New approaches to standard of care in early-phase myeloproliferative neoplasms: can interferon-α alter the natural history of the disease?

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Abstract

The classical *BCR::ABL*-negative myeloproliferative neoplasms (MPN) include polycythemia vera, essential thrombocythemia, and primary myelofibrosis. They are acquired clonal disorders of hematopoietic stem cells leading to hyperplasia of one or several myeloid lineages. MPN are caused by three main recurrent mutations, *JAK2*^{V617F} and mutations in the calreticulin (*CALR*) and thrombopoietin receptor (*MPL*) genes. Here, we review the general diagnosis, the complications, and the management of MPN. Second, we explain the physiopathology of the natural disease development and its regulation, which contributes to MPN heterogeneity. Thirdly, we describe the new paradigm of MPN development highlighting the early origin of driver mutations, decades before the onset of symptoms, and the consequence of early detection of MPN cases in the general population for prompt diagnosis and better medical management. Finally, we present interferon-α therapy as a potential, early disease-modifying drug after reporting its good hematologic and molecular efficacies in polycythemia vera, essential thrombocythemia, and early myelofibrosis in clinical trials as well as its mechanism of action in pre-clinical studies. As a result, we may expect that, in the future, MPN patients will be diagnosed very early during the course of disease and that new selective therapies under development, such as interferon-α, JAK2^{V617F} inhibitors and CALR^{mut} monoclonal antibodies, will be able to intercept the mutated clones.

Generalities on classical myeloproliferative neoplasms

Diagnosis, complications and progression of myeloproliferative neoplasms

Myeloproliferative neoplasms (MPN) are heterogeneous clonal disorders of hematopoietic stem cells (HSC) characterized by increased production of myeloid cells. Classical *BCR::ABL1*-negative MPN include essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). Their diagnosis is based on the World Health Organization (WHO) 2016 and 2022 classifications.[1,2] ET, PV, and PMF are characterized by excess platelet production, excess red blood cell production and megakaryocytic hyperplasia with bone marrow fibrosis, respectively. Although they have different phenotypes, they are a continuum of diseases as ET can progress into PV and ET/PV into secondary myelofibrosis (SMF). Moreover, since the WHO 2016 classification, overt PMF has been distinguished from prefibrotic PMF, which is an intermediate phenotype between ET and overt PMF.[3] Lastly, leukemic transformation, with its dismal prognosis, is a common complication of classical MPN. The other main non-hematologic complications are vascular events (mainly in ET and PV) and symptoms such as pruritus, fatigue, and night sweats, which alter the patients' quality of life.

MPN are due to the acquisition of a driver mutation in an HSC that constitutively activates the JAK2/STAT signaling pathway. These driver mutations affect three genes encoding thier respective proteins, *JAK2*, calreticulin (*CALR*) and the thrombopoietin receptor (*MPL*).[4-7] Their presence is a major diagnostic criterion in the WHO classifications.

JAK2^{V617F} is found in 95% of cases of PV and around half of patients with ET and PMF. Approximately 100 different *CALR* mutations (*CALR*^{mut}) have been described to date and ob-

served in 20-30% of cases of ET and PMF.[4-7] Among them, the most frequent are a deletion of 52 base pairs (type 1) and the insertion of five base pairs (type 2). These mutations are found at nearly the same frequencies in ET but the type 1 mutation is highly enriched in PMF. Three to five percent of ET and PMF patients harbor *MPL* mutations, which mainly occur in the W515 residue of the protein.

General management of myeloproliferative neoplasms

The main goal of the management of ET and PV patients is the prevention of vascular events (thrombosis and hemorrhages) and relies on rigorous treatment of cardiovascular risk factors in all patients, and the administration of low-dose acetylsalicylic acid and cytoreductive therapy depending on individual vascular risk. International guidelines[8,9] recommend using the revised International Prognostic Score of Thrombosis for Essential Thrombocythemia (IPSET-thrombosis) scoring system to stratify the vascular risk of ET patients.[10,11] All ET patients should receive acetylsalicylic acid, apart from very low-risk, asymptomatic ET patients with *CALR*^{mut} for whom observation only is recommended.[12] Cytoreductive treatment is indicated in patients >60 years with *JAK2*^{V617F} mutation and/or cardiovascular risk factors, in patients with a history of vascular events and in patients with extreme thrombocytosis (platelet count >1,500x10^9/L).[8,9,12] Current international guidelines recommend that all PV patients should receive acetylsalicylic acid and undergo phlebotomies to maintain their hematocrit ≤45%.[8,9,13,14] High-risk PV patients, defined by age ≥60 years and/or a prior history of thrombosis, are eligible for cytoreductive drugs.[8,9,13,14] The risk of complications in PV does not only rely on the increase of the hematocrit level.[15] Several criteria reflecting a higher thrombotic risk (e.g., leukocytosis, inadequate hematocrit control with phlebotomies, relevant cardiovascular risk factors), a bleeding risk (thrombocytosis >1,500×10^9/L), as well as disease-related symptoms (e.g., splenomegaly, pruritus) are additional indications for cytoreductive treatment in low-risk PV patients.[8,9,16-18] For both ET and PV, the recommended first-line agents are hydroxyurea and pegylated interferon alpha (IFNα). Anagrelide and ruxolitinib are second-line therapeutic options in ET and PV, respectively.[9,15-17]

