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Therapy-associated leukemic transformation in myeloproliferative neoplasms – What do we know?

Danielle Cuthbert[a], Brady Lee Stein[b,*]

[a] McGaw Medical Center of Northwestern University, Department of Internal Medicine, 676 North St. Clair Street, Arkes Suite 2330, Chicago, IL 60611, USA

[b] Northwestern University Feinberg School of Medicine, Division of Hematology/Oncology, Department of Medicine, 645 N. Michigan Avenue, Suite 1020, Chicago, IL 60611, USA

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ABSTRACT

Myeloproliferative Neoplasms (MPNs) are a group of progressive diseases that share a common pathogenesis, clinical and laboratory features, as well as a spontaneous risk of secondary AML. Certain MPN therapies have been associated with an increased risk of leukemic conversion, with robust data highlighting the highest rates with ^{32}P, chlorambucil, and pipobroman. Herein, we review risk factors for leukemic transformation, including therapy-related MPN-BP, with a focus on the debate surrounding the potential leukemogenicity of hydroxyurea. Lastly, we discuss emerging studies on the association between ruxolitinib and high grade B-cell lymphomas. We conclude that statistical associations have not implicated hydroxyurea monotherapy as leukemogenic. However, it is difficult to definitely disprove an association, as large prospective, controlled studies with decades of follow-up would be needed to draw conclusions. Overall, the concept of therapy-related neoplasms remains important to the field, and mandates judicious selection and sequencing of therapies for MPN patients.

1. Introduction

Polycythemia Vera (PV), Essential Thrombocytosis (ET) and Myelofibrosis (MF) share a number of clinical and laboratory features, including mature blood cell proliferation, a thrombo-hemorrhagic tendency, extramedullary hematopoiesis, often manifest as splenomegaly, variable degrees of bone marrow fibrosis and acute leukemic transformation. Dr. Dameshek recognized these shared features, and classified these entities together as "Myeloproliferative Disorders." Dr. Dameshek also speculated upon a shared pathogenesis, which was confirmed nearly 55 years after his landmark article [1]. It is now known that the majority of patients with MPNs will have 1 of 3 driver mutations, which universally activate the JAK-STAT pathway. *JAK2* V617F was the first of Dameshek's "myelostimuatory" factors to be discovered. *JAK2*, a tyrosine kinase gene found on chromosome 9, is essential to normal hematopoiesis, but when mutated, results in constitutive activation and overactive signaling of the JAK-STAT pathway [2,3]. *JAK2* is mutated in nearly 99% of PV cases and 60% of patients with ET and MF [4,5]. Subsequently, mutations in the *MPL* gene that encodes the thrombopoietin receptor, and lead to its activation, were described in 2006 [6]. *MPL* mutations are only seen in ET and MF – approximately 3% and 7% of cases, respectively [5]. In 2013, two groups described recurrent mutations of the *CALR* gene, which codes for calreticulin, an intracellular regulatory protein involved in signaling and transport within the endoplasmic reticulum [7,8]. The contribution of mutant *CALR* has been recently described, with binding and activation of *MPL* as the proposed mechanism

* Corresponding author.
E-mail addresses: danielle.cuthbert@northwestern.edu (D. Cuthbert), bstein@nm.org (B.L. Stein).

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leading to JAK-STAT activation [9,10]. *CALR* is mutated 15–35% of all patients with ET and MF [5]. Interestingly, approximately 10% of patients of ET and MF can lack mutations in *JAK2*, *CALR* or *MPL* and are known as "triple negative MPNs." [11,12].

In addition to these three "driver" mutations, additional somatic mutations have been noted in ET, PV, and MF patients. The prognostic impact is established in MF, as mutations involving *ASXL1*, *IDH1/2, EZH2,* and *SRSFR2* considered to be high risk molecular markers, especially so if more than 1 is present [13]. The adverse impact in ET and PV is under evaluation, but there is a suggestion that certain somatic mutations impact transformation and accordingly, prognosis. Recently, with long term molecular follow-up, additional non-driver mutations in ET and PV predicted for: cytopenias during hydroxyurea therapy (*DNMT3A*, *SRSF2*, *IDH1/2*, *TP53*); genetic instability (*SRSF2, RUNX1*); MF transformation (*SF3B1, IDH1/2*); AML (HR 12.2; *ASXL1, TP53, SRSF2, IDH1/2, RUNX1*), and decreased survival (*DNMT3A*, *SRSF2*, *SF3B1*, *IDH1/2*, *RUNX1*) [14].

