FINAL DEGREE PROJECT

PEGYLATED INTERFERON AS A FIRST LINE TREATMENT IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA PATIENTS: A QUASI-EXPERIMENTAL STUDY

January, 2022

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Primeiro de todo, agradecer á Dra. Anna Angona a axuda e compromiso con este proxecto. Ao servicio de Hematoloxía do ICO Girona pola súa atención e dedicación ás estudantes, en especial ao Dr. Gallardo que sempre nos recibe con simpatía e amabilidade. Todas as médicas e médicos do equipo son exemplos para min de trato humano e respectuoso, con pacientes e compañeiras.

Agradecer ao Dr. Rafael Marcos e ao Dr. Rafael Ramos a súa axuda e supervisión. Tamén ao profesor Marc Sáez, un exemplo de dedicación cara ao alumnado.

Ás personas que fixeron de Girona unha casa durante este tempo:

Paula, Macarena, Loretta, Ainara, Andrea; as vosas risas lévoas gardadas conmigo para sempre.

Laura, gracias por aprender a entendernos, por todo o escoitado, por todo o aprendido. Por ser tan leal ás túas amigas.

Inês, por ser miña irmá. Conmigo sempre terás unha casa.

Ao resto de persoas que me acompañaron, gracias por estes 6 anos.

Á Arousa; ás miñas amigas que son o meu lugar feliz. Á miña familia, o gran pilar da miña vida. Ás miñas primas, sempre. A Xoel e Mía por ser a miña alegría.

A Mirari, gracias pola túa valentía.

A meu pai que me deixou claro que o orgullo non son os resultados senón o esforzo.

A ti Mami, que estás en min. Gracias por confiar neste camiño antes de que o comezase.

Por último a Víctor, a verdadeira sorte da miña vida. Os nosos logros sempre serán compartidos; este traballo tamén é teu.

INDEX

FIGURE INDEX

TABLE INDEX

ABBREVIATIONS

- **AML**: Acute myeloid leukemia
- **ASA:** Acetylsalicylic acid
- **ASH**: American Society *of* Hematology
- ***ASXL1*****:** *Additional Sex Combs-Like 1*
- **BM**: Bone marrow
- ***CALR***: *Calreticulin*
- **CEIC**: Comitè Ètic d'Investigació Clínica
- **CI**: Confidence interval
- **CML**: Chronic myeloid leukemia
- **CR**: Crude rate
- **CVRF**: Cardiovascular risk factors
- ***DNMT3A***: *DNA methyltransferase 3 A*
- **ELN:** European Leukemia Net
- **ET:** Essentia trombocythemia
- **EPO**: Erythropoietin
- ***EZH2*****:** *Enhacer of zeste homolog 2*
- **G-CSF**: Granulocyte-colony stimulating factor
- **GC**: General coordinator
- **GEMFIN**: Grupo Español de Enfermedades Mieloproliferativas Crónicas Filadelfia Negativas
- **HBV**: Hepatitis B virus
- **HCV**: Hepatitis C virus
- **HIV**: Human immunodeficiency virus
- **HJT**: Hospital Universitari Josep Trueta
- **HSC**: Hematopoietic Stem Cell
- **Hto**: Hematocrit
- **HU**: Hydroxyurea
- **ICO**: Institut Català d'Oncologia
- **ICS**: Institut Català de la Salut

- ***IDH1/2***: *Isocitrate dehydrogenase 1/2*
- **IPSET-thrombosis**: International Prognostic Score for Thrombosis in Essential Trombocythemia
- **JAK**: Janus kinasas
- ***JAK2***: *Janus kinasa 2*
- **MPL**: Myeloproliferative Leukemia Protooncogen
- **MPN**: Myeloproliferative neoplasms
- **c-MPL**: myeloproliferative leukemia protein / Thrombopoietin receptor
- **MPN Ph (-)**: Philadelphia chromosome-negative myeloproliferative neoplasms
- **NGS**: Next Generation Sequencing
- **PMF**: Primary myelofibrosis
- **PV:** Polycythemia vera
- **RC:** Relative contraindications
- ***TET2*:** *Ten-Eleven Translocation-2*
- **TK**: Tirosin kinasa
- **TPO**: Thrombopoietin
- **STAT**: Signal transducer and activator of transcription
- **CR**: Crude incidence rate
- **WBC**: White Blood Cells
- **WES**: Whole Exome Sequencing
- **WHO**: World Health Organization
- **WT**: Wild-type

ABSTRACT

TITLE: Pegylated Interferon as a first line treatment in polycythemia vera and essential thrombocythemia patients: a quasi-experimental study

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BACKGROUND: Polycythemia vera (PV) and essential thrombocythemia (ET) are myeloproliferative disorders that are characterized by a proliferation of the erythroid and platelet series, respectively. These patients' survival is long and therefore drugs that are safe and effective in the long term are needed. In this respect, pegylated interferon (peg-IFNα-2a) is proposed as an optimal drug for first-line treatment. With the new formulations of the molecule, the tolerability profile has improved considerably and there is evidence of clinicohematologic and molecular responses that are not only comparable to classical treatment (hydroxyurea), but also more sustained over time. In some cases, it is even becoming possible to discontinue treatment without disease progression.

OBJECTIVE: The aim of this study is to evaluate the effect of peg-IFNα-2a on clinicohematologic and molecular response, incidence of thrombotic/bleeding events and toxicity after 24 months of treatment.

DESIGN: A multicenter ambispective quasi-experimental study has been designed.

PARTICIPANTS: Patients with PV or ET diagnosis that are candidates to receive peg-IFNα-2a as a first line treatment in Catalan hospitals.

METHODS: Data will be obtained both retrospectively and prospectively from the medical history of the patients and their regular visits on the Hematological departments. Statistical representation of the variables results will also be performed through a univariate, bivariate and multivariate analysis.

KEYWORDS: polycythemia vera, essential thrombocythemia, pegylated interferon

INTRODUCTION

1. Definition of the concept "myeloproliferative neoplasms"

Myeloproliferative neoplasms (MPN) are a group of hemopathies of the myeloid lineage whose common pathogenic basis is the disruption at the level of the hematopoietic stem cell (HSC), leading to excessive proliferation of one or more of the myeloid lineages: erythroid, granulocytic or platelet, as well as expansion and accumulation of their derived cells. Unlike other hematological malignancies, there are no stops in cell maturation in any of these syndromes (1).

These diseases share a common clinical and biological phenotype, defined by the finding of a hypercellular bone marrow (BM), splenomegaly, increased risk of thrombotic and hemorrhagic events and progression between entities, especially to primary myelofibrosis (PMF) and, in the longer term, to acute myeloid leukemia (AML) (**Figure 1**) (2).

In a very summarized and introductory way we will differentiate the two most relevant entities for this paper: polycythemia vera (PV), defined by the presence of erythrocytosis high hemoglobin and hematocrit (Hto) levels and essential thrombocythemia (ET), defined by thrombocytosis. However, as we will see in later sections, these diseases should not be considered independently and the entities may intersperse characteristics.