Despite recent improvements in the management of myelofibrosis, allogeneic stem cell transplantation remains the only curative treatment for these patients.[19] The significant risk of treatment-related morbidity and mortality makes rigorous selection of eligible patients essential. Prognostic scoring systems and individual factors guide decision-making regarding transplantion.[8,9,18,20] However, due to advanced age and comorbidities, only a minority of patients with expected poor survival are eligible for allogeneic stem cell transplantation. Therefore, the management of patients with myelofibrosis remains mainly symptomatic. Myelofibrosis patients have heterogeneous phenotypes, from absence of symptoms through pronounced constitutional symptoms, bulky splenomegaly and deep cytopenias. Observation alone is recommended for asymptomatic patients.[8,9,18] Hydroxyurea is a therapeutic option for the treatment of splenomegaly in patients with low-risk or intermediate-1-risk myelofibrosis and in the case of leukocytosis or thrombocytosis needing cytoreduction.[8,9,18] JAK inhibitors are effective for the treatment of constitutional symptoms and splenomegaly.[21-26] Ruxolitinib is approved by the Food and Drug Administration for intermediate- and high-risk myelofibrosis patients with platelet counts >50x10^9/L, fedratinib for ≥ intermediate-2-risk myelofibrosis patients with platelet counts >50x10^9/L and pacritinib for ≥ intermediate-risk myelofibrosis patients with platelet counts <50x10^9/L.[18] Conventional therapies for myelofibrosis-related anemia have insufficient and transient efficacy,[27] however, a fourth JAK inhibitor, momelotinib, was recently approved for the treatment of ≥intermediate-risk myelofibrosis patients with anemia.[28,29]

Ruxolitinib administration significantly improves survival compared to historical treatment and decreases *JAK2*^{V617F} variant allele frequency (VAF) and fibrosis in some myelofibrosis patients,[30] as well as *JAK2*^{V617F} VAF in some PV patients.[31] However, there is little evidence of a disease-modifying role for either ruxolitinib or any of the other molecules currently used to treat MPN. IFNα appears to be the only molecule with a distinctive effect on MPN cells in clinical and preclinical studies.

Natural history of myeloproliferative neoplasms

Function of driver mutations in myeloproliferative neoplasms

JAK2^{V617F} activates the oncogenic JAK2/STAT signaling downstream of the homodimeric type 1 cytokine receptors (erythropoietin receptor [EPOR], MPL, G-CSFR), while CALR mutants act as rogue chaperones and ligands by binding, oligomerizing and activating MPL at the cell surface.[32-36] *JAK2*^{V617F}, *CALR* and *MPL* mutations have been proven to be drivers of disease in different mouse models.[37-40] Importantly, inactivation of JAK2^{V617F} through deletion of *JAK2* in an innovative conditional mouse model led to the abrogation of MPN disease, demonstrating the monogenic character of MPN development.[41]

Although these driver mutations occur in HSC and activate JAK2/STAT signaling, they have distinct effects on hematopoiesis. JAK2^{V617F} gives a major proliferative advantage during hematopoietic differentiation of the three myeloid lineages but less at the HSC level. Heterozygous *JAK2*^{V617F} mutation, which is mainly observed in ET patients, induces a mild advantage in the HSC compartment but strongly amplifies the myeloid differentiation resulting in lower VAF in HSC than in peripheral blood cells.[42-44] Homozygous clones have a stronger competitive advantage over heterozygous clones even in the HSC compartment.[43,45] ET patients generally

have low *JAK2*^{V617F} VAF in their blood (around 20% or less), compared to 50% in PV patients and 70-80% in post-ET/PV myelofibrosis due to *JAK2*^{V617F} homozygosity, in line with the continuum of diseases. In contrast, in *CALR*^{mut} ET patients, VAF was around 30-50% in HSC, with very mild increases during hematopoietic differentiation. During the progression of ET to myelofibrosis, the *CALR*^{mut} VAF may increase slightly to around 50%.[45] Homozygous *CALR*^{mut} clones are observed in ET patients with *CALR* type 2 mutations and in advanced myelofibrosis with *CALR*^{mut} type 1. Altogether, these data suggest that *CALR*^{mut} give a stronger clonal advantage to HSC than *JAK2*^{V617F} in ET patients or that *CALR*^{mut} disease occurs when the HSC are nearly all mutated[46-48] (Figure 1). Anyway, this difference points to an important concept to be considered when determining the interplay between driver mutation identity, VAF and the resultant disease phenotype. The different effects on hematopoiesis could be due to distinct intensity in the activation of JAK2/STAT signaling or to JAK2/STAT-independent pathways involving CALR^{mut}-mediated endoplasmic reticulum stress, unfolded protein response[49-51] and immunosuppression.[52]

The natural history of MPN, i.e., the evolution of ET/PV towards SMF or ET towards SMF, can be modified by additional somatic mutations as well as germline variants and extrinsic factors.

Intrinsic and extrinsic regulators responsible for the heterogeneity of myeloproliferative neoplasms

Together with driver mutations, MPN patients can harbor additional somatic mutations of epigenetic regulators, the spliceosome machinery and transcription factors, which are also found in other myeloid malignancies. Their type, number and order of appearance are important as disease-modifying factors. They contribute to disease onset, the type of MPN, the risk of progression and resistance to therapy.[53,54] Additional mutations can appear before or after *JAK2*^{V617F} but generally after *CALR*^{mut}. Mutations in *TET2* (5-methylcytosine dioxygenase) and *DNMT3A* (DNA methyltransferase) induce a strong HSC advantage and, in combination with *JAK2*^{V617F}, accelerate progression into SMF.[55,56] When acquired before *JAK2*^{V617F}, these co-occurring mutations can also lead to a higher clonality than *JAK2*^{V617F} and contribute to the disease onset.[57] Several other mutations are associated with the progression of ET into SMF by skipping the development of PV. *ASXL1*, *EZH2* and several splicing mutations are associated with PMF. *ASXL1* mutations are also linked to a higher risk of leukemic transformation, except when associated with type 1 *CALR*^{mut} mutation, and poorer survival.[27,58,59] *IDH1/2* neomorphic mutations are also at high risk of inducing leukemic transformation of *JAK2*^{V617F} MPN.[60] *SRSF2* and *U2AF1* mutations are of poor prognosis. *SRSF2* mutations

