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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 (219 words)

The classical *BCR::ABL*-negative myeloproliferative neoplasms (MPN) include Polycythemia Vera (PV), Essential Thrombocytemia (ET), and Primary Myelofibrosis (PMF). They are acquired clonal disorders of the hematopoietic stem cells (HSC) 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 the 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 the MPN development highlighting the early origin of driver mutations decades before the onset of symptoms and the consequence on early detection of MPN cases in the general population for early diagnosis and better medical management. Finally, we present the interferon alpha (IFNα) therapy as a potential early disease-modifying drug after reporting its good hematological and molecular efficacies in ET, PV and early MF 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 IFNα, JAK2^{V617F} inhibitors and CALR^{mut} monoclonal antibodies, would be able to intercept the mutated clones.

1- Generalities on classical myeloproliferative neoplasms (MPN)

Diagnosis, complications and progression of MPN

MPN are heterogeneous clonal disorders of the 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 upon the WHO 2016 and 2022 classifications [1,2]. Excess of platelet production, excess of red blood cells (RBC) production and megakaryocytic hyperplasia with bone marrow fibrosis characterize ET, PV, and PMF, 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 intermediary phenotype between ET and overt PMF [3]. At last, leukemic transformation of dismal prognosis is a common complication of classical MPN. The other main non-hematological complications are vascular events (mainly in ET and PV) and symptoms like pruritus, fatigue, night sweats that alter the patients' quality of life (QoL).

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 3 genes encoding *JAK2*, the calreticulin (*CALR*) and the thrombopoietin receptor (*MPL*) [4–7]. Their identification is a major diagnostic criterion in the WHO classifications.

JAK2^{V617F} is found in 95% of PV and around half of ET and PMF. Around 100 different *CALR* mutations (*CALR*^{mut}) have been described to date and observed in 20-30% of ET and PMF [4–7]. Among them, the most frequent are the deletion of 52 base pairs (type 1) and the insertion of 5 base pairs (type 2). They 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 on the W515 residue of the protein.

General management of MPN

Management of ET and PV patients has for main goal the prevention of vascular events (thrombosis and hemorrhages) and relies on a rigorous treatment of the cardiovascular risk factors (CVRF) for all patients, and the administration of low dose acetyl salicylic acid (ASA) and cytoreductive therapy depending on individual vascular risk. International guidelines [8,9]

recommend using the revised IPSET-thrombosis (IPSET-th) scoring system to stratify the vascular risk of ET patients [10,11]. All ET patients should receive ASA apart from very low risk (LR) asymptomatic ET patients with *CALR*^{mut} for whom observation is only recommended [12]. Cytoreductive treatment is indicated in patients > 60 years with *JAK2*^{V617F} mutation and/or CVRF, in patients with 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 ASA and phlebotomies to maintain their hematocrit ≤ 45% [8,9,13,14]. High-risk (HR) PV patients, defined by age ≥ 60 years and/or 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 (i.e. leukocytosis, inadequate hematocrit control with phlebotomies, relevant CVRF), 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 LR PV patients [8,9,16–18]. For both ET and PV, the recommended first-line agents are hydroxyurea (HU) and pegylated IFNα. Anagrelide and ruxolitinib (RUX) are second-line therapeutic options in ET and PV, respectively [9,15–17].

Despite recent improvements in the management of MF, allogeneic stem cell transplantation (ASCT) remains the only curative treatment for these patients [19]. The significant risk of treatment-related morbidity and mortality implies a rigorous selection of eligible patients. Prognostic scoring systems and individual factors guide the transplant decision-making [8,9,18,20]. However, due to advanced age and comorbidities, only a minority of patients with expected poor survival are eligible for ASCT. Therefore, the management of MF patients remains mainly symptomatic. MF 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]. HU is a therapeutic option for the treatment of splenomegaly in patients with LR or int-1 risk MF and in case of leukocytosis or thrombocytosis needing cytoreduction [8,9,18]. JAK inhibitors (JAKi) are effective for the treatment of constitutional symptoms and splenomegaly [21–26]. RUX is approved by the FDA in intermediate (int-) and high-risk MF patients with platelet count > 50.10^9/L, fedratinib for ≥ int-2 risk MF patients with platelet count > 50.10^9/L and pacritinib for ≥ int-risk MF patients with platelet count < 50.10^9/L [18]. Conventional therapies for MF-related anemia have insufficient and transient efficacy (reviewed in [27]), however a

fourth JAKi, Momelotinib, was recently approved for the treatment of ≥ int-risk MF patients with anemia [28,29].