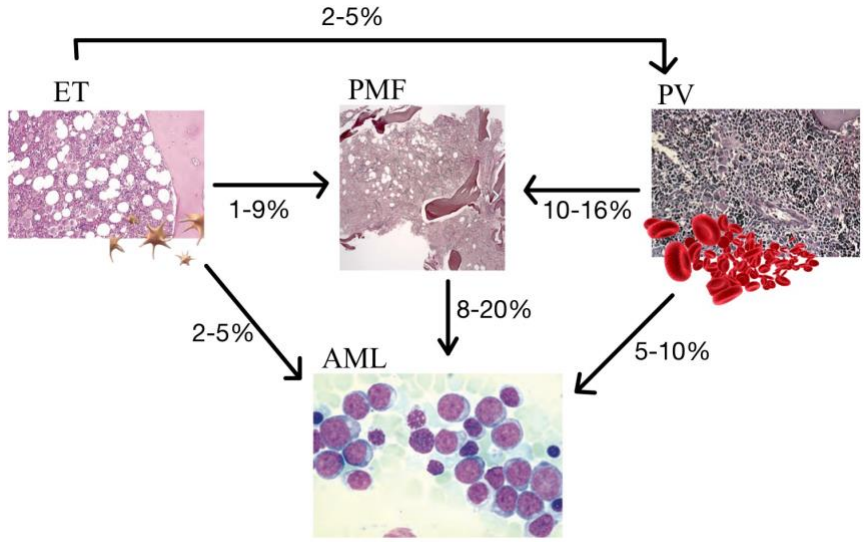


Figure 1. Percentages of progression between entities (3–5)

2. The different classifications of MPNs

2.1. The classical differentiation according to the Philadelphia Chromosome

The existence of a clonal population derived from a somatic mutation of the progenitors has been demonstrated in all MPNs. The first mutation that was defined, in the 1970s, was the t(9;22) that occurs in chronic myeloid leukemia (CML), called the Philadelphia Chromosome and whose molecular expression is the BCR-ABL gene (6,7).

Thus, historically and until this day, MPNs were divided according to whether they were "Philadelphia Chromosome Positive MPN", i.e. CML, or "Philadelphia Chromosome Negative MPN" [Ph(-) MPN], which includes the aforementioned entities that will be the main object of study in this work: PV and ET.

2.2 Latest classification proposed by the WHO

The WHO has developed different classifications of myeloid neoplasms which, over time, have been adjusted to the new information available on clinical, morphological, cytogenetic, molecular and immunophenotypic characteristics that define the entities.
The latest version dates from 2016 and it is a revision of the previous one published in 2008 and it is shown in **Table 1** (8,9).

Table 1. WHO classification for Myeloproliferative neoplasms (MPN)
Polycythemia vera (PV)
Essential trombocythemia (ET)
Primary myelofibrosis (PMF)
PMF, prefribotic/early stage
PMF, overt fibrotic stage
Chronic myeloid leukemia (CML), BCR-ABL1
Chronic neutrophilic leukemia (CNL)
Chronic eosinophilic leukemia, not otherwise specified (NOS)
MPN, unclassifiable

3. Epidemiology of MPNs

When we talk about the incidence of MPNs, we must be aware that the data collected on these pathologies at both national and international level are poorly characterized. This is why, first of all, we stress the need for new, more exhaustive and up-to-date studies on these entities.

According to data collected by the "Red Española de Registros de Cáncer" (REDECAN) in an incidence study carried out between 2002 and 2013, the most frequent subtypes of myeloid neoplasms were ET (13.7%), CML (7.6%) and PV (6.1%). This registry used data from 13 Spanish provinces and 3 islands, covering 26% of the Spanish population (10).

Myeloproliferative neoplasms are rare pathologies, but nevertheless remain the most common of the myeloid neoplasms in our environment. Data on incidence and age at presentation are shown in **Table 2** (10).

Table 2. Distribution of incident cases, median age at diagnosis and incidence rates of myeloproliferative neoplasms (10)

	%	Median age	Crude incidence rate (CR)	95% CI
Myeloproliferative neoplasms	100%	69	4.68	(4.56-4.8)
Chronic myeloid leukemia	22.55%	62	1.06	(1-1.11)
Polycythemia vera	18.12%	71	0.85	(0.8-0.9)
Essential thrombocythemia	40.89%	71	1.91	(1.84-1.99)
Primary myelofibrosis	7.78%	68	0.36	(0.33-0.4)
Chronic neutrophilic leukamia/ Chronic eosinophilic	0.9%	66	0.04	(0.03-0.05)
Myeloproliferative neoplasms unclassifiable	8.34%	75	0.39	(0.36-0.43)
Mastocytosis	1.41%	54	0.07	(0.05-0.08)

CR: crude rate = rate by 100.000 individuals/year; CI: Confidence interval

Regarding to the prognosis of MPN patients, data are in some cases contradictory between studies. In general, ET is considered the MPN with the best prognosis; despite this, there appears to be a lower overall survival than the rest of the population. The age of presentation is also lower, with some studies reporting >50% of patients younger than 60 years and even 20% younger than 40 years (3,11).

PV has a worse prognosis, followed by PMF. In a Mayo Clinic cohort of 826 patients, the median survivals were as follows: 19.8 years for ET, 13.5 for PV and 5.9 for PMF; for younger patients (<60 years) they were 32.7 for ET, 23.8 for PV and 14.6 for PMF (3).

4. Etiopathogenesis of MPN; driver and non-driver mutations

MPNs share mutations that maintain activated the hematopoiesis-stimulating pathway. The hypothesis that the entities have a common and clonal origin has been postulated since they were first described, based on clinical similarities and progression from one condition to the next (12,13).

Over the years, there have been discovered different "driver mutations" involved in the etiopathogenesis of these diseases, by altering various proteins involved in the processes of apoptosis and cell proliferation. As well as acting as a starting point for the different MPNs, they have implications for diagnosis, clinical phenotype, prognosis and response to treatment.

4.1. Chronology of the discovery of the different driver mutations

It was in 2005 that the first of the driver mutations was defined: the *V617F* mutation in the *Janus kinasa 2* gene (from now on *JAK2*), which results from the change of a guanine to a thymidine at position 1894 in exon 14 of the *JAK2* gene involving a change from valine (V) to phenylalanine (F) at amino acid 617.

The discovery was done simultaneously by different working groups in the field, demonstrating the presence of the mutation in 95% of patients with PV, and around 50% of patients with ET and PMF (14–18). Subsequently, in 2007, another group demonstrated mutations in exon 12 of the *JAK2* gene in those patients in whom *V617F* was not detected (19).

It was also between 2006 and 2007 when mutation was discovered in exon 10 of the *MPL* (*Myeloproliferative Leukemia Protooncogene*) gene, which codes for the thrombopoietin receptor (c-MPL) (20–22).

Finally and more recently, in 2013, the presence of heterogeneous mutations in the *Calreticulin* gene (from now on *CALR*), which codes for the calreticulin protein, was

demonstrated in a considerable proportion of patients with MPN Ph (-), all of them non-mutated *JAK2* (*JAK2* wild-type -WT-), as they are exclusionary mutations (23,24).

4.2. Incidence of each driver mutation

Below we explain the incidences of driver mutations in the different entities, the percentages of which can be seen in **Annex 1** (3,25).

PV is only associated with mutations in *JAK2*, mostly *JAK2V617F*. In ET and PMF, any of the three driver mutations may be present but at markedly different frequencies. As we can see, the *JAK2V617F* mutation continues to be the most implicated, followed by mutations in *CALR* and, to a lesser extent, *MPL*. There is a proportion of patients who will not have any of the three driver mutations, which we will call "triple negatives" (3,25).