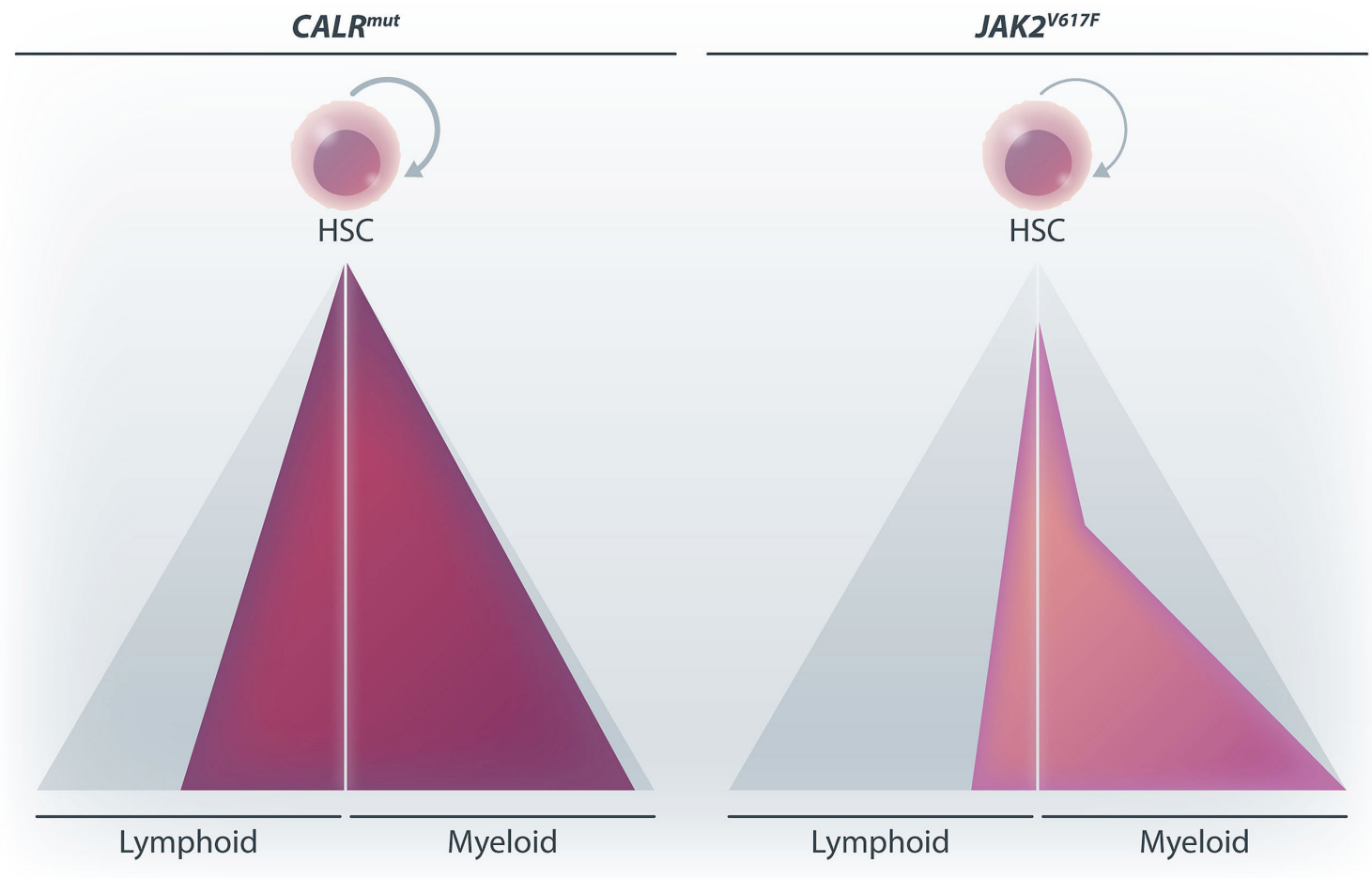

Figure 1. Impact of driver mutations in hematopoiesis. In patients with essential thrombocythemia, *CALR*^{mut} show strong clonal dominance in all the hematopoietic stem cell (HSC) compartment. *CALR*^{mut} induce a mild amplification during hematopoietic differentiation, being present with almost similar variant allele frequencies in granulocytes and in the HSC compartment. Moreover, *CALR*^{mut} are also present in lymphoid cells, including T cells. In contrast, in patients with essential thrombocythemia, *JAK2*^{V617F} is generally present with low clonal dominance in the HSC compartment but is strongly amplified during myeloid differentiation. *JAK2*^{V617F} is generally not present or present at very low variant allele frequencies in lymphoid cells.

are strongly involved in leukemic transformation and *U2AF1* mutations are associated with anemia and thrombocytopenia.[27] Several mutations in transcription factors contribute to leukemic transformation, such as *TP53* alterations in 20% of mutated cases.[61,62]

Genetic factors also contribute to the heterogeneity of MPN. Common genetic variants in the general population have been identified in large genome-wide association studies as predisposing to the development of sporadic cases of MPN (haplotype 46/1, *MECOM*, *GFI1B*, *MYB*, *GATA2*, *TET2*, *CHEK2*, *TERT*, *ATM*, *TP53*, etc.).[63,64] More recently, polygenic risk scores underlying common hematologic traits were identified as important for *JAK2*^{V617F} clonal expansion.[65] Pan-genomic approaches in some MPN families identified very rare germline predisposing factors, such as *RBBP6* and *EPOR*, and a structural copy number variation (duplication) located at 14q32 which is associated with high penetrance of the disease and with ET rapidly progressing to myelofibrosis and to leukemia in half of cases.[66,67]