RUX administration significantly improves survival compared to historical treatment and decreases of *JAK2*^{V617F} variant allele frequency (VAF) and fibrosis in some MF patients (reviewed in [30]), as well as *JAK2*^{V617F} VAF in some PV patients [31]. However, there is little evidence for a disease-modifying role for either RUX or any of the others 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.

2- Natural history of MPN

Function of MPN driver mutations

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 a rogue chaperone and ligand by binding, oligomerizing and activating MPL at the cell-surface [32–36]. The *JAK2*^{V617F}, *CALR*^{mut} and *MPL* mutation have been proven to be drivers of the disease in different settings of mouse modeling [37–40]. Importantly, inactivation of JAK2^{V617F} through deletion of *JAK2* in an innovative conditionally mouse model led to the abrogation of the MPN disease, demonstrating the monogenic character of MPN development[41].

Although these driver mutations occur in HSC and activates the JAK2/STAT signaling, they have distinct effects on the 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 that 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 the peripheral blood cells (PBC) [42–44]. The homozygous clones have a stronger competitive advantage over the heterozygous clones even in the HSC compartment [43,45]. ET patients have generally low *JAK2*^{V617F} VAF in their blood (around 20% or less), compared to 50% in PV patients and to 70-80% in post-ET/PV MF due to *JAK2*^{V617F} homozygosity, in line with the continuum of diseases. In contrast, in *CALR*^{mut} ET patients, the VAF were found at around 30-50% at the HSC level with very mild increase during hematopoietic differentiation. During the progression of ET to MF, *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 MF 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 the *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. These different effects on hematopoiesis could be due to distinct intensity in the activation of the 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 ET/PV towards SMF or ET towards SMF, can be modified by additional somatic mutations as well as germline and extrinsic factors.

Intrinsic and extrinsic regulators responsible for MPN heterogeneity

In association to the driver mutations, MPN patients can harbor additional somatic mutations of the epigenetic regulators, the spliceosome machinery and the transcription factors, which are also found in other myeloid malignancies. Their type, number and order of appearance are of importance as disease-modifying factors. They contribute to the disease onset, the type of MPN, the risk of progression and the 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 the 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 disease by skipping the development of PV. *ASXL1*, *EZH2* and several splicing mutations are associated with PMF. *ASXL1* mutations are also linked to higher risk of leukemic transformation, except when associated with type 1 *CALR*^{mut} mutation, and poorer survival [27,58,59]. *IDH*1/2 neomorphic mutations are also at high-risk of inducing leukemic transformation of *JAK2*^{V617F} MPN [60,61]. *SRSF2* and *U2AF1* mutations are of poor prognosis. *SRSF2* mutations are strongly involved in leukemic transformation and *U2AF1* mutations are associated with anemia and thrombocytopenia [27]. Several mutations in transcription factors contribute to the leukemic transformation such as *TP53* alterations in 20% of mutated cases [62].

Genetic factors also contribute to the MPN heterogeneity. Common genetic variants have been identified in the general population in large genome-wide association studies (GWAS) as predisposing to the development of sporadic MPN patients (haplotype 46/1, MECOM, GFI1B, MYB, GATA2, TET2, CHEK2, TERT, ATM, TP53, etc.) [63,64]. More recently, polygenic risk scores underlying common hematological traits were identified as important for *JAK2*^{V617F} clonal expansion [65]. Pan-genomic approaches in some MPN families identified very rare germline predisposing factors like *RBBP6*, *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 MF and to leukemia in half of cases [66,67].