4.3. *JAK2* mutations and phenotype implications

The *JAK2* gene is located on chromosome 9p24 and codes for a group of proteins belonging to the "non-receptor" tyrosine kinases (TK): JAK1, JAK2, JAK2 and Tyk2. Their role is to transmit signals from external stimuli by activating enzymatic cascades when specific ligands bind to their receptors. These enzymatic cascades will be able to activate the Signal transducer and activator of transcription (STAT) (26–28).

The ligands that activate the JAK-STAT signaling pathway are different hematopoietic cytokines and growth factors, which is why they play a vital role in the growth, survival, development and differentiation of myeloid cells. They therefore play as well an important role in the development of MPNs. Examples of such ligands include erythropoietin (EPO), granulocyte-colony stimulating factor (G-CSF) or thrombopoietin (TPO) (**Figure *2***) (29,30).

The *JAK2V617F* mutation causes non-inhibition of the JAK-STAT pathway and therefore a gain of function in it as well as a state of constitutional activation of the receptor in the absence of ligand, as can be seen in **Figure *2***. This mutation has been found in erythroid, megakaryocytic and granulomonocytic colonies as well as in hematopoietic precursor stem cells, which is why thrombocytosis, erythrocytosis and leukocytosis can occur at the same time in MPN (**Figure *2***) (14,15,18).

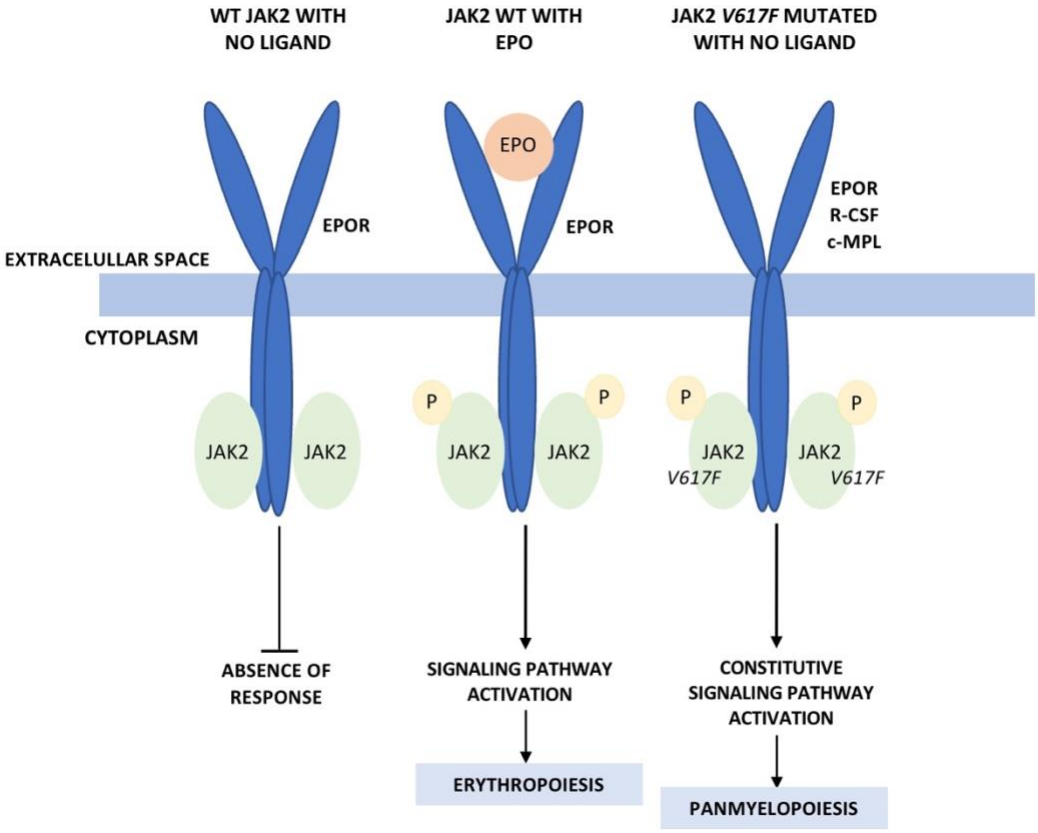


Figure 2. ***JAK2* mechanism**. *Adapted from* (31).

WT: Wild type; EPO:Erythropoietin; EPOR:Erythropoietin receptor; R-CSF: Colony-stimulating-factor receptor; c-MPL: Thrombopoietin receptor

Here we can see how the signaling pathways aren't activated when the erythropoietin receptor (EPOR) is not binded with its ligand. Then, once the EPO is bound, EPOR is activated and conduces to JAK2 phosphorylation and the signaling pathway starts.

However, when *JAK2* is mutated (*JAK2V617F*), the signaling pathway is constitutively activated without any ligand binded and then the three series are stimulated.

JAK2 exon 12 mutations involve punctual mutations at this location that have a similar translation in signaling pathways to *JAK2V617F*.

Phenotype implications

Mutated *JAK2* MPNs have been understood in recent years not so much as separate entities but as a biological continuum, especially ET and PV, with PMF being considered a late event. In fact, ET with the *JAK2V617F* mutation can progress to PV, with no reverse progression (32).

When the *JAK2V617F* mutation is present, at the BM level we find increased total (and specific granulocyte and erythrocyte) cellularity, leading to raised peripheral hemoglobin levels and increased erythrocyte, neutrophil and total white blood cell (WBC) counts. These are characteristics that would be more associated with PV. In contrast, isolated thrombocythemia is more frequently seen in patients without this mutation (see **Figure 4**). In addition, it has been defined that patients with the *JAK2V627F* are older at presentation (32,33).

There are factors that can influence the phenotype and make the entity more prone to PV or ET. They are explained below and are represented in **Figure 3**:

- Sex: PV is more common in men and ET in women and may be due to hormonal and metabolic differences (34,35).
- Iron reserves: lower iron stores are associated with a ET phenotype (17,32).
- *JAK2V617F* homozygosity: It has been shown that homozygosity for the mutation is associated with a PV-like phenotype, and that the acquisition of this status may in some cases lead to progression from ET to PV (36–38).
- STAT phosphorylation levels: STAT1 activation in the signaling cascade promotes megakaryocytic differentiation and inhibits erythroid differentiation and is therefore related to ET (39).
- Genetic predisposition: Polymorphisms have been shown to predispose to the presentation of certain phenotypes (40).
- Additional mutations: The addition of other mutations that we can detect by Next Generation Sequencing (NGS) technique can modulate the phenotype. Some of these will be addressed later in the paper in section 4.5 Role of non-driver mutations, NGS panels (40). (32)

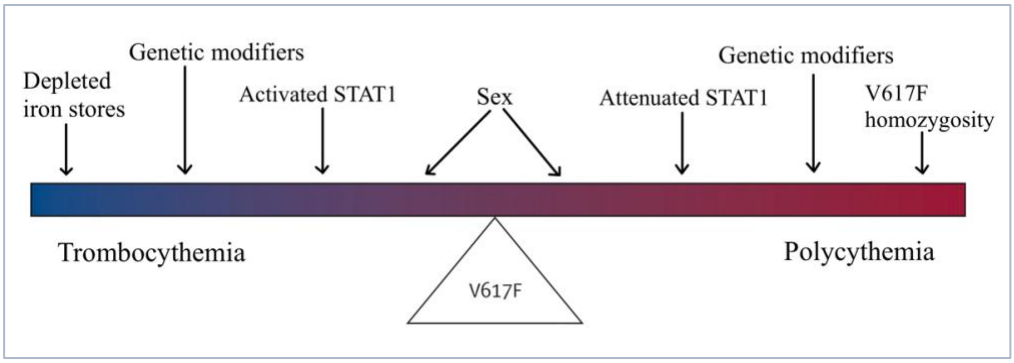


Figure 3. Continuum model for *V617F*-positive ET and PV. *Adapted from* (32)

On the other hand, mutation in exon 12 of *JAK2* results in a more erythroid phenotype meaning higher hematocrit and hemoglobin levels but almost normal leukocytes and platelets counts (41).