Treatment goals in the MPNs include palliation of a pronounced symptom burden – driven by micro- and macrovascular distance, cytokine excess, organomegaly, and either cytosis or cytopenia. In PV, standard therapy includes anti-platelet therapy and phlebotomy. In both ET and PV, cytoreductive therapies are reserved for those with prior thrombosis, or advanced age [4]. Contemporary options include hydroxyurea, interferons and JAK-inhibitors (ruxolitinib is a second-line option for PV) [15]. In MF, stem cell transplant is curative, but high risk, and only an option for selected patients [4]. Otherwise, ruxolitinib is the only approved therapy. Androgens, epo-stimulating agents, and immunomodulatory drugs are options for anemic MF patients [16].

The MPNs are progressive diseases, and transformation of ET/PV to secondary MF impacts longevity; while physicians aim to prevent or delay transformation, there are no proven therapies to do so. As mentioned, there is a spontaneous risk for secondary AML, which is highest in MF patients. Historically, certain MPN therapies have been associated an increase in the risk of secondary AML. Herein, we review risk factors for leukemic transformation of chronic phase MPNs. We also review the impact of cytoreductive therapies, including hydroxyurea, on this risk. Last, a recent report described an association between high grade lymphoma and ruxolitinib in MF patients. We therefore review the association between MPN, ruxolitinib, and lymphoproliferative disorders.

2. Inherent risk of leukemic transformation

Patients with MPNs are at risk of spontaneous conversion to an MPN-blast phase (MPN-BP), characterized by 20% or more blasts in the peripheral blood or bone marrow [17]. Unfortunately, MPN-BP is an aggressive disease with a dismal prognosis. The risk of disease transformation differs among the subtypes of MPN, and is influenced by various risk factors including the molecular genetic profile (Table 1).

As mentioned, the incidence of MPN-BP is substantially higher in patients with MF in comparison to PV and ET. Studies estimate that the incidence of MPN-BP transformation among patients with MF is 10%–20% within the first 10 years after diagnosis [18]. In contrast, the incidence among patients with PV is 2.3% and 7.9% at 10 and 20 years, respectively [19]. ET has the lowest risk, with ~1% of patients developing AML within a decade after diagnosis [20]. Typically, ET/PV transforms to MPN-BP, through an interim MF phase, but direct transformations (ET/PV to MPN-BP) are occasionally observed. One study demonstrated transformation to AML in 4.1% of ET cases, 6.7% of PV patients, and 12.7% of patients with MF [10]. Interestingly, the incidence of AML is similar in post-ET and post-PV MF compared to patients with primary MF [21].

There are a multitude of clinical risk factors associated with leukemic transformation among patients with MPN. In addition to MPN subtype and disease duration, advanced age (> 60–65 years) has been associated with progression to MPN-BP [22]. Laboratory values including leukocytosis ($> 15–30 \times 10^9$/L), as well as (in MF) anemia with transfusion-dependence, thrombocytopenia, and the percentage of circulating blasts influence MPN-BP transformation [18,23–26]. In addition, inflammatory cytokines, including IL-

Table 1
Risk factors for leukemic transformation.

Risk Factor	Comments
Subtype of MPN	MF (↑↑↑) PV (↑↑) ET (↑)
Age	≥60–65 years old
Laboratory derangements	Leukocytosis ($> 15–30 \times 10^9$/L) Transfusion-dependent anemia (MF) Thrombocytopenia (MF) Increase % of circulating blasts (MF) Inflammatory Cytokines (IL-8, IL-2R, CRP)
Cytogenetic Abnormalities	Monosomal Karyotypes ≻ 1q, 7q, 5q, 6p, 7p, 19q, 22q, 3q, −5, −7 Chromosome 17 derangements (del17p[a]) Complex unfavorable karyotypes (11q23)
'Driver' Somatic Mutations	*JAK2* V617F *MPL* *CALR* (best prognosis) 'triple negative MPNs' (worst prognosis)
'Non-driver' Somatic Mutations	*ASXL1, IDH 1/2, SRSF2, RUNX1, PPM1D, DNMT3A, EZH2* (MF, ET), *TP53 (PV, ET), SH2B3 (ET), SF3B1 (ET), U2AF1 (ET)*

[a] del17p associated with TP53 mutations.

8, IL-2R and CRP correlate with disease progression [27].