Several extrinsic factors may also modify initiation, phenotype and progression of MPN such as aging and inflammatory cytokines (TNFα, IL1β, IL-6, IL-13, IL-8) [68]. MPN are mainly diseases of the elderly and age is associated with "inflammaging" phenomenon that can drive DNA damages and the exit of quiescence of HSC [69], allowing the selection of *JAK2*^{V617F} HSC [70]. Acute or chronic infections [71] and exposure to toxicants such as chemotherapy are environmental stressors that may modify the HSC homeostasis or the composition of the bone marrow microenvironment to play a role in the selection and the expansion of the mutated clone (for example *ASXL1*, *EZH2* and *RAS* selection with RUX). Conversely, *JAK2*^{V617F} alone or in combination with others 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 ROS [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.

3- New paradigm of MPN development: early origin of the driver mutations and diagnosis

From clonal hematopoiesis of indeterminate potential (CHIP) to MPN development

The concept of clonal hematopoiesis emerged with the identification of people without hematological 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 10% of healthy people older than seventy and in 1% of younger individuals [75–77]. This clonal hematopoiesis without disease was named 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 hematological malignancies, coronary heart disease, and ischemic stroke. Recently, CHIP was defined by the WHO/ICC classification as a precursor state of myeloid disease [1,3]. *JAK2*, *CALR*, and *MPL* CHIP have been associated with higher platelet count and hemoglobin level, and an increased risk of developing an MPN [78]. Therefore, these CHIP (VAF>2%) could be considered as a pre-MPN state since *JAK2*, *CALR*, and *MPL* mutations alone without associated mutations, are sufficient to drive the disease. Even if *JAK2*^{V617F} CHIP were 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 corelated 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 these sensitive methods in the general population contrasts with the low penetrance of the MPN disease with an incidence of 1/100,000 at an advanced age (median of 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 are a sufficient number of mutated HSC (around 30 HSC), while the penetrance of the disease is less than 1% of the 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 diseases, especially in patients harboring with the 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. Therefore, the early screening of the driver mutation could be an asset in those cases to improve clinical care and to avoid inaugural thrombo-hemorrhagic events

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

Early origin of the driver mutations

The discovery of clonal hematopoiesis expansion over time, suggests that the acquisition of the driver mutation may occur long before the onset of the disease symptoms. The reconstruction of the 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 patient age [48]. Occurrence of *JAK2*^{V617F} in the fetal life was confirmed by its identification in a cord blood unit used for ASCT in an AML patient [84]. *CALR*^{mut} was inferred to occur 10 years later than JAK2^{V617F} (average acquisition at 25 years versus 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 MF at ages 37 and 38 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 presents the challenge of the 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 data set 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 to avoid identifying it too late (after the development of the symptoms) or too early, with the risk of false-negative results, even using methods with a sensitivity of 0.01% sensitivity. This mathematical method revealed that early screening of *JAK2*^{V617F} mutation in people at risk to develop MPN might be feasible at an optimal age of 30 while early screening of *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 higher 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 was to predict MPN disease in people with CHIP (VAF>2%). Whole exome sequencing data from 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 an early detection of MPN. Understanding the regulation of mutated HSC dynamic 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 eventually, to prevent the development of MPN using molecules targeting the malignant clones or the vascular events (Figure 2C).

4- Interferon alpha (IFNα): a disease-modifying drug?

IFNα signaling

IFN are cytokines produced in response to infectious and inflammatory stimuli. They have antiviral, immunomodulatory and/or antioncogenic properties. The IFNα receptor consists in 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/homodimers formation and results in the transcription of IFN stimulating genes (ISGs) and IFN-γ-activated factor (GAF) [88,89]. Further diversity of IFNAR signaling is achieved by the activation of non-STAT pathways, including the p38 MAPK and PI3K pathway. The IFNα response is negatively controlled by phosphatases, protein inhibitors of activated STAT (PIAS) and two well-known ISGs: SOCSs and USP18 [88].

Cellular and molecular mechanism of IFNα in MPN

Interestingly, hematological response and *JAK2*^{V617F} deep molecular responses (MR) were reported in MPN patients treated with IFNα [90]. In mice, IFNα induces the 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 (MK)/myeloid HSC with lower long-term reconstitution capacities [38,91]. In primary *JAK2*^{V617F} MPN samples, Tong *et al.* found by single cell transcriptomic that IFNα induces quiescence of homozygous *JAK2*^{V617F} MK-biased HSC and apoptosis of heterozygous *JAK2*^{V617F} MK-biased HSCs [92]. A mathematical model developed on a prospective longitudinal cohort of 48 MPN patients treated with IFNα demonstrated that IFNα selectively targets mutated HSC through a process compatible with their exhaustion by differentiation, in line with previous work showing that IFNα increased the percentage of HSPCs in the active phase of the cell cycle [93]. These effect depends on the type of the 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, the *CALR*^{mut} HSC appear to be more resistant to IFNα than *JAK2*^{V617F} HSC. Targeting of the mutated HSC was slow over several years suggesting altering disease outcome involves long-term exposure to IFNα.