The mutational burden of JAK2

Clones with mutated *JAK2* coexist with *JAK2* WT cellularity and therefore we can find variable proportions of mutated alleles in myeloid precursors. This mutational load can be quantified and several studies have correlated it with phenotypic, progression and prognostic characteristics (42,43).

On the one hand mutational load may decrease during the course of the disease due to the treatment effect. On the other hand, increases in mutational load have been seen by a change in status from heterozygosity (only one allele mutated) to homozygosity (both alleles mutated by recombination in mitosis), leading to clonal dominance. Other reason for increasing allele burden are extra mutations (16,42,44,45).

Numerous studies have been done assessing the dose effect of this mutational load. Here we will summarize some conclusions to help us understand why allelic load quantification is important:

- Quantification >50% indicates a homozygous clone, and more progression to PMF has been demonstrated in patients with the mutation at this stage (36,42,43,45).
- Homozygous mutation, and thus higher allele load, has also been linked to higher hemoglobin concentrations (36,42,43).
- An increased allele burden in ET patients may be associated with a phenotype shift towards a more PV-like phenotype (44).
- In general, a higher allelic load of *JAK2V617F* has been associated with more aggressive and symptomatic manifestations in MPNs, especially with more pruritus and splenomegaly (36,43,46).

4.4. *CALR* and *MPL* mutations and phenotype implications

The *CALR* gene is located on chromosome 19p13 and codes for the calreticulin protein. It plays a role in different biological systems in relation to antigen presentation, complement activation and also in cancer immunogenicity and apoptosis (47).

Mutations in *CALR* have been shown to induce constitutive ligand-independent activity in c-MPL leading to dimerization of the JAK2 TK domain and its activation (48).

The phenotype implications of ETs with *CALR* mutations are summarized in **Figure 4**. It can be said that the phenotype will be similar to that of patients with mutated *MPL*, associated with a predominant expansion of the megakaryocytic lineage, as in PMF (49).

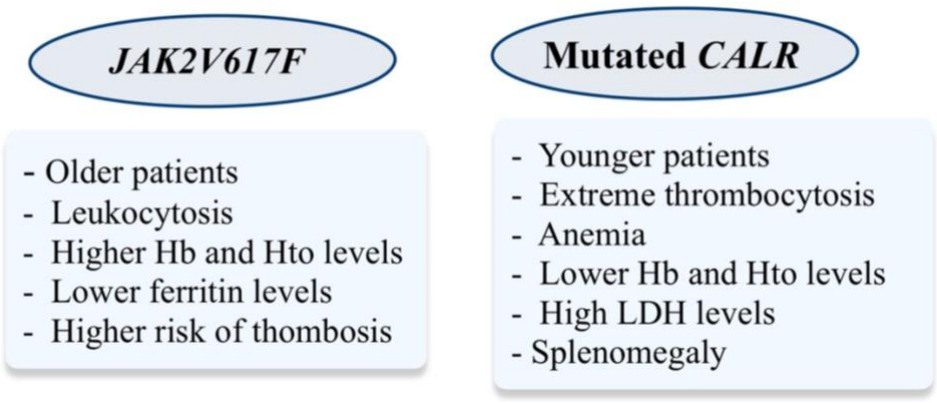


Figure 4. Comparative diagram of the main features of mutated *JAK2V617F* and *CALR* ET

MPL is responsible for coding for c-MPL. When the mutation in exon 10 *MPLW515L* is present, there is constitutional activation of the receptor in the absence of ligand and thus continuous stimulation of spontaneous megakaryocytic differentiation (22).

As already mentioned, these patients' phenotype is very similar to that of patients with mutated *CALR* and therefore we will not go into specifics here (50,51).

4.5. Role of non-driver mutations, NSG panels

When we want to obtain information on numerous genetic variants, disease-targeted NGS panels are currently the most practical technique. These provide data on the mutational profile of the patient and may also play a role in the diagnosis, classification and prognosis of the disease. A panel of 79 mutations (**Annex 2**) associated with hematological diseases is currently performed in Catalan centers on all patients with a MPN before starting treatment.

There are certain mutations for which prognostic and treatment response implications are relevant to our study, which will be briefly defined below (52):

- *TET2*: Acquired at the HSC level, they are mutations associated with epigenetic processes that confer clonal advantage in patients with the *JAK2V617F* mutation and have been linked to the leukemization process (53).

- *ASXL1*: Different studies have reported that variants in this gene had an adverse prognostic effect with shortened overall survival (54).
- *EZH2*: *EZH2* mutations result in a loss of function of the protein and lead to an MDS-like phenotype. They have also been associated with progression to ET-PMF and PV-PMF (53,55,56).
- *DNMT3A*: Similar to mutations in TET2, these appear to confer a clonal advantage to cells and alter the disease phenotype (53,57).
- *IDH1*/*IDH2*: These mutations are associated with MPN transformation as well as clonal dominance in a similar way to *TET2*.

It has been defined that patients that present some of these high-risk mutations have a lower molecular response (decrease on clonal load) to treatment (52).

5. Clinical manifestations of myeloproliferative neoplasms

The quality of life of patients with MPN may be affected due to the accompanying symptomatology of the entities. The pathophysiology of the clinic is still uncertain in some cases, but it is generally associated with splenomegaly, anemia and the neoplasic condition itself. It is very important to carry out a complete anamnesis of the respective symptomatology of the patients as it can sometimes indicate an underlying progression of the disease (1,58).

The most common symptom is fatigue or asthenia, which can be related to possible underlying anemia, cachexia, tumor burden or drug toxicity. Other constitutional symptoms include unwanted weight loss, fever and night sweats (58,59).

Symptoms possibly associated with splenomegaly are abdominal pain or discomfort and postprandial fullness (in some cases perceived by patients as loss of appetite). These are more frequent in PMF as splenomegaly is inherent to it; however, we must not forget that patients with PV and ET can also present it and it could be indicating an entity transformation (58,59).

Pruritus, and particularly aquogenic pruritus, is another of the most prevalent symptoms, being more intense in patients with PV (58–60).

Finally, we will mention symptoms that we include in the "microvascular clinic" group, which are: paresthesia, vertigo, headache, erythromelalgia and visual disturbances. Of these, headache is the most prevalent one (1,58,59).

6. Diagnostic criteria for MPN

The latest diagnostic criteria for MPN are those proposed by the WHO in 2016. These take into account analytical determinations, mutations and BM study that may be essential (in ET) or not (certain cases of PV). These can be seen in **Table 3** and **Table 4** for PV and ET respectively.

2016 WHO criteria for PV
Major criteria
1. Hemoglobin > 16.5 g/dl in men / Hemoglobin >16.9 g/dL in women
or,
Hematocrit >49% in men / Hematocrit > 48% in women
or,
Increased red cell mass (RCM)*
2. BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
3. Presence of *JAK2V617F* or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion †

*More than 25% above mean normal predicted value.

† Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5g/dL in men (hematocrit, 55,5%) or >16.5g/dL in women (hematocrit, 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

Table 3. WHO criteria for PV. *Adapted from* (9)

2016 WHO criteria for ET
Major criteria
1. Platelet count ≥ 450 x 10^9/L
2. BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for *BCR-ABL*, CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR* or *MPL* mutation
Minor criterion
Presence of a clonal marker or absence of evidence for reactive thrombocytosis
Diagnosis of ET requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion.

Table 4. WHO criteria for ET. *Adapted from* (9)

7. Current treatment scheme for MPNs according to risk stratification

The goals of treatment in MPNs are, in general (1,61):

- Prevention of thrombotic and hemorrhagic complications
- Disease-associated symptomatology control
- Minimization of the risk of transformation to AL or PMF.

The choice of treatment and its monitoring will be conditioned not only by the patient's characteristics [age, cardiovascular risk factors (CVRF), comorbidities, pathological history…] but also the disease's (present driver mutation, platelet count, symptomatology…).

First, we must control CVRFs by means of both hygienic-dietary measures and pharmacological measures if necessary, including in some cases antiplatelet treatment with acetylsalicylic acid (ASA), which will be discussed in more detail in section **7.1**.

In the case of PV, phlebotomies are also an important measure for the control of Hto, aiming for values <45%, as they have shown a lower incidence of thrombotic events compared to higher values. In very low-risk patients, they may be the only treatment along with ASA and

they can also be used concomitantly with cytoreductive therapy to optimize Hto response (1,62,63).

In addition, depending on the assessment of thrombotic risk, cytoreductive therapy may be indicated to achieve clinicohematologic and molecular control of the disease. The conventional way to stratify risk in ET and PV is based on patient age and previous history of thrombosis. New scoring systems have now been developed and validated, such as the IPSET-thrombosis for ET (International Prognostic Score for Thrombosis in Essential Trombocythemia). The European Leukemia Net (ELN) recommendation is to follow the "classical" system for PV and IPSET-thrombosis for ET (**Table 5**) (62).

Conventional prognostic system; recommended for PV	
Risk factors	**Risk group**
Age <60 and no history of thrombosis	Low-risk
Age ≥ 60 or prior thrombosis	High-risk
IPSET-thrombosis score; recommended for ET	
Risk factors and points	**Score and risk category**
Age ≥60 (1 point)	Low-risk: 0-1 point
CVRF (1 point)[a]	Intermediate-risk: 2 points
Previous thrombosis (2 points)	High-risk: ≥3 points
JAK2617F mutation	

[a]CVRF: smoking habit, metabolic syndrome, diabetes mellitus, arterial hypertension, hypercholesterolemia.

Table 5. Risk stratification for PV and ET following ELN recommendations.

Recently, a new risk group has been described in ET characterized as "very low risk", which is composed of patients without a history of thrombosis, who do not have the *JAK2* mutation and who are under 60 years of age (64).

Although platelet count does not enter into the risk stratification discussed above, it may influence the treatment decision. Extreme thrombocythemia levels (>1500 x 10^9) have been shown to correlate with an increased risk of bleeding (associated with acquired von Willebrand disease) (1,61).

7.1 Indications for ASA therapy

Treatment with ASA is beneficial for the prevention of thrombotic events as well as for the control of symptomatology, especially microvascular one. Indications are summarized in Table 6 (1,61,62).

Indications for ASA therapy in ET	
Low-intermediate risk patients	- Aged ≥ 60, OR - no control of CVRF - *JAK2V617F* mutation
High-risk patients	Always recommended [a]
[a] Individualize in those with extreme thrombocythemia because of bleeding risk	
Indications for ASA therapy in PV	
Always, irrespective of a history of thrombosis [b]	
[b] It should be noted that an extreme platelet count significantly increases the risk of bleeding and will therefore be one of the indications for initiating cytoreductive treatment.	

Table 6. Indications for ASA therapy

7.2 Cytoreductive treatment, an overview

Hydroxyurea

Hydroxyurea (HU) is an antimetabolite that arrests the cell cycle in the G1/S phase by interfering with DNA repair and it is the most widely used cytoreductive drug in PV and ET.

The main undesirable effects of this drug, which may affect its tolerability, derive from its cytostatic effect on all cells in the body, especially those with high cell turnover: gastrointestinal discomfort and mucocutaneous lesions (especially aphthous lesions on the oral mucosa and painful ulcers on the legs) (1,65,66).

Also important is the risk of unwanted myelosuppression that may lead to discontinuation of treatment and dose reduction, especially due to a tendency to thrombocytopenia and anemia in elderly patients (67,68).

On the one hand, possible leukemogenic effects have been reported in different series with this drug, as well as an association with the development of skin cancer (non-melanoma type). Its leukemia-inducing character has not yet been confirmed by prospective studies, and is therefore a controversial point (62,65,69–71).

On the other hand, impact on fertility is another important consideration on this drug use, especially in those young patients who might require life-long treatment such as ours (66).

However, hydroxyurea has been shown to be effective in lowering platelet and leukocyte counts, preventing thrombotic events, alleviating pruritus and allows suitable control for these entities with a considerably great tolerance spectrum (66,72,73).

Pegylated interferon-α2a (peg-IFNα-2a)

IFN-α is a biological response modifier with a wide range of properties such as (74):

- Induction of toxic effects in transforming cells
- Immune response to malignant cells booster
- Pro-apoptotic genes induction
- Angiogenesis inhibition
- Ability to inactivate malignant stem cells' cycles

It then exerts a myelosuppressive action on excessively proliferative cell lineages, making it a useful molecule as a therapeutic option for myeloid malignancies such as MPN. The use of the pegylated form of IFN-α has now led to improved drug tolerance and its use has been commonly used for both ET and PV as first or second line therapy depending on patient characteristics (see sections **7.3** y **7.4**). A new formulation already approved by the FDA called ropeginterferon α-2b appears to further improve even more the profile and convenience of administration of IFN- α (74,75).

The most common unwanted effects of peg-IFNα-2a are musculoskeletal pain, asthenia, skin toxicity (allergic reaction) and gastrointestinal symptoms (76).

Other less frequent but important undesirable effects are the development of autoimmune diseases (hypothyroidism, hepatitis), increased liver markers and development of mood disorders (77,78).

Treatment with peg-IFNα-2a has been shown to be associated with a significant molecular response rate (decrease in mutational load). Two meta-analyses recently presented at the American Society of Hematology (ASH) Congress concluded an overall molecular response of 64% and 84% for PV and ET respectively (46,79,80).

In addition, it appears that peg-IFNα-2a offers the option in some cases to discontinue treatment without affecting the maintenance of response over time, which would offer

patients greater independence from their disease, especially in those with long expected survival times, such as the youngest patients (76,81).

PROUD-PV and CONTINUATION-PV are randomized, controlled trial where peg-IFNα-2b and HU were randomly assigned. Higher hematological, clinical and molecular responses as well as longer response duration group were reported on the peg-IFNα-2b (81).

The dosage of peg-IFNα-2a will initially be weekly starting at 45-90µg/week and adjusting upwards or downwards according to platelet counts. Subsequently, it can be changed to a bi-weekly administration and it is recommended to do it at night to minimize the associated flu-like symptoms (meaning asthenia and musculoskeletal pain) (1).

