resolving unmet clinical needs in MPNs

A number of unmet clinical needs, i.e. strategies whose non-performance is a leading cause of reduced survival and increased morbidity, has emerged in the last few years challenging the traditional therapeutic strategies in MPNs.

Treating low-risk patients with ET or PV

Clinical guidelines and expert opinions recommend to approach an individual patient with ET or PV by first identifying her/him potential risk to develop major thrombotic complications [2-5]. Risk stratification today is based on well established risk factors according which the incidence of cardiovascular complications is higher in patients aged more than 60 years or with a history of thrombosis. Recommendations for treatment in PV and ET include chemotherapy in high-risk disease, while asymptomatic low-risk patents, i.e. with an age lower than 60 years and no prior thrombosis can be safely observed. These recommendations are based on prospective trials showing that low-risk patients had a thrombotic risk comparable to that of the control population [6,7]. None of these trials, however, has a statistical power adequate to derive conclusive incidence figures for thrombosis. Thus, concern has been raised on the possibility that the population at low-risk could include a subset of patients for whom the currently used risk stratification is not completely fitting. The continuous search for new prognostic factors has brought up the hypothesis that two other factors could be taken into consideration in risk stratification, i.e. *JAK2* V617F mutational status and leukocytosis.

Two systematic literature reviews and meta-analyses were carried out to compare the frequency of clinically significant outcomes between *JAK2* V617F positive and wild-type patients with ET [8*,9*]. Both analyses included 17 studies up to February 2008. In the first analysis [8], incidence figures for thrombosis varied from 17% to 43%, and *JAK2* V617F positivity varied from 37% to 71%. A significant association of *JAK2* mutation with thrombosis was evident in half of these studies whereas no such correlation was documented in the remaining. Meta-analysis of 2,905 patients with ET and 778 patients with thrombosis showed that *JAK2* V617F patients have a twofold risk of developing thrombosis (OR = 1.84, 95%CI 1.40–2.43) with significant heterogeneity between studies. *JAK2* V617F patients were older at diagnosis, had higher hemoglobin levels, higher leukocyte counts and lower platelet counts. Given the exaggerating effect of smaller studies, larger series (>100 patients)

were analyzed separately (8 studies, 2,394 patients, 627 with thrombosis) and the effect remained significant (OR = 1.77, 95%CI 1.46–2.15).

The second meta-analysis [9] reported 2436 patients of whom 56% were found to be positive to *JAK2* mutation. *JAK2* V617F positivity was associated with clear increase in the odds of thrombosis (OR = 1.83, 95% CI 1.32-2.53) and much higher odds of transformation to PV (OR = 7.67, 95% CI 2.04-28.87). The mean difference of the WBC count between *JAK2* positive and negative patients was associated with an increased odds ratio for thrombosis.

These analyses represent the cumulative evidence on *JAK2* association with thrombosis in ET, with all its inherent biases and weaknesses. Therefore, these studies cannot prove direct causality. The effect on *JAK2* mutation is probably mediated through a distinct prothrombotic phenotype, that includes leukocytosis, older age and thrombosis at presentation, features that are well-established risk factors of thrombosis even in the pre-*JAK2* era.

Recent ad hoc studies have added new evidence to leukocytosis as an independent risk factor for thrombosis in PV and ET. Gangat et al showed that leukocyte count greater than 15×10^9 per liter was an independent predictor of inferior survival, leukemic transformation and venous thrombosis in patients with PV [10]. Three large cohort studies reported similar results in ET [11-13]. In addition, Carobbio and colleagues [14**] demonstrated that ‘low-risk’ ET patients with leukocytosis have the same probability to develop a vascular event in the follow-up of ‘high-risk’ patients without leukocytosis. This paper deserves high consideration since it documented that using a prognostic score that included leukocytosis had more discrimination accuracy over the previous one, namely, the new score better differentiated between individual survivors and non survivors.

Now, prospective thorough validation is needed before leukocytosis could be confidently applied to clinical practice as a criterion for selecting low-risk ET and PV patients in need for cytoreductive treatment.

Pursuing clonal remission in PV

Hydroxyurea, the most commonly used agent to treat PV and ET patients requiring cytoreductive therapy, is unable to eradicate the disease malignant clone. This goal has been recently approached in PV patients placing high expectation on the therapeutic efficacy of interferon. Interferon alpha (IFNα) was since a long time considered for the treatment of patients with MPNs because this agent suppresses the proliferation of hematopoietic progenitors. In addition, IFNα, has proven to induce reversion from monoclonal to polyclonal patterns of hematopoiesis in some cases [15-17]. IFNα however, may also have immunological properties by inducing immune responses to candidate tumor antigens, among which the recently identified PV-associated tumor antigens may represent targets for immune effectors [18].