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

Clinical trials of IFNα in MPNs

IFNα has been shown to be efficacious in MPN for decades. A single center retrospective study of 470 PV patients that compared IFNα to either HU or phlebotomy reported longer myelofibrosis-free survival (MFS) and overall survival (OS) with IFNα, suggesting a disease-modifying role of this drug [99]. However, frequent sub-cutaneous injections and short-term side effects lead to high treatment discontinuation rate [100,101]. The most frequent adverse events (AEs) are flu-like symptoms, weight loss, bone pain, depression, and autoimmune complications. Development of pegylated recombinant IFNa2 (Peg-IFNα), Pegasys® (Peg-IFNa2a) and PegIntron® (Peg-IFna2b), with weekly injections [102] and more recently of ropeginterferon alfa-2b (ropeg-IFNα) with a fortnightly administration has improved tolerance and compliance.

Phase 2 clinical trials of pegylated IFNα in monotherapy in high-risk ET and PV

Several phase 2 clinical trials of Peg-IFNa2 monotherapy in HR ET and PV patients have reported a high rate of complete hematological response (CHR) rate in around 80% of patients [90,101,103–106], even in patients who are refractory or intolerant to HU [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 reported similar responses [108]. It is worth noting that hematological and molecular responses increased over the time and were durable. A bone marrow response was also reported in a subset of patients. The rate of thromboembolic events was uniformly low (reviewed in [109]). The multicenter PEGINVERA phase 1/2 clinical trial of ropeg-IFNα in PV patients had similar results [110].

Randomized phase 3 clinical trials of pegylated IFNα in monotherapy in high-risk ET and PV

The MPD-RC 112 [111], DALIAH [112] and PROUD/CONTINUATION–PV [111] phase 3 trials randomized pegylated IFNα to HU in HR adult ET and PV patients (Table 1).

The MPD-RC 112 trial compared Peg-IFNa2a to HU in HR ET and PV patients. The study enrolled 168 patients (81 ET and 87 PV) out of the targeted number of 300 patients due to the lack of availability of Peg-IFNa2a, which limited its statistical power. In the DALIAH trial patients aged over 60 were randomized to either Peg-IFNa2a, Peg-IFNa2b or HU and patients ≤ 60 years of age to Peg-IFNa2a or Peg-IFNa2b, resulting in imbalance between the two arms with 38 patients assigned to the HU 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 MF. The PROUD/CONTINUATION–PV trial randomized ropeg-IFNα versus HU in 257 PV patients who required cytoreduction with no prior treatment or with no CHR after less than 3 years of HU.

Despite some limitations, these studies concluded to comparable efficacy of pegylated IFNα and HU with no difference in term of hematological and molecular response rates at 12 months. Then, the overall response rate (ORR) and CHR gradually increased among patients treated with pegylated IFNα and decreased in patients treated with HU, reaching statistical significance at the later evaluation time points. From 24 months, patients treated with pegylated IFNα had significant higher, deeper and longer molecular responses.

In the DALIAH trial, the Peg-IFNα arm compared to HU had a significant higher rate of discontinuation due to toxicity (30% of the PV patients), probably reflecting a lower clinical tolerability of Peg-Intron®. Tolerability data were indeed better in patients treated with Peg-IFNa2a and ropeg-IFNα. In the MPD-RC 112 trial, > or = grade 3 AEs were more frequent with Pegasys® but the discontinuation rate for AE was similar between arms (11% *versus* 15%). Only 11% of patients in the ropeg-IFNα arm stopped their treatment due to drug-related toxicity in the PROUD/CONTINUATION-PV study.