Other cytoreducing drugs

- Anagrelide: It is a quinazoline-derived drug that reduces platelet levels in ET patients and inhibits platelet aggregation through its anti-AMP cyclic pathway activity. Decreased platelet counts have been shown to be associated with the interference in megakaryocyte colonies at the BM level (82,83). Nowadays it is used in ET patients as first or second line treatment. The most common toxicities associated with this drug are cardiac (arrhythmias, cardiac insufficiency), renal and hepatic failure (1).
- Busulfan: is an alkylating agent that induces myelosuppression and can trigger bone marrow aplasia. It is therefore only recommended for elderly patients who cannot tolerate HU (1,84).
- Ruxolitinib: is a JAK inhibitor drug used as a first line therapy for PMF. However, it should be reserved for patients resistant or intolerant to HU in PV (1).

7.3 Indications for cytoreductive treatment in ET

It is not recommended to initiate cytoreduction in patients with low-intermediate risk and good CVRF control.

All patients at high risk according to the IPSET-thrombosis score are candidates for cytoreductive treatment. It should be remembered that extreme thrombocytosis may be a risk factor for hemorrhagic events and should therefore be considered as another criterion for initiating platelet-lowering treatment (61,62).

Persistence of microvascular symptomatology despite treatment with ASA is also considered a criterion for initiating cytoreductive therapy (1).

Cytoreductive treatment should always be initiated when a patient changes risk group, either due to the occurrence of a thrombotic phenomenon, change of age group (≥60 years) or when platelet levels exceed 1,500 x 10^9 (61).

7.4 Indications for cytoreductive treatment in PV

All high risk patients are candidates for cytoreductive treatment (**Table 5**) (1,62).

Other indications for starting cytoreductive treatment in PV include (62):

- Poor tolerance to phlebotomy
- Descend of Hto due to iron therapy when iron deficiency is associated
- Symptomatic or progressive splenomegaly
- Severe symptoms that are not controllable with ASA
- Platelet counts greater than 1500 x 10^9/L

7.5 First line of cytoreductive treatment in ET and PV

Three drugs are generally considered for cytoreductive treatment of ET: hydroxyurea, peg-IFNα-2a and anagrelide. However, the ELN expert panel recommends leaving anagrelide as a second-line treatment and considers hydroxyurea and peg-IFNα-2a as first-line options for both ET and PV (62).

The decision to start treatment with HU or peg-IFNα-2a is made primarily on the basis of the patient's age, as well as possible contraindications to one or the other (**Table 7**). The ELN recommends a cautious use of HU in young patients due to the possible leukemogenic and long-term fertility effect of this drug that has been reported (61,62,66).

HU contraindications	Peg-IFN contraindications
- Pregnancy (RC) - Antecedent cutaneous cancer (RC) - High risk to develop cutaneous toxicity (RC) - Known hypersensitivity to HU	- History of psychiatric disorder such as major depression, bipolar disorder, maniac episodes… - Autoimmune disorders - Significant hepatic and renal dysfunction - Uncontrolled cardiac dysfunction - Known hypersensitivity to peg-IFNα-2a

Table 7. Contraindications on HU and peg-IFN treatment.

RC: Relative contraindications

Although the latest guidelines of the "Grupo Español de Enfermedades Mieloproliferativas Crónicas Filadelfia Negativas" (GEMFIN) recommend the use of peg-IFNα-2a as first line in patients under 50 years of age, the reality in clinical practice in Catalan hospitals is different. Based on professional experience, other centers' experience and new evidence, this drug is being administered as a first therapeutic strategy to older patients. A sub-analysis of the PROUD-PV and CONTINUATION-PV studies showed that there was no difference in efficacy and tolerability at 24 months in patients older and younger than 60 years (considering that those >60 years remained mostly in between 60 and 70) (85,86).

Thus, in summary, we can conclude that in our environment in patients with PV and ET requiring cytoreductive treatment, we will opt for the administration of peg-IFNα-2a in patients <60 years of age; HU in those of advanced age, from 70 years of age; the administration of one or other treatment will be assessed according to comorbidities in the range of 60 to 70 years of age (**Table 8**).

Patients aged <60	- peg-IFNα-2a is the recommended treatment if there aren't CI - HU is the recommended treatment if there're CI to peg-IFNα-2a
Patients aged between 60-70	- Comorbidities should be considered in order to choose between peg-IFN and HU if there aren't CI to any.
Patients aged >70	- HU is recommended as first line treatment if there aren't any CI - Anagrelide is considered in case there are CI to HU[a]

[a] In case of patients with ET; [b] In case of patients with PV; CI: Contraindications

Table 8. First line cytoreductive treatment on patients diagnosed with ET.

7.6 Second-line cytoreductive treatment

Second-line therapy in PV and ET are very similar: It's recommended treatment with HU as a substitute for peg-IFNα-2a and peg-IFNα-2a as a substitute of HU, along with anagrelide in case of ET (1,61,62).

Ruxolitinib may be considered in older symptomatic patients with diagnose of PV that present intolerance or resistance to HU. Busulfan may be considered in elder patients that do not tolerate HU in both PV and ET (61,62).

JUSTIFICATION

Despite having a low incidence, ET and PV are among the 5 most frequent myeloid neoplasms in our environment. With proper disease control the survival rates are high, but according to the latest evidence, they do not reach those of the general population (3,10).

Moreover, in their course they may progress to diseases with markedly worse prognoses such as PMF, AML and MDS. This means that a greater variety of available treatments is necessary, both to offer each patient the most appropriate option for their characteristics, and to opt for effective second lines of treatment if necessary (3).

Although in another disease profile, IFNα was approved more than 30 years ago but due to a low tolerance profile and the emergence of new, more effective drugs, its use was reduced to a limited number of patients. However, its spectrum of action is very broad, acting on the biology of tumors at various levels: amplification of the immune response towards malignant cells, inhibition of angiogenesis, direct toxicity on transformed cells, etc, which makes it a candidate drug for numerous diseases, including myeloid neoplasms (74).

With the new formulations (pegylated interferon and ropeginterferon) the toxicity and tolerance profiles have improved remarkably, representing a renaissance of this drug, especially (but not only) in the treatment of Philadelphia Chromosome-negative MPNs (74).

It is estimated that in MPNs 12.5% of patients are diagnosed under the age of 40. The percentages for PV and ET are 12% and 20% respectively. As mentioned in the introduction, some large series report >50% of their patients as under 60 years of age (3,11).

Taking into account the issues related with leukemogenic effects, fertility and non-melanoma skin cancer described in relation to hydroxyurea treatment, the European Leukemia Net recommends a cautious use of this drug in young people. This is why in recent years the use of peg-IFNα-2a has been preferred in this patient profile and is reflected in the GEMFIN recommendations. Moreover, based on the results of new studies such as PROUD-PV and CONTINUATION-PV, the experience of our professionals and other teams (e.g. the Austrian

Society of Haematology and Oncology), the age of administration of peg-IFNα-2a has been extended to 70 years in Catalan hospitals (85,86).

Peg-IFNα-2a has been shown to be associated with an promising clinical hematological response. Moreover, the molecular response (allelic load of mutations) appears to be better and more sustained than the one associated with hydroxyurea. Furthermore, complete molecular responses maintained for 30 months after cessation of treatment have even been reported (76,81).