Cytogenetic abnormalities also associate with leukemic transformation. Chromosomal abnormalities are most commonly identified in MF; monosomal karyotypes (1q, 7q, 5q, 6p, 7p, 19q, 22q, and 3q, del17p, −5, −7), chromosome 17 derangements (del17p) and other unfavorable karyotypes including 11q23, are all poor prognostic markers [28].

The driver mutational profile also impacts the risk of leukemic transformation in MF. In a large 2018 study by Tefferi et al., 826 patients at the Mayo Clinic and 755 patients in Italy were followed until death [29]. The median survivals were 19.8 years in ET, 13.5 years in PV, and 5.9 years in MF. The same study also confirmed that both survival time and leukemic transformation were affected by mutational status, with *CALR* mutations carrying the best prognosis and triple-negative disease indicating a poor outcome. Specifically, the leukemia-free survival was the worst in those with triple-negative disease, compared to patients with *CALR*, *JAK2*, or *MPL*-mutant MF. Moreover, patients with *CALR* mutations had an average survival of 16 years compared to a 2.3-year survival in patients with triple-negative disease.

Additional somatic mutations have also been implicated as key drivers in the progression from MPNs into AML. High molecular risk mutations in MF include *ASXL1, IDH 1/2, SRSF2, RUNX1, EZH2, DNMT3A* [12]. Studies in ET and PV have also identified adverse variants with an impact on prognosis. In PV, these have included *ASXL1, SRSF2*, and *IDH2*; while there was an impact on survival and MF transformation, an impact of leukemia-free survival was not confirmed in the validation cohort. In ET, adverse variants included *SH2B3, SF3B1, U2AF1, TP53, IDH2,* and *EZH2*; in a validation cohort, these variants influenced MF and LFS [30]. In another cohort of ET and PV patients, mutations in *ASXL1*, *TP53*, *SRSF2*, *IDH1/2*, and *RUNX1* associated with an increased risk of MPN-BP [13].

Prognostic scoring systems continue to evolve, especially in MF, and a more contemporary tool (MIPSS70/MIPSS70-plus) includes these previously mentioned variables, including the presence of symptoms, anemia, leukocytosis, circulating blasts ≥ 2%, ≥ Grade 2 fibrosis, absence of *CALR* type 1, high molecular risk mutational status, and karyotype [31]. Extending on this, a large genomic study, including 2035 MPN patients, identified 8 distinct genetic subgroups with distinct features and risk of transformation [32]. Among these groups, those with *TP53* abnormalities, 17p loss of heterozygosity, and/or chromosome 5 abnormalities had a particularly increased risk of AML transformation. Interestingly, Protein Phosphatase 1D (*PP1MD*) mutations, which have been identified in those with therapy-related neoplasms, were identified in ∼2% of patients, including some following hydroxyrea treatment and will be further discussed below. The abundant genomic data was used with clinical data to calculate a personalized prognosis, including leukemia-free survival (https://cancer.sanger.ac.uk/mpn-multistage/).

3. MPN and therapy-related neoplasms

Various treatments for MPN have been implicated in the conversion to MPN-BP. (Table 2). Such treatments include Phosphorus-32 (^{32}P), and alkylating agents including chlorambucil, and pipobroman. Busulfan has a controversial association with therapy-related BP. The Polycythemia Vera Study Group (PVSG) played a major role in identifying these leukemogenic therapies. In one of the seminal studies, patients were randomized to receive phlebotomy alone, chlorambucil plus phlebotomy, or ^{32}P combined with phlebotomy [32]. Results of the study found no statistically increased risk of leukemia between ^{32}P compared to phlebotomy alone ($P = > .10$). However there was a significant risk of progression to AML observed in patients treated with chlorambucil which was 2.3 times higher compared to ^{32}P and 13 times higher than patients who received phlebotomy alone ($P = < .002$). Subsequently, use of chlorambucil has been discouraged.

During the same period, the European Organization for Research on Treatment of Cancer (EORTC) randomized 293 PV patients to receive either ^{32}P or busulfan [33]. This study found that patients who received busulfan experienced a longer first remission ($P = < .001$) and longer overall survival ($P < .02$). However, conversion to acute leukemia was only observed in 2 of 140 patients treated with ^{32}P, and 3 of 145 patients who received busulfan. Authors concluded that leukemogencity was comparable in both groups, and overall selected bulsulfan as a favorable treatment over ^{32}P.

A 2005 prospective observational study conducted by Finazzi et al. analyzed the progression to AML/MDS in 1638 PV patients [34]. Of 22 patients who developed AML/MDS, they were more likely to have been treated with more than one cytoreductive agent

Table 2
Leukemic potential for MPN therapies.