Combination therapy of RUX and pegylated IFNα in high risk PV

In contrast to its inhibitory effect on JAK1, adding RUX to IFNα increased efficacy and tolerance to treatment by reducing inflammation [113]. In the phase 2 COMBI-I study, 32 patients with active PV received a combination of RUX and Peg-IFNα. Eighty-one percent of them achieved complete PBC normalization within 6 months with a median time to response of 1 month [114]. At the 2 years final analysis, 22% of patients achieved a partial response (including absence of symptoms, normal spleen size, normal PBC, no progression or vascular event) and 9% achieved CHR (PR with histological remission). The *JAK2*^{V617F} VAF significantly decreased at all time points. Although the majority of patients were intolerant to IFNα before enrollment, only 31% of them stopped Peg-IFNα. There were few ≥ grade 3 hematological AEs. Grade 3-4 pneumonias were reported in 15.6% of patients [115]. The COMBI-II trial is currently evaluating the efficacy and safety of RUX and Peg-IFNα combination in 25 newly diagnosed PV patients. Preliminary data show high CHR rate after 1 month of treatment as well as a significant decrease of *JAK2*^{V617F}, with an acceptable toxicity profile [116].

Pegylated IFNα in low-risk PV

The randomized phase 2 Low-PV study compared ropeg-IFNα and phlebotomy *versus* phlebotomy alone in low-risk PV patients [117]. Addition of ropeg-IFNα led to higher response rate and improvement of symptoms and PBC. Response to ropeg-IFNα was durable [117,118]. There was no difference for grade 3 AE between arms.

Clinical trials of pegylated IFNα in MF

Several case series and preliminary results of the phase 2 P1101MF trial reported efficacy of pegylated IFNα in monotherapy in MF patients with resolution of symptoms, reduction of spleen size, PBC improvement and decrease of *JAK2*^{V617F} VAF with acceptable safety profile [119–122]. In the P1101MF study, among the 46 patients who had serial bone marrow biopsies, eight patients (17.4%) had resolution of marrow fibrosis by 48 weeks. MF patients seem to benefit from IFNα in monotherapy at early stage of the disease. Eighty MF patients enrolled in the COMBI I study and 37 MF patients in the phase 1/2 RUXOPEG (preliminary results) received combination of Peg-IFNα and RUX. They obtained rapid clinical, hematological, molecular and histological responses with acceptable toxicities [114,115,123]. Molecular response in *CALR*^{mut} patients was more infrequent than for *JAK2*^{V617F} patients.

Clinical trials of pegylated IFNα in MPN: conclusion

Clinical trials confirm the high rate of hematological and molecular responses particularly in *JAK2*^{V617F} patients as well as the safety of IFNα for the treatment of ET, PV, and early PMF. Response to IFNα is more gradual compared to HU but is deeper and more durable but needs long-term exposition to IFNα to obtained full efficiency. These trials point IFNα as a disease-modifying therapy that could counteract disease progression and even cure *JAK2*^{V617F} MPN, suggesting the possibility of IFNα discontinuation in patients with long-term and deep molecular responses. Eligibility criteria for IFNα interruption have still to be clearly defined but works have been done based on mathematical modeling and statistical inference [124–126]. Association of IFNα with RUX led to fast response without limiting toxicity. Therefore, improvement of IFNα treatment could rely on the combination with other drugs. Pre-clinical models have suggested a synergistic association between IFNα and arsenic [127] as well as IFNα and 5-azacytidine that could specifically overcome resistance to treatment due to *DNMT3A* loss [128,129]. 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 these combinations could be a limiting factor. Moreover, additional non-driver mutation should be taken into account, especially *TP53* mutation that is a risk factor for leukemia transformation [61] and can be selected upon IFNα treatment [130]. Therefore, in presence of *TP53* mutation, treatment with IFNα should be carefully followed or avoided.

Conclusions and perspectives

In conclusion, MPN are due to driver mutations occurring in HSC during fetal life or early childhood and that leads to slow clonal expansion until the disease reveals itself decades after, mainly after sixty, opening new avenues for the early detection of MPN in the general population and for the early diagnosis and treatment of MPN diseases. Pioneer works have started but should be more deeply refined in view of the large diversity of MPN development (age, type of disease, progression). In the future, we can hope to intercept the MPN development with selective therapies (IFNα, $CALR^{mut}$ monoclonal antibodies and $JAK2^{V617F}$ inhibitors) that will decrease of 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 early MPN patients and remain a challenge in people without any symptoms such as in pre-MPN state.