Much of the evidence collected on peg-IFNα-2a is based on patients from other countries and especially from the USA. This is why the GEMFIN recommends that knowledge of patients treated with this drug in our setting should be expanded. The clinical impression of our professionals is optimistic, but an exhaustive study is needed to reflect the reality of Catalan patients suffering from PV and ET.

If our hypotheses are correct, peg-IFNα-2a would be confirmed as a very suitable first-line option for young patients as well as for those not so young but whose comorbidities make peg-IFNα-2a a more suitable profile.

HYPOTHESIS AND OBJECTIVES

1. HYPOTHESIS

Based on the available literature on MPN treated with peg-IFNα-2a as well as on the experience of clinical practice, our main hypothesis are:

1) >70% of patients on treatment with peg-IFNα-2a acquire complete or partial clinicohematologic response in the first 24 months from the start of the treatment.
2) <5% of patients on treatment with peg-IFNα-2a suffer a major thrombotic event in the first 24 months from the start of the treatment.
3) >85% of patients on treatment with peg-IFNα-2a acquire a total or partial molecular response in the first 24 months from the start of the treatment.

Secondary hypothesis that we want to present are:

4) Discontinuation of treatment due to toxicity occurs in <10% of patients in the first 24 months from baseline.
5) The percentage of patients that acquire a total molecular response after 24 months of treatment is smaller in those who present high-risk mutations on NSG panels at baseline than those without additional mutations.

2. OBJECTIVES

Primary objectives

In order to confirm our hypothesis, our main objectives are:

1) To analyze the clinicohematologic response on patients with PV and ET diagnosis after 24 months on treatment with peg-IFNα-2a and compare it to their characteristics at baseline.
2) To analyze the incidence of major thrombotic events in patients treated with PV and ET diagnosis with peg-IFNα-2a.
3) To analyze the molecular response in patients with PV and ET diagnosis after 24 months on treatment with peg-IFNα-2a and compare it to their characteristics at baseline.

Secondary objectives

4) To describe the tolerance to treatment by analyzing the percentage of patients that require discontinuation of treatment due to side effects.
5) To analyze and compare the molecular response achieved in patients that showed high-risk mutations on NGS panels at baseline and those without additional mutations.

METHODOLOGY

1. STUDY DESIGN

It has been designed a longitudinal, ambispective, analytic, quasi-experimental study. There will be then a before-and-after evaluation of the intervention.

The study will be coordinated by the ICO (Institut Català d'Oncologia) Girona using the data recorded by Catalan hospitals, including ICO centers and other collaborating hospitals.

2. STUDY POPULATION

The study population is based on hematological patients diagnosed in Catalan centers with PV or ET diagnosis who have received peg-IFNα-2a as a first-line cytoreductive treatment between 1st January 2018 and 31st December 2022.

2.1 Inclusion criteria

- Age ≥ 18 years old
- Patients who sign the informed consent
- Patients with a diagnosis of PV or ET according with the 2016 WHO criteria
- Patients who have receive or are candidates to receive exclusively peg-IFNα-2a as a first line therapy on cytoreductive treatment
- Patients who have received or are candidates to receive peg-IFNα-2a as a first line therapy after no more of 2 months of treatment with HU as a inducer treatment
- NGS panel performed at diagnosis (pre-treatment)
- Allele burden study at diagnosis (pre-treatment) in *JAK2* or *CALR* mutated patients

2.3 Exclusion criteria

- Diagnosis of PV post-ET
- Patients that reject to participate in the study
- Patients who have received peg-IFNα-2a as a first line therapy but after more than 2 months of treatment with HU as inducer treatment.
- Contraindications for peg-IFNα-2a treatment

2.4 Withdrawal criteria

- Patients who drop out of treatment due to poor tolerance
- Patients with disease progression to PMF, MDS or AL

3. SAMPLING

3.1 Sample size

In a bilateral test, with a significance level (α) of 5% and a statistical power of 80%, assuming an incidence of 0,85 (CR) and 1,91 (CR) for PV and ET respectively, we would need 87 subjects. However, assuming a drop-out rate of 10%, we will need a sample size of 96 patients.

The computations were carried out with the Prof. Dr. Marc Saez' software based on the package "pwr" of the free statistical evironment R (version 4.1.2).

3.2 Sample collection

A non-probabilistic consecutive method of recruitment will be used in this study. All patients with PV or ET diagnosis that are candidates for the treatment (peg-IFNα-2a) as a first line therapy who meet the inclusion criteria and none of the exclusion ones proposed in the study will be asked to participate.

The patients will be recruited from ICO Girona, ICO Badalona, ICO Tarragona, ICO Bellvitge and other collaborating Catalan hospitals that accept to participate in the study.

All patients will be informed about the purpose of the study. The information document and the informed consent of the study will be given to all participants (**Annex 3- 6**). It's important that the physician highlights to the patients the voluntarily and confidential aspects of their participation, as well as their right to withdrawal from the study at any time.

4. STUDY VARIABLES

4.1. Independent variable

Treatment with peg-IFNα-2a as first line cytoreductive therapy. It's a dichotomous qualitative variable, being "no" before the intervention and "yes" at the point of 24 months.

4.2 Dependent variable of the main objectives

4.2.1 Clinicohematologic response

The dependent variable of the first objective is the clinicohematologic response of the patient associated to the treatment. We define it by following and adapting the recommendations of the ELN for both PV and ET. The criteria are represented in **Table 9** and **Table 10**.

This is a qualitative ordinal variable, considering three categories: complete response, partial response or no response.

Response Grade	**Definition of Response in Polycythemia Vera**
Complete response	1. Hematocrit <45% without phlebotomy, AND 2. Platelet count ≤400 x 10^9/L, AND 3. WBC count ≤ 10 x 10^9/L, AND 4. No palpable splenomegaly, AND 5. No disease-related symptoms [a]
Partial response	In patients who do not fulfill the criteria for complete response: 1. Hematocrit <45% without phlebotomy, OR 2. Response in ≥ 3 of the other criteria
No response	Any response that does not satisfy partial response

*Disease-related symptoms include microvascular disturbances, pruritus and headache

Table 9. Definition of clinicohematologic response in PV
WBC: White Blood Cell

Response Grade	**Definition of Response in Essential Thrombocythemia**
Complete response	1. Platelet count ≤ 400 x 10^9/L, AND 2. No disease-related symptoms [a], AND 3. No palpable splenomegaly, AND 4. WBC count ≤10 x 10^9
Partial response	In patients who do not fulfill the criteria for complete response: Platelet count ≤ 600 x 10^9 /L or decrease of >50% from baseline
No response	Any response that does not satisfy partial response

*Disease-related symptoms include microvascular disturbances, pruritus and headache

Table 10. Definition of clinicohematologic response in ET
WBC: White Blood Cell

4.2.1 Incidence of a major thrombotic/bleeding event

The dependent variable of the second objective is the incidence of a major thrombotic or bleeding event, understood as the occurrence of any of those described in **Table 11**.

This is a dichotomous qualitative variable being "no" if no thrombotic evets occurred and "yes" if any of them occurred during the first 24 months from the start of the treatment.