Several extrinsic factors may also modify the initiation, phenotype and progression of MPN, such as aging and inflammatory cytokines (tumor necrosis factor-α, interleukins 1β, -6, -13, and -8).[68] MPN are mainly diseases of the elderly and age is associated with the "inflammaging" phenomenon that can drive DNA damage and the exit of HSC from a state of quiescence,[69] enabling the selection of *JAK2*^{V617F} HSC.[70] Acute or chronic infections[71] and exposure to toxic agents, such as chemotherapy, are environmental stressors that may modify HSC homeostasis or the composition of the bone marrow microenvironment to play a role in the selection and expansion of the mutated clone (for example *ASXL1*, *EZH2* and *RAS* selection with ruxolitinib). Conversely, *JAK2*^{V617F} alone or in combination with other mutations could fuel the disease towards progression by maintaining an inflammatory state due to the secretion of various pro-inflammatory cytokines and the production of reactive oxygen species.[68,72,73]

Although MPN are due to driver mutations, they are heterogeneous depending on how the different intrinsic and extrinsic regulators affect the initiation, promotion and selection of the malignant clone.

New paradigm of the development of myeloproliferative neoplasms: early origin of the driver mutations and diagnosis

From clonal hematopoiesis of indeterminate potential to the development of myeloproliferative neoplasms

The concept of clonal hematopoiesis emerged with the identification of people without a hematologic malignancy but with abnormal X-linkage ratios in the circulating blood increasing over time.[74] In 2014, several groups reported *DNMT3A*, *TET2*, *ASXL1* and *JAK2*^{V617F} mutations in more than 10% of healthy people over the age of 70 years and in 1% of younger individuals.[75-77] This clonal hematopoiesis without disease was named clonal hematopoiesis of indeterminate potential (CHIP) or age-related clonal hematopoiesis (ARCH) since the frequency of the mutations correlates with the age of the general population. CHIP was associated with an increased risk of hematologic malignancies, coronary heart disease, and ischemic stroke. Recently, CHIP was defined by the WHO/International Consensus Classification as a precursor state of myeloid disease.[1,3] *JAK2*, *CALR*, and *MPL* CHIP have been associated with higher platelet counts and hemoglobin levels, and an increased risk of developing an MPN.[78] These CHIP (VAF≥2%) could, therefore, be considered as a pre-MPN state since *JAK2*, *CALR*, and *MPL* mutations alone, without associated mutations, are sufficient to drive the disease. Although *JAK2*^{V617F} CHIP was reported in around 0.2% of the general population,[75,76] the use of more sensitive methods allowed the identification of a 3 to 30 times higher prevalence of *JAK2*^{V617F} (3.1%) and *CALR*^{mut} (0.16%) in other cohorts.[79,80] In these later studies, the VAF of the clonal hematopoiesis was correlated with age and blood count, in line with the hypothesis of a continuum from CHIP to MPN. Moreover, the VAF of *JAK2*^{V617F} and *CALR*^{mut} clonal hematopoiesis was initially identified in young healthy individuals at very low allele burden, but expanded slowly over time (Figure 2A). This high frequency of *JAK2*^{V617F} and *CALR*^{mut} clonal hematopoiesis found with sensitive methods in the general population contrasts with the low penetrance of MPN disease with an incidence of 1/100,000 at an advanced age (median, 65 years old), suggesting that *JAK2*^{V617F} and *CALR*^{mut} can only induce a MPN disease in particular conditions when they give robust HSC clonal amplification. In agreement, the disease develops in mouse models when there is a sufficient number of mutated HSC (around 30 HSC), while the penetrance of the disease is less than 1% in mice when only a single mutated HSC is engrafted.[81] Interestingly, *JAK2*^{V617F} clonal hematopoiesis has been detected in familial MPN cases before the development of the disease, especially in patients harboring a 14q32 duplication or the *EPOR*^{P488S} mutation,[67,82] strongly suggesting that genetic factors could contribute to an earlier and/or faster amplification of mutated HSC. Early screening for the driver mutation could, therefore, be an asset in those cases to improve clinical care and to avoid inaugural thrombo-hemorrhagic events.

Collectively, all these data suggest that MPN driver mutations occur quite frequently in individuals, expand slowly with age but induce the disease with a low penetrance. The heterogeneity in MPN development might be due to intrinsic and extrinsic factors that regulate the age of acquisition of the mutations, the amplification of the mutated HSC or the function of normal HSC.

Early origin of the driver mutations

The discovery of expansion of clonal hematopoiesis over time suggests that the acquisition of the driver mutation