Leukemogenic Risk	Therapeutic Agents
Non-leukemogenic	Anagrelide Interferons Ruxolitinib[a]
Lower Risk	Hydroxyurea (controversial) Busulfan [b]Erythropoiesis stimulating agents (ESAs) [b]Splenectomy
Higher Risk	Phosphorus-32 (^{32}P) Chlorambucil Pipobroman

[a] Possible association with lymphoproliferative disorders.
[b] Not reproducible risk factors, and confounded by advanced disease/severe underlying disease.

(18.2% vs. 3.3%, P = .0002). More specifically, patients with disease conversion to MPN-BP had higher rates of exposure to MPN treatments compared to patients without disease progression including ^{32}P (12.6% VS 2.5%; p = .0014), busulfan (18.2% vs. 3.5%; P = .0003), pipobroman (18.2% vs. 6.3%; P = .0246). Overall, patients who had received ^{32}P or alkylating agents had a 4 – to 8 – fold increased risk of progression to AML/MDS (HR 4.69; 95% CI, 1.42–15.46; P = .0110).

A 2007 single-center study by Gangat et al. analyzed the risk of leukemic conversion in patients with ET [35]. Of the 605 patients studied, 20 (3.3%) had progression to MPN-BP. The study identified three treatment groups to further risk stratify patients; a group only exposed to non-cytotoxic therapies such as anagrelide or interferon alpha (n = 244), patients who received single or combination cytotoxic agents other than hydroxyurea such as ^{32}P or busulfan (P = 68), and those who received hydroxyurea alone or with anagrelide or interferon alpha (N = 293). The study found a significant difference (P = .03) in favor of anagrelide and interferon, however there was no comparison of the leukemic conversion between specific cytotoxic agents such as ^{32}P versus busulfan.

4. The potential leukemogenicity of hydroxyurea

Hydroxyurea is an anti-metabolite agent used to treat ET, PV, and sickle cell disease. It acts by inhibiting the enzymatic activity of ribonucleotide reductase, thereby inactivating DNA synthesis [36]. The drug increases the amount of functioning fetal haemoglobin in patients with sickle cell disease, which in turn decreases the number of vaso-occlusive crises these patients experience. When used for the treatment of MPNs, hydroxyurea is the most frequently used cytoreductive agent used due to its perceived efficacy and tolerability. Despite a relatively favorable side effect profile, myelosuppression, cutaneous and gastrointestinal toxicities are the most common reported. The increased risk of secondary dermatologic malignancies such as basal cell carcinoma and squamous cell carcinoma has been well established with hydroxyurea use [37]. However, there remains significant controversy regarding the potential leukemogenic risk of hydroxyurea, which is reviewed below.

4.1. Arguments in favor of leukemogenicity

Studies of the genetics of BP transformation have raised concern about prior HU exposures [38]. Thoennissen's group utilized high-density single-nucleotide polymorphisms (SNP) arrays to analyze genetic abnormalities in a total of 148 patients with MPN, including 159 samples −88 (55%) in chronic phase and 71 (45%) in blast phase [39]. Interestingly, the study found that chromosome 17p deletions (*TP53*) were more likely in blast-phase patients who had been treated with hydroxyurea, with or without prior exposure to alkylating agents (n = 5, P = .035). Beer et al. conducted a study of 16 patients with leukemic conversion after preceding *JAK2*- positive MPN – seven patients with *JAK2*-mutant AML and nine individuals with *JAK2*-wild type AML [40]. The study found that development of *JAK2*-wild type AML was not a result of mitotic recombination, gene conversion or deletion of the mutant allele. Focusing on these patients with wild-type AML, all nine had prior exposure to hydroxyurea, including six treated with hydroxyurea alone. Of the six patients treated with hydroxyurea only, four individuals had *TP53* mutations. Overall, the authors stated that the data regarding the association between hydroxyurea and leukemic transformation remains inconclusive.