In a multicenter phase II trial of Peg-IFN-α2a in 27 PV patients, Kiladjian et al. [19**] showed a decrease of *JAK2* mutant expression in 24 cases (89%) and in 1 patient mutant

JAK2 was no longer detectable after 12 months of therapy. A profound and sustained molecular response with a *JAK2* V617F allele burden below 1.0% was further reported in two patients with PV treated with IFN-α.2b. Discontinuation of the drug in one of the patients was followed by a sustained long-lasting (12 months of follow-up) major molecular response [20].

These results open new perspectives in the management of PV and questions the therapeutic strategy of limiting cytoreductive therapy to patients at high-risk of vascular events.

Reducing the risk and discomfort of unnecessary hematocrit-lowering strategies in PV

Only the elevated hematocrit (Hct) in PV is a mandatory therapy target among the primary consequences of the myeloproliferative process of MPNs. This derives from scientific evidence testifying a relation between the increased Hct values and the risk of thrombosis [21,22], while the same is not documented for elevated platelet count in ET. This prompted to generally recommend to keep PV patients at a Hct level below 45% in man and 42% in women by phlebotomies or cytoreductive therapy [3,23]. However, in the ECLAP study [24], a randomized controlled study comparing aspirin versus placebo in PV, only 48% of patients had Hct values below the recommended threshold, whereas 39% and 13% of patients remained between 45% and 50%, and greater than 50%, respectively. Moreover, a multivariate analysis considering all the confounders failed to show any correlation between these Hct values and thrombosis. The uncertainty described above has raised concern on the unnecessary over exposition of patients to phlebotomy or cytoreductive drugs, and on the logistic, economic, and medical consequences of such intensive strategy, like severe iron deficiency or risk of chemotherapy-induced late malignancy. The recognition of this unmet clinical need has prompted Italian Investigators to launch a prospective, randomized clinical study (CYTO-PV).

Molecularly targeted therapies for MPNs

The era of molecularly targeted therapy, that implies to use drugs that contrast molecular hallmarks for which a direct influence on tumorigenesis is proven, has received a drive in light of the efficacy of imatinib and second generation abl kinase inhibitors for the treatment of chronic myeloid leukemia. In the classical Ph-negative MPNs, the lack of a clear documentation that malignancy depends from a well defined molecular mechanism, and that *JAK2* mutations only marginally impact on the diseases progression and outcome, has empirically extended the paradigm of targeted therapy to potential targets not specifically engaged in the malignancy.

Therapies against non specific molecular targets

In the last two years, a number of non specific molecular hallmarks of malignancy have been the target of experimental therapies for MPNs.

The farnesyltransferase-inhibitor tipifarnib inhibits *in vitro* proliferation of myeloid progenitors from patients with MPNs. In a phase II clinical trial, single-agent oral tipifarnib (300 mg twice daily × 21 of 28 days) was given to 34 symptomatic patients with either PMF or post-PV/ET MF [25*]. Response rate was 33% for hepato-splenomegaly and 38% for

transfusion-requiring anemia. No favorable changes occurred in bone marrow fibrosis, angiogenesis or cytogenetic status. Clinical response did not correlate with either degree of colony growth or measurable decrease in quantitative *JAK2* V617F levels seen in pretreatment samples.

Hematopoietic cells from patients with PMF have been identified as having activation of the NF-kB pathway which in turn activates the production of TGF-β, a stimulator of fibrogenesis and osteogenesis [26]. Based on these observations and results obtained in a murine model [27], clinical trials have been launched to test the efficacy and safety of bortezomib, a proteasome inhibitor with anti-TGF-β properties, in patients with PMF. In a phase II clinical study in 11 patients [28*], therapy with bortezomib was found substantially ineffective and according to the IWG-MRT criteria [29], no patient achieved even a clinical improvement. In another phase I-II study performed by the MPD Research Consortium [30], 12 patients with PMF, refractory or not suitable to first line chemotherapy, were treated with bortezomib given at day 1, 4, 8, and 11 at the dose of 0.8-1.3 mg/m^2, every 21 days × 6 cycles. The maximum-tolerated dose was 1.3 mg/m^2 for 4 days every 3 weeks. No complete, major or moderate responses according to the EUMNET response criteria [31] were documented.

In patients with PMF, hypermethylation in the p15INK4b, p16INK4a, calcitonin, RARß2, and CXCR4 gene has been demonstrated [32-34**]. Shi and co-workers [35] documented that exposure of CD34+ cells derived from PMF patients to a DNA methyltransferased inhibitor 5-Aza-2'-deoxycytidine (5-Aza) in combination with histone deacetylase inhibitor, trichostatin A (TSA) resulted in a significant reduction of both *JAK2* V617F-positive hematopoietic colonies and the number of colonies that contained chromosomal abnormalities in two JAK2 V617F-negative PMF patients. Bogani and coworkers [34**] documented that following incubation with 5-Aza, the percentage of PMF CD34+ cells expressing CXCR4 increased 3-10 times, whereas CXCR4 mRNA level increased approximately 4 times. 5-Aza-treated PMF CD34+ cells displayed almost complete reversal of CpG1 island 1 hypermethylation and showed enhanced migration in vitro in response to SDF-1. These data provided a rationale for therapy with chromatin-modifying agents for patients with PMF.