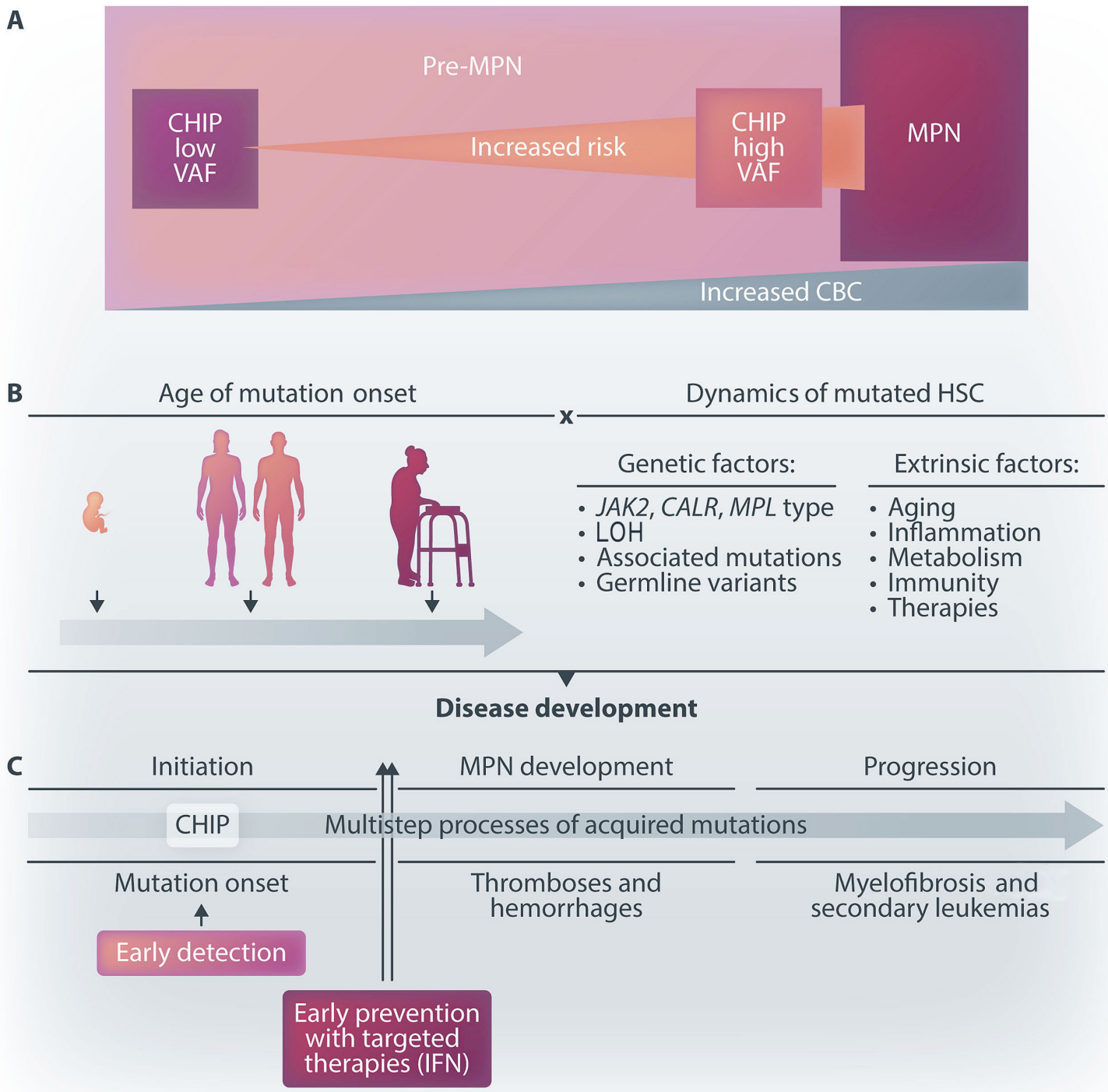

Figure 2. Early detection, early diagnosis and early prevention. (A) Clonal hematopoiesis of indeterminate potential (CHIP) corresponds to a state in which driver mutations (variant allele frequency >2%) are present without manifest disease. Nevertheless, as *JAK2*^{V617F} alone can be responsible for myeloproliferative neoplasms (MPN), CHIP with *JAK2*^{V617F} can be considered as a pre-MPN state. The variant allele frequency of *JAK2*^{V617F} CHIP correlates with complete blood counts and with an increased risk of triggering a MPN. (B) The onset of MPN disease is the result of many factors: the age of acquisition of MPN driver mutations at the level of HSC and the dynamics of mutated HSC. The latter depends on many intrinsic and extrinsic factors. (C) The natural evolution of MPN diseases and treatment approaches. Given that driver mutations appear decades before the onset of symptoms, early detection of MPN in the general population is important to improve medical care and follow-up. Preventive medicine, which involves intercepting mutated clones using targeted therapy such as interferon-α or new selective treatments, could be a breakthrough in preventing the development, progression and complications of MPN. VAF: variant allele frequency; CBC: complete blood count; LOH: loss of heterozygosity; IFN: interferon.

may occur long before the onset of disease symptoms. The reconstruction of HSC phylogenetic trees using somatic mutations as a molecular clock to time the origin of the driver mutations strikingly reveals that the *JAK2*^{V617F} mutation can occur in the first or second decade of life[44] and even during fetal development.[83] The early onset of *JAK2*^{V617F} was also confirmed using a mathematical model that retrospectively inferred the number of mutated HSC from the initial mutation to the sampling over time using clonal architecture of the patients' progenitors and the patients' age.[48] Occurrence of *JAK2*^{V617F} in fetal life was confirmed by its identification in a cord blood unit used for an allogeneic stem cell transplant in a patient with acute myeloid leukemia.[84] *CALR*^{mut} was inferred to occur 10 years later than *JAK2*^{V617F} (average acquisition at 25 years *vs*. 15 years for *JAK2*^{V617F}),[48] in line with the absence of *CALR*^{mut} MPN in children under 10, unlike *JAK2*^{V617F} MPN.[85] However, in an exceptional case of monozygotic twins diagnosed with myelofibrosis at the

ages of 37 and 38 years and carrying the same *CALR*^{mut}, a whole-genome sequencing lineage tracing indicated that the *CALR*^{mut} was acquired during fetal life by twin-to-twin transplacental transmission.[86]