The French Polycythemia Study Group (FPSG) conducted a randomized control trial assigning 285 patients with PV aged 65 years or less to receive either hydroxyurea alone (n = 136, 48%) or pipobroman alone (n = 149, 52%). Kiladijan et al. published a final update in 2011, after a median follow-up time of 16.3 years since the initiation of the study [41]. Findings from the trial revealed that at 10, 15 and 20 years since treatment initiation, the progression to AML/MDS was 7.5%, 14.3% and 22%, respectively in patients treated with hydroxyurea. For patients who received pipobroman, the cumulative incidence of MPN-BP at the same time intervals was 12.2%, 37.5% and 56%, respectively. These findings were statistically significant (P = .008). Interestingly, there was a statistically higher cumulative incidence of MF in the hydroxyurea treatment arm compared to pipobroman at the same time intervals (P = .02); 15.4% vs. 5.1% at 10 years, 24.3% vs. 9.8% at 15 years, and 31.6% vs. 21.3% at 20 years. Overall, this study concluded that the cumulative incidence of transformation to AML/MDS is 23.6% at 15 years, higher than expected with the previously reported 5%–16%. Furthermore, leukemic conversion represents the main cause of death (54%) in PV patients younger than 65 years old at time of diagnosis. The study concludes that pipobroman should no longer be used as a first-line treatment for PV given the previously indices of leukemic transformation, thereby favoring the use of hydroxyurea. However, rates of AML/MDS transformation were higher than expected even in the HU group, possibly suggesting a time-dependent increase in risk.

A study which highlighted the potentially harmful effects of hydroxyurea was a 2016 single-center retrospective cohort study by Hansen et al. which sought to analyze secondary malignancies in MPN patients treated with hydroxyurea alone, interferon (IFN) monotherapy or both agents [42]. Of 196 patients with MPN identified, 90 were treated with hydroxyurea alone, 38 with IFN monotherapy, and 68 patients received IFN in addition to hydroxyurea. The study found a statistically significant higher risk of secondary malignancies in patients treated with hydroxyurea compared to IFN alone (P = .023). Focusing on hematologic cancers, a total of nine (4.6%) of patients developed AML (n = 8) or MDS (n = 1); four patients treated with hydroxyurea alone, one treated with IFN monotherapy, and four patients treated with both agents. The above findings were not statistically significant (P = .629). An additional three patients developed other hematologic cancers including B and T cell lymphomas. Moreover, seventeen patients (8.7%) developed skin cancers, predominantly in the two groups who received hydroxyurea. Additional secondary malignancies were identified including breast, prostate and gastrointestinal cancers. The study concluded that patients treated with hydroxyurea had a higher risk of developing all secondary malignancies, however importantly for our review, the risk of hematologic cancers was not statistically significant.

A recent study by Grinfeld et al. developed genomic classification and prognostic models for patients with MPN [31]. The study included a total of 2035 patients; 1321 with ET, 356 with PV, 309 with MF and 49 with other diagnoses of MPN. Mutations in *JAK2,* *MPL* and *CALR* totaled 1831 driver mutations and were the only mutations found in 45% of patients. A total of 1075 additional driver mutations were identified in other genes. One of these included Protein Phosphatase 1D (*PPM1D*), a protein involved in cell cycle regulation through interactions with *TP53* which has been identified as a key mutation in breast and ovarian cancer patients after exposure to chemotherapy. This study found a total of 45 truncating mutations in *PPM1D* in 38 patients (1.9%), and was the eighth most commonly mutated gene in MPNs. Interestingly, ten patients with *PPM1D* mutations had prior hydroxyurea exposure. The significance of this finding is unclear, because twenty individuals were found to have mutations at or within one month of their MPN diagnosis.

4.2. Arguments against leukemogencity

Finazzi et al. performed a long term follow-up of a randomized controlled trial in ET patients to analyze the risk of secondary cancers based on prior treatment exposures [43]. The original study randomized patients to receive hydroxyurea (n = 56) versus no myelosuppressive treatment (n = 58). Importantly, 15 patients had been treated with busulfan prior to the study initiation. Analysis revealed 8 patients (7%) who developed a secondary malignancy. Seven of these individuals were in the hydroxyurea group, with 5 having had prior exposure to busulfan as well. Furthermore, 4 of these secondary malignancies were AML/MDS, and all leukemia cases had received hydroyxurea ± busulfan. The difference in cancer-free survival was statistically significant between the hydroxyurea plus busulfan group versus the untreated group ($P < .0001$), but not between hydroxyurea alone in comparison to the untreated cohort. The authors concluded that results supported a high rate of second malignancies in patients treated with sequential busulfan and hydroxyurea, however rare in those treated with hydroxyurea alone. Coupled with a beneficial effect in reducing thrombosis, they support the continued use of hydroxyurea in ET patients.