In a phase-II trial, 34 patients with either relapsed/refractory or newly diagnosed PMF with poor prognosis were treated with 5-Aza at the dose of 75 mg/m^2 subcutaneously daily for 7 days, every 4 weeks [36*]. The median duration of 5-Aza therapy was 5.5 months and a clinical response was observed in 8 (24%). Only one patient met all the criteria for complete remission except for the achievement of bone marrow histological remission. Responses were observed both in patients with and without *JAK2* V617F mutation. Therapy with 5-Aza resulted in gradual and significant decline of the methylation from the baseline but the degree of global DNA hypomethylation achieved during treatment was not different between responders and non-responders. The outcome of 10 other patients treated with an abbreviated 5-day 5-Aza, reaffirmed the limited benefit of this agent in PMF [37*].

Fifteen patients treated with low-dose thalidomide were audited and the responses evaluated according to the EUMNET criteria [38*]. Three of 7 transfusion-dependent patients had a fall in transfusion requirement (43%), two became transfusion independent.

In three consecutive patients with del(5q)-associated PMF or post-PV MF (all three patients were *JAK2*V617F-positive), lenalidomide, an immunomodulatory, antiangiogenic and antineoplastic drug structurally related to thalidomide, produced clinical and hematological complete response [39*]. Based on this limited experience, the Authors encouraged screening for del(5q) in all patients with PMF or post-PV MF; whenever found, lenalidomide therapy should be offered and continued indefinitely, if tolerated.

In summary, trials with new non specific targeted therapies have confirmed that their use in PMF is of modest clinical activity. Tipifarnib may be added to conventional therapeutic instruments for symptomatic PMF. Lenalidomide has a role in del(5q) variant of PMF.

Anti JAK2 targeted therapy

At present, several groups are developing small molecules that selectively target JAK2 kinase, including *JAK2* V617F, or agents which were previously used for non-MPN, but considered with significant therapeutic potential in MPNs due to their “off-target” JAK2 inhibitory activity (Table 1). These drugs demonstrated the *in vitro* ability to significantly inhibit *JAK2* V617F positive cell lines, or hemopoietic progenitor cells from *JAK2* V617F positive MPN patients [40-50].

A number of such JAK2 inhibitors have been tested in *in vivo* animal models of *JAK2* V617F-induced hematopoietic diseases. In a mouse xenograft model with Ba/F3-V617F cells, administration of TG 101207 rescued the animals from a fully penetrant fatal hematopoietic malignancy [51]. In a murine bone marrow transplant assay of established PV, animals treated with TG 101348 showed a dose dependent reduction in the degree of splenomegaly, extramedullary hematopoiesis and reduction of bone marrow reticulin with survival advantage [52].

Currently the following agents are being studied in patients with MPNs: MK-0456, CEP-701, INCBO1842, XL 019, AT 9283. In a phase I/II trial with INCB018424, a potent and selective inhibitor of JAK1 and JAK2, a dose of 25 or 50 mg PO twice daily resulted in a rapid and marked reduction in splenomegaly, marked and durable improvement in constitutional symptoms and resulted in striking reduction of systemic cytokine levels. However, minor reduction of V617F:WT *JAK2* ratio was noted in both peripheral blood and bone marrow. Reversible thrombocytopenia was the dose limiting toxicity [53].

The results of anti-JAK2 targeted therapies are encouraging as regards symptoms reduction but not disease clonal remission.

Conclusion

To face unmet clinical needs in MPNs, the scientific community has reacted by engaging an extensive experimental approach. However, neither the concern of leaving untreated low-risk ET and PV patients presenting leukocytosis or *JAK2* V617F mutation, nor pursuing clonal

remission in PV by IFNα, or reducing the risk and discomfort of unnecessary hematocrit-lowering strategies in PV, have enough evidence to be translated into strategies for clinical practice. The preliminary results with anti-JAK2 agents reinforce the concept that clinical development of these agents requires a new set of skills and a greater degree of complexity when compared with conventionally developed chemotherapeutic agents, like incorporation of novel endpoints of clinical benefit and precise selection of patients.

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thrombocythemia. In the model with conventional risk factors alone, C statistic ratio for total thrombosis was 0.63 and when leukocytosis was added, the change was small (C = 0.67). In contrast, in younger and asymptomatic patients (low-risk category), C statistic value indicated an high risk for thrombosis in patients with leukocytosis, similar to that calculated in conventionally defined high-risk group (C = 0.65). The best leukocyte cutoff values for predicting the events was found to be 9.4×10^9/L.

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