Early screening for an early diagnosis

The early onset of *JAK2*^{V617F} and *CALR*^{mut}, decades before the disease, raises the challenge of early detection of MPN in the general population. To address this issue, our laboratory determined the common characteristics of a MPN patient population by mathematical modeling using the dataset actually observed from clonal architecture of the mutations in progenitors.[48] The different possible clonal expansion dynamics in individuals who developed MPN were determined as well as the probability of distribution of the clonal fraction of mutated HSC at any age. The best age to detect the mutation was determined in order to avoid identifying it too late (after the development of the symptoms) or looking for it too early, with the risk of false-negative results, even using methods with a sensitivity of 0.01%. This mathematical method revealed that early screening for *JAK2*^{V617F} mutation in people at risk of developing MPN might be feasible at an optimal age of 30 years while early screening for *CALR*^{mut} seems more difficult to establish. Interestingly, the parameter of *JAK2*^{V617F} clonal advantage inferred from the MPN cohort was stronger than that obtained by Watson *et al.* who studied CHIP in normal individuals,[87] highlighting the greater fitness of *JAK2*^{V617F} HSC in MPN. These results need further validation, but they suggest the possibility of detecting the *JAK2*^{V617F} mutation from a certain age in true cases of pre-MPN (Figure 2B). Another strategy is to predict MPN disease in people with CHIP (VAF ≥2%). Whole-exome sequencing data from healthy people who developed myeloid neoplasms over a 15-year period showed that the diagnosis could be predicted years before the development of a symptomatic disease, enabling the development of a robust time-dependent Cox proportional hazards model to predict the risk of myeloid malignancies, including MPN.[78]

These results suggest the possibility of early detection of MPN. Understanding the regulation of mutated HSC dynamics in larger cohorts of patients would improve the power of these mathematical models. The detection of people at risk of MPN in the general population would allow them to be carefully monitored and, possibly, prevent the development of MPN using molecules targeting the malignant clones or vascular events (Figure 2C).

Interferon-α: a disease-modifying drug?

Interferon-α signaling

IFN are cytokines produced in response to infectious and inflammatory stimuli. They have antiviral, immunomodulatory and/or anti-oncogenic properties. The IFNα receptor consists of two subunits, IFNAR1 (signal-transducing unit) and IFNAR2 (ligand binding/signaling unit), which are members of the class II cytokine receptor superfamily. IFNAR1 is associated with TYK2 and IFNAR2 with JAK1. The interaction of IFNα with its receptor activates STAT1 and STAT2 hetero/homodimer formation and results in the transcription of IFN-stimulating genes and IFN-γ-activated factor.[88,89] Further diversity of IFNAR signaling is achieved by the activation of non-STAT pathways, including the p38 MAPK and PI3K pathways. The IFNα response is negatively controlled by phosphatases, protein inhibitors of activated STAT (PIAS) and two well-known IFN-stimulating genes: *SOCS* and *USP18*.[88]

Cellular and molecular mechanism of interferon-α in myeloproliferative neoplasms

Interestingly, hematologic responses and *JAK2*^{V617F} deep molecular responses were reported in MPN patients treated with IFNα.[90] In mice, IFNα induces depletion of the *JAK2*^{V617F} disease-initiating HSC,[37,38] by increasing their cell cycle and inducing their differentiation leading to the expansion of megakaryocyte-biased HSC with lower long-term reconstitution capacities.[38,91] In primary *JAK2*^{V617F} MPN samples, Tong *et al*. found by single-cell transcriptomic analysis that IFNα induces quiescence of homozygous *JAK2*^{V617F} megakaryocyte-biased HSC and apoptosis of heterozygous *JAK2*^{V617F} megakaryocyte-biased HSC.[92] A mathematical model developed on a prospective longitudinal cohort of 48 MPN patients treated with IFNα showed that IFNα selectively targets mutated HSC through a process compatible with their exhaustion by differentiation, in line with previous findings that IFNα increased the percentage of hematopoietic stem and progenitor cells in the active phase of the cell cycle.[93] These effects depend on the type of driver mutation and on IFNα doses: IFNα was found to be more efficient on homozygous *JAK2*^{V617F} HSC than on heterozygous *JAK2*^{V617F} HSC, then on type 2 *CALR*^{mut} HSC and finally on type 1 *CALR*^{mut} HSC.[94] Thus, *CALR*^{mut} HSC appear to be more resistant to IFNα than *JAK2*^{V617F} HSC. Targeting the mutated HSC was slow, over several years, suggesting that long-term exposure to IFNα is required to alter disease outcome.

The signaling mechanisms by which IFNα specifically targets *JAK2*^{V617F} or *CALR*^{mut} HSC are still largely unknown. In mouse *Jak2*^{V617F} models, IFNα preferentially targets *JAK2*^{V617F} HSC through several mechanisms such as cell cycle activation, induction of reactive oxygen species and accumulation of DNA damage.[95] The IFNα-induced PKCδ-ULK1-P38 MAPK pathway was recently shown to be antiproliferative in *JAK2*^{V617F} MPN cells.[96] The effects of IFNα appear to be dependent on p53.[97] Finally, it has been observed that *JAK2*^{V617F}, but not *CALR*^{mut}, primes IFNα via transcriptional induction of STAT1.[95,98]

Clinical trials of interferon-α in myeloproliferative neoplasms

Decades of research have shown that IFNα is efficacious in MPN. A single-center, retrospective study of 470 PV patients

that compared IFNα to either hydroxyurea or phlebotomy reported longer myelofibrosis-free survival and overall survival with IFNα, suggesting a disease-modifying role of this drug.[99] However, frequent subcutaneous injections and short-term side effects lead to high treatment discontinuation rates.[100,101] The most frequent adverse events are flu-like symptoms, weight loss, bone pain, depression, and autoimmune complications. The development of pegylated recombinant IFNα2 (Peg-IFNα), Pegasys® (Peg-IFNα2a) and PegIntron® (Peg-IFNα2b), which is injected weekly,[102] and more recently of ropeginterferon alfa-2b (ropeg-IFNα), with fortnightly administration, has improved tolerance and compliance.