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Table 1 : Latest clinical trials with IFNα

Clinical Trials	Type	Treatments	Diseases	Hematological/Molecular responses	Refs
MPD-RC 112	Randomized phase 3	Comparison of Peg-IFNa2a with HU	High risk ET and PV patients	After 12 months: no difference in CHR nor MR After 36 months: increased CHR and MR in IFN	[111]
DALIAH trial	Randomized phase 3	Peg-IFNa2a, Peg-IFNa2b or HU	MPN patients	From 36 to 60 months: increased CHR and MR in IFN in patients per protocol	[112]
PROUD/CONTINUATION–PV trial	Randomized phase 3	Ropeg-IFNa versus HU	Early stage PV (no history of cytoreductive treatment or < 3 years of previous hydroxyurea treatment	After 12 months: no difference in CHR nor MR After 36 months: increased CHR and MR in IFN	[111]
COMBI-I study	Phase 2	RUX and Peg-IFNa	Active PV patients	After 6 months: CHR in 80% of patients After 24 months: 22% of patients achieved partial response (PR) and 9% achieved CHR MR decreased at all time points	[115]
COMBI-II trial	Phase 2	RUX and Peg-IFNa combination in 25 newly diagnosed PV patients	Newly diagnosed PV patients	After 1 month: CHR After 24 months: significant MR	[116]
Low-PV study	Randomize	Ropeg-IFNa	Low-risk PV	After 2 years: higher	[117,118]

	d phase 2	and phlebotomy versus phlebotomy alone	patients	response rate and improvement of symptoms and blood counts with Ropeg-IFNa and phlebotomy	
P1101MF trial	Phase 2	Ropeg-IFNa	Prefibrotic primary myelofibrosis or low- or intermediate -1–risk myelofibrosis	After 48 weeks: decreased symptoms, reduction of spleen size, CBC improvement, resolution of marrow fibrosis (17.4%) and significant MR	[116]
COMBI I study	Phase 2	Peg-IFNa and RUX	MF	Rapid clinical, hematological, molecular and histological responses with MR	[114,115,123]
RUXOPEG	Phase 1/2	Peg-IFNa and RUX	MF	Rapid clinical, hematological, molecular and histological responses with MR	[114,115,123]

New approaches to standard of care in early phase MPN – can IFNα alter disease natural history?

Figures legend:

Figure 1 : Impact of driver mutations in hematopoiesis.