The 2004 European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) Study was a double-blind, placebo-controlled randomized control trial which compared treatment with aspirin alone (n = 253) to placebo (n = 265), with primary endpoints including fatal and nonfatal cardiovascular events, stroke and major thrombotic events such as pulmonary embolism [44]. The results of this trial revealed that low-dose aspirin safely reduces risk of thrombosis in patients with PV. In 2005, Marchioli et al. conducted a subsequent study of 1,638 patients initially screened for inclusion in the ECLAP trial [45]. The study analyzed the clinical history of PV, including risk of cardiovascular death and thrombosis similar to the original ECLAP study, in addition to the rate of conversion to leukemia, MDS and MF. Focusing on risk of hematologic progression, the authors concluded that the use of cytoreductive therapies, excluding both hydroxyurea and interferon, was associated with a statistically significant risk of AML/MDS (hazard ratio, 10.99%; 95% CI, 2.85 to 42.33; P = .0005). Hydroxyurea alone did not statistically increase the risk of leukemic conversion (hazard ratio, 1.58; 95% 0.40 to 6.21). The authors note that leukemic deaths constituted 8.2% and 34.5% among patients receiving hydroxyurea alone versus other cytoreductive treatments (excluding interferon), respectively. Interestingly, the study highlights the difficulty with discerning the risk of leukemic transformation due to the natural course of the disease long-term cytoreductive treatment, given the tendency for more aggressive therapies in high-risk disease.

A 2011 nested case-control trial conducted by Björkholm et al. evaluated the risk of MPN progression to AML/MDS based on treatment exposures [46]. They identified a total of 162 patients who had disease progression −153 to AML and nine to MDS, compared to 242 matched controls. Twenty-five percent of patients with transformation to AML/MDS had never been exposed to alkylating agents, radioactive treatment (^{32}P) or hydroxyurea, in keeping with an intrinsic risk of evolution. Of those who were treated, exposure to ^{32}P and alkylating agents were associated with a statistically significant risk of transformation (P = .030), whereas treatment with hydroxyurea was not (P = .510).

A 2013 multi-center international study by Tefferi et al. sought to investigate leukemic transformation and survival among 1545 PV patients [18]. When analyzing the effects of cytoreductive drugs, the authors grouped patients based on prior exposure to agents generally thought to be leukemogenic (^{32}P or chlorambucil), those more recently associated with leukemic transformation (pipobroman) and drugs with a controversial association (hydroxyurea or busulfan). A total of 50 patients (3%) underwent leukemic transformation at a median time of 10.8 years after diagnosis. Univariate analysis revealed significant associations between leukemic transformation and exposure to ^{32}P or chlorambucil alone (P = .007), pipobroman alone (P = .008), and pipobroman plus hydroxyurea or busulfan (P = .03). These findings remained significant with multivariate analysis. The authors found no association between leukemic transformation with hydroxyurea monotherapy (P = .23), single-agent busulfan (P = .091), or both busulfan and hydroxyurea (P = .044).

A meta-analysis by Samuelson et al. reviewed anagrelide versus hydroxyurea as treatment options for patients with ET [47]. More specifically, the authors focused on rates of arterial and venous thrombosis, bleeding and death between these two treatment groups. Two randomized controlled trials met criteria for review. The study found no significant difference between rates of thrombosis between the two drugs (RR 0.86, 95% CI 0.64–1.16), however there was a difference between arterial (RR 0.64, 95% CI 0.45–0.90) and venous (RR 2.67, 95% CI 1.26–6.11) events, which favored hydroxyurea for reduced rates of arterial thrombosis and a superiority of anagrelide in reducing venous events. Major bleeding was lower in patients treated with hydroxyurea (RR 0.37, 95% CI 0.18–0.75). There was no significant difference in rates of death between the treatment groups, however hydoxyurea showed a better risk reduction when the composite of thrombosis, bleeding and death was analyzed. Focusing on risk of leukemic transformation, there was no significant difference in rates of AML between patients treated with hydroxyurea versus anagrelide (RR 1.50, 95% CI 0.43–5.29), but there was a decreased rate of MF in patients who received hydroxyurea (RR 0.33, 95% CI 0.13–0.83). Based on the above, study authors favored the use of hydroxyurea as a first-line agent for ET patients.