Phase II clinical trials of pegylated interferon-α monotherapy in high-risk essential thrombocythemia and polycythemia vera

Several phase II clinical trials of Peg-IFNα2 monotherapy in high-risk ET and PV patients have documented high rates of complete hematologic response, occurring in about 80% of patients,[90,101,103-106] even among those refractory or intolerant to hydroxyurea.[107] A decrease of *JAK2*^{V617F} VAF was observed in 40% to 90% of patients, with some deep molecular responses. Patients with *JAK2*^{V617F} mutation seemed to have better responses than patients harboring additional driver mutations. A prospective study on 123 ET patients and 136 PV patients found similar responses.[108] It is worth noting that hematologic and molecular responses increased over time and were durable. A bone marrow response was also reported in a subset of patients. The rate of thromboembolic events was uniformly low.[109] The multicenter PEGINVERA phase I/II clinical trial of ropeg-IFNα in PV patients had similar results.[110]

Randomized phase III clinical trials of pegylated interferon-α monotherapy in high-risk essential thrombocythemia and polycythemia vera

The MPD-RC 112,[111] DALIAH[112] and PROUD/CONTINUATION–PV[113] phase III trials randomized high-risk adult ET and PV patients to Peg-IFNα or hydroxyurea (Table 1).

The MPD-RC 112 trial compared Peg-IFNα2a to hydroxyurea in high-risk ET and PV patients. Of the target number of 300 patients, the study enrolled 168 patients (81 ET and 87 PV) due to the lack of availability of Peg-IFNα2a, which limited its statistical power. In the DALIAH trial patients aged >60 years were randomized to Peg-IFNα2a, Peg-IFNα2b or hydroxyurea and patients ≤60 years of age were randomized to Peg-IFNα2a or Peg-IFNα2b, resulting in an imbalance between the two arms with 38 patients assigned to the hydroxyurea arm and 135 patients to the Peg-IFNα arm; patients were significantly younger in the Peg-IFNα arm. Of note, 20% of the patients had myelofibrosis. The PROUD/CONTINUATION–PV trial was a randomized trial of ropeg-IFNα *versus* hydroxyurea in 257 PV patients who required cytoreduction with no prior treatment or with no complete hematologic response after less than 3 years of hydroxyurea.

Despite some limitations, these studies concluded that Peg-IFNα and hydroxyurea had comparable efficacy with no differences in term of hematologic and molecular response rates at 12 months. The overall and complete hematologic response rates gradually increased among patients treated with Peg-IFNα and decreased in patients treated with hydroxyurea, with the difference reaching statistical significance at the later evaluation timepoints. From 24 months, patients treated with Peg-IFNα had significantly higher rates of molecular responses, which were also deeper and longer. In the DALIAH trial, there was a significantly higher rate of discontinuation due to toxicity (30% of the PV patients) in the Peg-IFNα arm than in the hydroxyurea arm, probably reflecting a lower clinical tolerability of Peg-Intron®. Tolerability data were indeed better in patients treated with Peg-IFNα2a and ropeg-IFNα. In the MPD-RC 112 trial, grade ≥3 adverse events were more frequent with Pegasys® but the discontinuation rate for adverse events was similar in the two arms (11% *vs*. 15%). In the PROUD/CONTINUATION-PV study, only 11% of patients in the ropeg-IFNα arm stopped their treatment due to drug-related toxicity.

Combination therapy with ruxolitinib and pegylated interferon-α in high-risk polycythemia vera

In contrast to its inhibitory effect on JAK1, adding ruxolitinib to IFNα unexpectedly increased the efficacy and tolerance of treatment by reducing inflammation.[114] In the phase II COMBI-I study, 32 patients with active PV received a combination of ruxolitinib and Peg-IFNα. Eighty-one percent of them achieved complete peripheral blood cell normalization within 6 months with a median time to response of 1 month.[115] At the 2-year final analysis, 22% of patients achieved a partial response (including absence of symptoms, normal spleen size, normal peripheral blood cells, no progression or vascular event) and 9% achieved a complete hematologic response (partial response with histological remission). The *JAK2*^{V617F} VAF decreased significantly at all timepoints. Although the majority of patients were intolerant of IFNα before enrollment, only 31% of them stopped Peg-IFNα. There were few grade ≥3 hematologic adverse events. Grade 3-4 pneumonia was reported in 15.6% of patients.[116] The COMBI-II trial is currently evaluating the efficacy and safety of the ruxolitinib and Peg-IFNα combination in 25 newly diagnosed PV patients. Preliminary data show a high complete hematologic response rate after 1 month of treatment as well as a significant decrease of *JAK2*^{V617F}, with an acceptable toxicity profile.[117]

Pegylated interferon-α in low-risk polycythemia vera

The randomized phase II Low-PV study compared ropeg-IFNα and phlebotomy *versus* phlebotomy alone in low-risk PV patients.[118] Addition of ropeg-IFNα led to a higher response rate and improvement of symptoms and peripheral blood cell counts. Responses to ropeg-IFNα were

Table 1. Latest clinical trials with interferon-α.