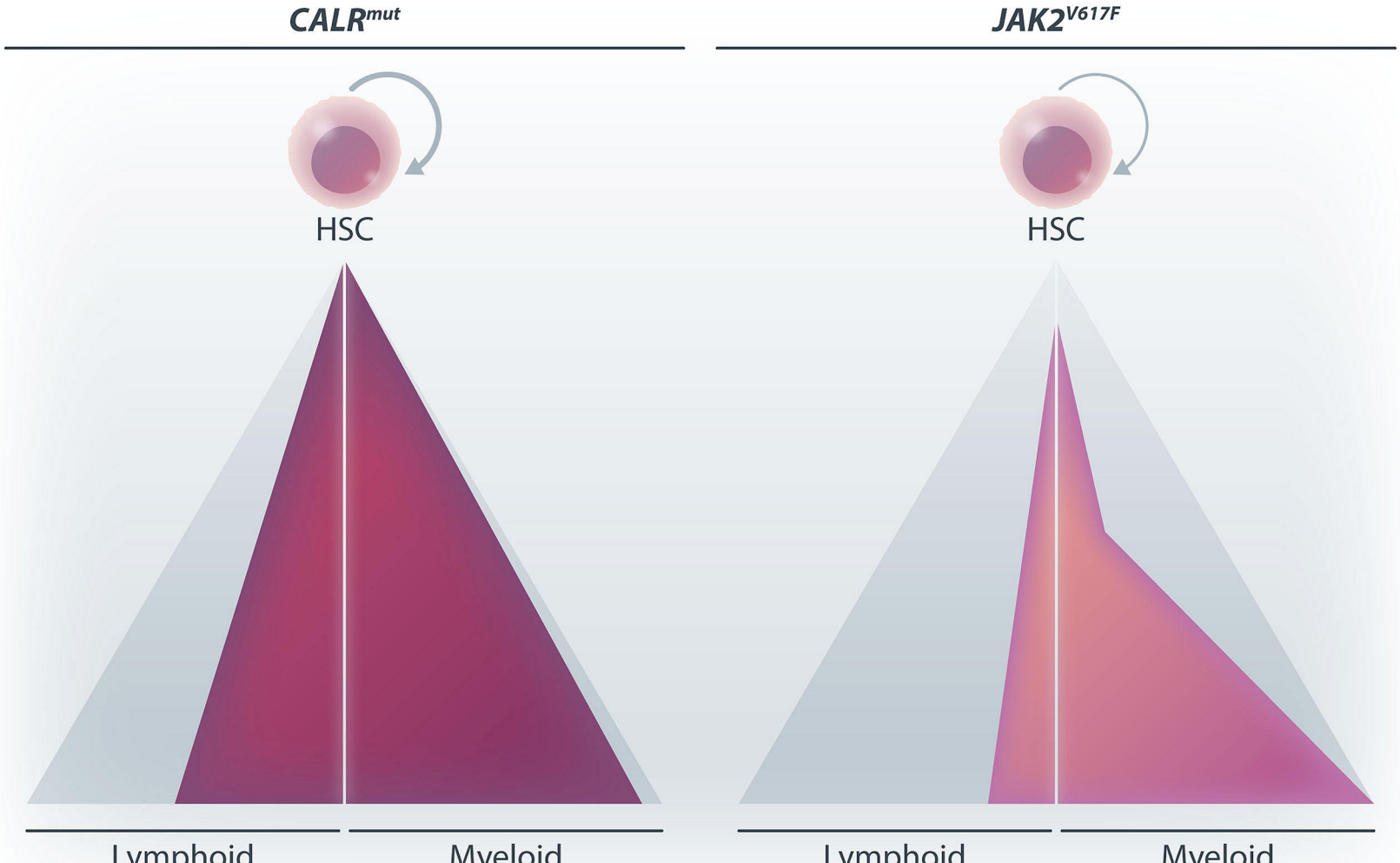
In ET patients, *CALR*^{mut} show strong clonal dominance in all the HSC compartment. *CALR*^{mut} induce a mild amplification during hematopoietic differentiation, being present with almost similar VAF in granulocytes than in the HSC compartment. Moreover, *CALR*^{mut} are also present in lymphoid cells including T cells. In contrast, in ET patients, *JAK2*^{V617F} is generally present with low clonal dominance in the HSC compartment but strongly amplifies during myeloid differentiation. *JAK2*^{V617F} is generally not present or at very low VAF in lymphoid cells.