In 2017, Barbui et al. identified a cohort of 1,042 PV patients screened in the aforementioned ECLAP study to analyze the therapeutic profile of hydroxyurea (n = 342) compared to phlebotomy alone (n = 681) over a follow-up period of 35 and 30 months, respectively [48]. The study focused on primary end-points including fatal and non-fatal cardiovascular events in addition to hematologic transformation. Focusing on the latter, hydroxyurea demonstrated a superiority to phlebotomy alone, with a rate of hematologic transformation of 0.1 vs. 1.1 per 100 patients, respectively (P = .006). More specifically, development of acute leukemia occurred in a total of three patients; two in the phlebotomy arm and one treated with hydroxyurea alone. Eight patients experienced disease conversion to myelofibrosis, all of who were in the phlebotomy treatment group. After further risk stratification, findings revealed that high-risk patients (age greater than 60 years and/or prior history of thrombosis) experienced lower rates of MPN-BP (0.1 vs. 1.5 per 100) and overall mortality (0.1 vs. 0.5 per 100) when treated with hydroxyurea monotherapy compared to phlebotomy alone, respectively.

A recent study by Senín et al. evaluated the role of non-driver mutations in the progression to MF and AML among 100 patients with *JAK2* PV or ET [13]. Moreover, the authors investigated if long-term treatment with hydroxyurea influenced non-driver mutations, and thus, promoted leukemic transformation. Overall, the study results showed a direct correlation between non-driver mutations and disease progression; 83% of patients with leukemic transformation had mutations in their first sample and had high rates of developing subsequent mutations during follow-up evaluation. Conversely, patients with more than ten years of molecular follow-up without disease progression had the lowest mutation frequency at the first sample, and lowest rate of new mutations during follow-up. Regarding the role of hydroxyurea, findings suggested no significant differences in the risk of new mutations among individuals who received hydroxyurea for longer than 5 years, those treated for less than 5 years, and patients who never received hydroxyurea (P = .794). Overall, study authors concluded that there were no genotoxic effects associated with hydroxyurea exposure.

Lastly, the safety and side effect profile of hydroxyurea has been studied in patients with sickle cell disease (SCD) due to its widespread use in this patient population. A 2012 meta-analysis by Strouse and Heeney reviewed 26 articles in order to extrapolate information to better treat children with SCD [49]. The authors favored the use of hydroxyurea for continued treatment of SCD given their review of its side effect profile, particularly in the BABY HUG trial [50]. Interestingly, exposure to hydroxyurea was not associated with increased genotoxicity, including chromosome breaks, variable-diversity-joining (VDJ) recombination events or micronuclei assay results. A 2008 systematic review by Lanzkron et al. synthesized the data regarding efficacy, effectiveness and toxicity of hydroxyurea in adults with SCD [51]. The authors conducted a thorough review of the literature and found 3 reported cases of leukemia in 4 observational studies. One of these included a large study on cancer development in 16,613 patients with SCD, which revealed a total of 49 cases of cancer, of which 7 were leukemia and only 1 patient was previously exposed to hydroxurea. Lanzkron and colleagues also found an additional 2–3 case reports about individuals developing leukemia after hydroxyurea treatment. Overall, the authors concluded that based on their review of the literature, there is little evidence to suggest that hydroxyurea treatment in adults with SCD increases the risk of leukemia.

5. MPN and lymphoproliferative disorders

The co-occurrence between MPN and lymphoproliferative disorders (LPD) has been reported by a number of investigators. In a study of 1915 Italian patients, the risk of developing a lymphoid neoplasm in MPN patients was 2.8 times that of the general population [52]. Recently, a systematic review including 214 patients revealed the development of lymphoproliferative disease after a median of 72 months following diagnosis, whereas 20% of patients had an antecedent or concurrent diagnosis [53]. Chronic lymphocytic leukemia was the most common LPD diagnosed prior to MPN, and most common LPD when diagnosed concurrently with MPN. A male predominance was noted in this group. Aggressive lymphomas were less common overall, but comprised 14% of LPD's diagnosed during follow-up of MPN. Interestingly, the most common molecular abnormality in those developing LPD was *JAK2 V617F*, identified in 75% of patients, including 83% with MF that developed LPD. The underlying pathogenesis regarding associations between MPNs and LPD has been unknown. Recently, Kennedy and colleagues conducted a detailed genetic analysis in 3 patients with MF and LPD [54]. In 2 patients, disease clones originated independently, and the authors speculated on the impact of environmental factors or germline predisposition that could explain parallel development of MPN and LPD. In a third of patients, there was suggestion of a shared clonal origin or genetic lesion given rise to MPN and LPD.