Clinical trials	Type	Treatments	Diseases	Hematologic/molecular responses	References
MPD-RC 112	Randomized phase III	Comparison of Peg-IFNα2a *vs.* HU	High-risk ET and PV	After 12 months: no difference in CHR or MR After 36 months: increased CHR and MR in IFN-treated pts	111
DALIAH trial	Randomized phase III	Peg-IFNα2a, Peg-IFNα2b or HU	MPN	From 36 to 60 months: increased CHR and MR in IFN patients per protocol	112
PROUD/ CONTINUATION–PV trial	Randomized phase III	Ropeg-IFNα *vs.* HU	Early-stage PV (no history of cytoreductive treatment or <3 years of previous HU treatment)	After 12 months: no difference in CHR or MR After 36 months: increased CHR and MR in IFN-treated pts	113
COMBI-I study	Phase II	RUX and Peg-IFNα	Active PV	After 6 months: CHR in 80% of patients After 24 months: 22% of patients achieved partial response and 9% achieved CHR MR at all time points	116
COMBI-II trial	Phase II	RUX and Peg-IFNα combination	Newly diagnosed PV	After 1 month: CHR After 24 months: significant MR	117
Low-PV study	Randomized phase II	Ropeg-IFNα and phlebotomy *versus* phlebotomy alone	Low-risk PV	After 2 years: higher response rate and improvement of symptoms and blood counts with ropeg-IFNα and phlebotomy	118,119
P1101MF trial	Phase II	Ropeg-IFNα	Prefibrotic primary MF or low- or intermediate-1-risk MF	After 48 weeks: decreased symptoms, reduction of spleen size, CBC improvement, resolution of marrow fibrosis (17.4%) and significant MR	123
COMBI I study	Phase II	Peg-IFNα and RUX	MF	Rapid clinical, hematologic, and histological responses with MR	115,116
RUXOPEG	Phase I/II	Peg-IFNα and RUX	MF	Rapid clinical, hematologic, and histological responses with MR	124

Peg: pegylated; IFN: interferon; HU: hydroxyurea; ET: essential thrombocythemia; PV: polycythemia vera; CHR: complete hematologic response; MR: molecular response; MPN: myeloproliferative neoplasm; RUX: ruxolitinib; MF: myelofibrosis; CBC: complete blood count.

durable.[118,119] There was no difference in grade 3 adverse events between the arms.

Clinical trials of pegylated interferon-α in myelofibrosis

Several case series and preliminary results of the phase II P1101MF trial documented the efficacy of pegylated IFNα monotherapy in myelofibrosis patients, with resolution of symptoms, reduction of spleen size, improvement of peripheral blood cell counts and a decrease of *JAK2*^{V617F} VAF with an acceptable safety profile.[120-123] In the P1101MF study, among the 46 patients who underwent serial bone marrow biopsies, eight patients (17.4%) had resolution of marrow fibrosis by 48 weeks. Myelofibrosis patients seem to benefit from IFNα monotherapy at an early stage of disease. Eighty myelofibrosis patients enrolled in the COMBI I study and 37 in the phase I/II RUXOPEG trial (preliminary results) received a combination of Peg-IFNα and ruxolitinib. They obtained rapid clinical, hematologic, molecular and histological responses with acceptable toxicities.[115,116,124] Molecular response was less frequent in *CALR*^{mut} patients than in *JAK2*^{V617F} patients.

Clinical trials of pegylated interferon-α in myeloproliferative neoplasm: conclusions

Clinical trials confirm the high rate of hematologic and molecular responses to IFNα particularly in *JAK2*^{V617F} patients, as

well as its safety for the treatment of ET, PV, and early PMF. Response to IFNα is more gradual compared to the response to hydroxyurea but is deeper and more durable; however, long-term exposure to IFNα is needed to obtained full efficacy. These trials indicate that IFNα is a disease-modifying therapy that could counteract disease progression and even cure *JAK2*^{V617F} MPN, suggesting the possibility of discontinuing IFNα in patients with long-term, deep molecular responses. Eligibility criteria for IFNα interruption have yet to be clearly defined but work has been done based on mathematical modeling and statistical inference.[125-127]

The association of IFNα with ruxolitinib led to fast responses without limiting toxicity. Therefore, improvement of IFNα treatment could rely on its combination with other drugs. Preclinical models have suggested a synergistic association between IFNα and arsenic,[128] IFNα and nutlin (an MDM2/P53 antagonist)[97, 129] as well as between IFNα and 5-azacytidine which could specifically overcome resistance to treatment due to *DNMT3A* loss.[130-132] The development of oral forms of arsenic and 5-azacytidine could make the administration of these drugs easier in MPN patients, but the toxicities of the combinations could be a limiting factor. Moreover, additional non-driver mutations should be taken into account, especially a *TP53* mutation which is a risk factor for leukemia transformation[61,62] and can be selected upon IFNα treatment.[132] Therefore, in the presence of a *TP53* mutation, treatment with IFNα should be followed carefully or avoided.

Conclusions and perspectives

In conclusion, MPN are due to driver mutations occurring in HSC during fetal life or early childhood which lead to slow clonal expansion until the disease reveals itself decades later, mainly after the age of 60 years. This very long evolution opens new avenues for the early detection of MPN in the general population and for the early diagnosis and treatment of MPN diseases. Pioneering work has started but should be pursued in more depth in view of the large diversity of MPN development (age, type of disease, progression). In the future, we can hope to intercept MPN development with selective therapies (IFNα, CALR^{mut} monoclonal antibodies and JAK2^{V617F} inhibitors) which will decrease the malignant clone. Furthermore, additional mutations occurring during the progression of the disease are likely to alter the response to conventional therapies, which is why it is also important to use therapies such as IFNα in the prevention of MPN progression at the time of presentation. Such therapies will be difficult to evaluate in patients with early MPN and remain a challenge in people without any symptoms such as those in a pre-MPN state.

Disclosures

No conflicts of interest to disclose.

Contributions

All the authors participated in the writing and critical reading of this review. IP led the writing. FP was more involved in the clinical part, JP in early detection, and JEM and SM in the natural history of the disease.

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