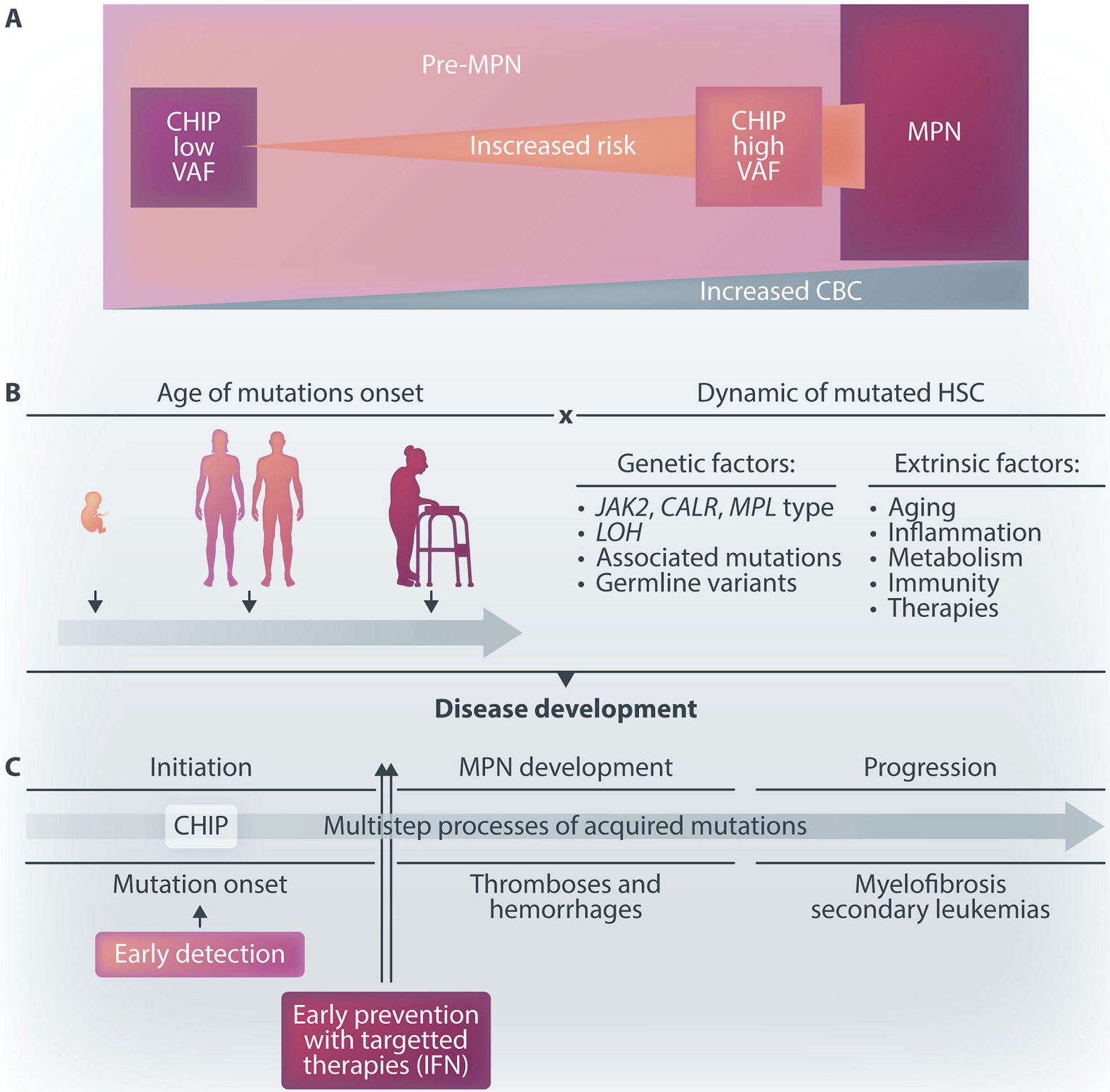
Figure 2 : Early detection, early diagnosis and early prevention

A- The clonal hematopoiesis of indeterminate potential (CHIP) corresponds to a state in which driver mutations (VAF>2%) are present without giving disease. Nevertheless, as JAK2^{V617F} alone can be responsible of MPN, CHIP with JAK2^{V617F} can be considered as a pre-MPN state. The VAF of JAK2^{V617F} CHIP correlates with complete blood count (cbc) levels and with the increased risk of triggering a MPN.

B- The MPN disease onset 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- Natural development of the MPN diseases and early standard of care approaches in early phase of MPN. 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 involve intercepting mutated clones using targeted therapy such as IFNα or new selective treatments, could be a breakthrough in preventing the development, progression and complications of MPN.


CALRmut
JAK2V617F
HSC
HSC
Lymphoid
Myeloid
Lymphoid
Myeloid


A
Pre-MPN
CHIP
low
VAF
Inscreased risk
CHIP
high
VAF
MPN
Increased CBC
B
Age of mutations onset
x
Dynamic of mutated HSC
Genetic factors:
• JAK2, CALR, MPL type
• LOH
• Associated mutations
• Germline variants
Extrinsic factors:
• Aging
• Inflammation
• Metabolism
• Immunity
• Therapies
Disease development
C
Initiation
MPN development
Progression
CHIP
Multistep processes of acquired mutations
Mutation onset
Thromboses and
hemorrhages
Myelofibrosis
secondary leukemias
Early detection
Early prevention
with targetted
therapies (IFN)