This baseline association is especially important given a recent report of aggressive B-cell lymphomas in patients treated with JAK1/JAK2 inhibitors [55]. Investigators identified B-cell lymphoma in 4 (5.8%) of 69 patients treated with JAK1/JAK2 inhibitors, compared to 2 (0.36%) of 557 conventionally treated patients (16 fold risk). Three of 4 MF patients had been previously treated with alkylating agents, and one with ruxolitinib alone. Another patient had received fedratinib as well. An independent cohort identified a prevalence of 3.5% in JAK-inhibitor treated patients, compared to 0.23% in conventionally treated patients (OR 15). The median time to development of lymphoma was 25 months. The authors suggested that the clinical features of lymphomas were similar to those observed in immunocompromised patients. The authors speculated that the immunosuppressive nature of JAK-inhibition could be a contributing factor. Further, a model of Stat $1-/-$ mice recapitulated a phenotype of coexisting MPN and B-cell transformation. As the authors state, *STAT1* deficiency leads to deficient immune surveillance, and potentially, emergence of malignant B cell clones. In this study, clonal immunoglobulin gene rearrangements were previously detected in the marrow in 16% of patients. This would suggest a specific at-risk patient population, and should favor into the risk-benefits discussion about JAK-inhibitor therapy.

6. Ruxolitinib and clonal evolution?

Clinical trials and experience in clinical practice suggests that loss of response to ruxolitinib occurs over time, with an estimated response duration of 3 years. Outcomes after ruxolitinib discontinuation have been reported. In a series including 62 patients who discontinued ruxolitinib and had molecular data at baseline and during follow-up, 22 (35%) acquired a new mutation (most commonly *ASXL1*) while under ruxolitinib treatment [56]. Those with clonal evolution were more likely to have transfusion-dependence and a shorter survival after discontinuation of ruxolitinib. Subsequently, Pacilli et al. investigated whether or not clonal instability was driven by ruxolitinib [57]. In this study of 71 MF patients, the mutational landscape was compared in ruxolitinib (N = 46) and HU (N = 25) patients. The authors concluded that ruxolitnib did not promote clonal evolution compared with hydroxyurea, but an increase in the variant allele frequency of pre-existing mutations, or acquisition of new mutations while under therapy negatively impacted treatment outcomes and survival. This suggests that progression while on ruxolitinib appears intrinsic to the disease, rather than a therapy-related consequence.

7. Conclusion

MPNs are inherently progressive diseases with a spontaneous risk of leukemic transformation, which in some cases, is exacerbated by therapy. Risk factors for transformation have been increasingly well-defined, especially in the current molecular genetic era. With regard to the role of therapies, in some instances, the evidence is consistent in its implications—there is agreement regarding a leukemogenic potential after exposure to ^{32}P, chlorambucil, and pipobroman. The data is less consistent with respect to the leukemogenic potential of busulfan.

There is more debate regarding the potential leukemogenicity of hydroxyurea. Certain studies have suggested a potential signal, highlighting HU exposure and chromosome 17 abnormalities or HU exposure and wild-type AML in those who have transformed to MPN-BP. Longer term follow-up studies showed higher than expected rates of AML/MDS in HU-treated patients. It is unclear if this reflects natural history in a cohort with longer observation times, or if there is a time-dependence to the association between HU and MPN-BP. More recently, *PPMD1* mutations were identified in a small number of HU-exposed patients.

Yet, when looking at contemporary studies, albeit largely retrospective by design, statistical associations have not implicated HU monotherapy as leukemogenic. However, a lack of association is difficult to definitively disprove, as large, prospective, controlled studies with decades of follow-up would be required to draw conclusions. Therefore, patients must be carefully informed about the risks and benefits of MPN-therapies, including ruxolitinib, given emerging data revealing aggressive B-cell lymphomas after treatment with this JAK1/JAK2 inhibitor.

The concept of therapy-related neoplasms remains important to the field, and mandates judicious selection and sequencing of therapies for MPN patients.

Practice points

- The main risk factors for leukemic transformation include the subtype of MPN, age, laboratory derangements, cytogenetic abnormalities, 'driver' and 'non-driver' mutations, as well as cytogenetic therapies.
- Among MPN therapies, there is the highest risk of leukemic conversion with Chlorambucil, ^{32}P and Pipobroman. Lowest risk agents include anagrelide, interferons and ruxolitinib. The leukemogenicity of hydroxyurea remains controversial.
- Emerging data points toward an association between ruxolitinib and high grade B-cell lymphomas, favoring a judicious use of ruxolitinib and a need to prospectively follow for risk of lymphoma.

Research agenda

- Large, prospective, controlled studies with decades of follow-up would be required to draw conclusions on the leukemogenicity of hydroxyurea in patients with myeloproliferative neoplasms.

Conflicts of interest

BLS has served as a consult with Incyte Corporation and Apexx Oncology.

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