Major thrombotic events	Deep venous thrombosis including cerebral thrombosis, abdominal veins thrombosis or Budd-Chiari syndrome.	
	Ischemic stroke	Angina pectoris
	Transient ischemic attack	Pulmonary embolism
	Myocardial infarction	
Major bleeding events	Fatal hemorrhages	
	Non-fatal intracranial bleeding	
	Any hemorrhages requiring surgery or red blood cell transfusion of at least 2 packed units.	
*Thrombotic and bleeding events will be recorded only if they occurred after the start of the treatment		

Table 11. Definition of major thrombotic and bleeding events.

4.2.3 Molecular response

The molecular response will be understood by the mutational allele burden found on each patient at the time of 24 months from the beginning of the treatment. The allele burden is measured by percentages and for this study we will categorize the variable.

Therefore is a qualitative ordinal variable, considering three categories:

Complete response: allele burden undetectable with the technique used
Partial response: decrease ≥50% of allele burden from baseline
Minor response: decrease <50% of allele burden from baseline

4.2 Dependent variable of the secondary objectives

4.2.1 Treatment discontinuation

We define treatment discontinuation as the need to either space out dosages or stop treatment for an interval of time. The decision must be related and motivated by low tolerance to treatment due to uncontrolled side effects.

The main side effects of peg-IFNα-2a have been described in the introduction of this paper but we will name the most common causes of discontinuation and withdrawal next:

- Skin toxicity
- Appearance of autoimmunity markers
- Hepatic toxicity
- Peripheral neuropathy

This is a dichotomous qualitative variable yes/no.

4.3 Covariables

- Sex: it will be considered as a qualitative dichotomous variable. Male or female.
- Age: It will be considered a quantitative variable measured in years.
- Diagnose: It will be considered a qualitative dichotomous variable: PV or ET.
- High-risk mutations: There will be considered high-risk mutations the following: *TET2, ASXL1, EZH2, DNMT3A, IDH1/2*.

  This is a dichotomous qualitative variable being “no” if none of the high-risk mutations are present and “yes” if ≥1 is registered.

- FRCV:
    - Smoker: using the WHO description, we will define smoker as the person who has smoked daily in the past month. It's a qualitative dichotomous variable yes/no.
    - Diabetic: as a qualitative dichotomous variable yes/no.
    - HTA: as a quantitative dichotomous variable yes/no.
    - Hypercholesterolemia: as a quantitative dichotomous variable yes/no.
- Socioeconomic level: proxied by occupation and education level. Qualitative polytomous variable: Class I, II, II, IV and V being class V the lowest.

5. METHODS OF MEASUREMENT

5.1 Blood test

Blood test is a regular petition on the follow-up of patients. We'll use the one that is closer to the 24 months from baseline period. The regular petitions are:

- Blood count: including hematocrit, WBC and platelet count.
- Biochemical test: including renal and liver function markers.

5.2 Anamnesis and medical history

We'll use anamnesis to evaluate the presence or absence of the disease-related symptoms that are mentioned on the clinicohematologic response criteria described by the ELN.

Same process will be used to evaluate the toxicities mentioned, and physical examination will be carried out to check the presence or absence of palpable splenomegaly and skin toxicity (if necessary).

5.3 Allele burden testing

JAK2: For this study we will need to perform an allele-specific polymerase chain reaction (PCR) test with TaqMan® for *JAK2V617F* using peripheral blood sample from the patients. Allele burden quantification is calculated as:

$$\frac{JAK2V617F \text{ allele burden}}{JAK2V617F \text{ allele burden} + JAK2 \text{ WT}} \times 100$$

CALR: For the study of exon 9 of the *CALR* gene we use PCR test followed by fragmentanalysis in a Genetic Analyzer 3130 (Applied Biosystems).

The recommendation that we follow is to repeat this test every 12 months from baseline. Thus, we will use the information of the one that is closer to the 24 months from baseline period to do the comparation.

5.4 NGS panel

For this study we will need to perform a NGS panel in every patient at diagnosis in order to identify high-risk mutations.

The panel performed in patients with MPN consists of 79 mutations related to hematological diseases that is approved by *CatSalut* (*Servei Caralà de Salut*) and it's depicted in **Annex 2.**

6. STUDY INTERVENTION

This study intervention consists on administrating peg-IFNα-2a as first line therapy cytoreductive treatment to patients with both PV and ET.

In some cases it is recommended to start cytoreductive-inducing treatment with HU at diagnosis since the response to peg-IFNα-2a is gradually stablished. Some of the scenarios in which this measure may be recommended are:

- Patients with extreme platelet counts.
- Very high risk of thrombosis due to comorbidities
- Previous thrombotic event

HU is a fast-acting treatment and the platelet counts normally lower between the first and second month from the start. We will include in the study those patients who have not needed more than two months of HU treatment to avoid confounding factors.

Treatment with peg-IFNα-2a can be initiate at the same time as HU or after the end of the HU period. If both are administrated at once, it is recommended to progressively lower the HU dose to achieve maintenance with peg-IFNα-2a .Dosage of peg-IFNα-2a should not vary either way, and it will be explained bellow.

The recommended starting dose is 45 µg/week and increases of 45 µg are considered if necessary, based on analytical and clinical progress as well as tolerance. The maximum weekly dosage is 180 µg.

Dose spacing may vary from weekly to every two weeks when disease control is achieved.

Peg-IFNα-2a is administered in the form of subcutaneous injections in the abdomen or thigh at the patient's discretion; practitioners will recommend alternating the location of injections. The first administration will be performed at the hospital to teach the patient how to handle the injection. A pharmacy technician will be responsible for educating the patient so that he or she can then administer the drug himself or herself.

The patient will be reminded of the possible side effects of the drug so that he/she can identify them and will be given a contact form for the service.

It should not be forgotten that many of these patients take ASA concomitantly and therefore the dosage of both drugs should be reviewed regularly so that there is no confusion in dosage.

7. FOLLOW-UP

In this project, as it was previously said, patients from Catalan hospitals will be used as the study population. The recommended follow-up for patients in the study is no different to the follow-up these hematological patients usually do and no additional visits or performance of techniques are needed. This is one of the reasons why an ambispective study can be carried out, as we expect that the retrospective information on the patients already included in the cohort is complete.

Prior to entering the study, a complete blood test is required, including:

- Blood count
- Peripheral blood smear to asses morphology
- Biochemical parameters, including renal and liver function markers.
- Thyroid function study
- Basic autoimmunity study: antinuclear antibodies (ANAs), rheumatoid factor (FR)
- Serologies, including: HCV, HBV and HIV

These parameters are the ones needed in order to receive treatment with peg-IFNα-2a so we expect all patients to have the information collected.

Measures that are carried out prior to the start of treatment are:

- Quantification of *JAK2* or *CALR* allele burden by the methods explained above
- Protocolary NGS panel for hematological diseases (**Annex 2**)
- Complete anamnesis to asses pathological antecedents such as psychiatric disorders, autoimmune diseases, cardiovascular disorders or any other cardiovascular risk factor. In addition, the clinical presentation of the patient should be collected, both symptoms and signs. A physical examination will also be performed, especially to check the size of the spleen.

At the start of treatment, the patient is seen every 15 days to adjust the dose and monitor possible adverse effects. Afterwards, when the disease is under control, visits can be spaced out to 3 months. On these regular visits a new blood test (blood count and biochemical markers) is carried out each time, as well as physical examination and anamnesis on symptoms from both of the disease and related to side effects.
Additionally, blood tests including thyroid function and autoimmunity markers are performed every 6 months.
The quantification of *JAK2* or *CALR* allele burden is repeated once a year.

8. DATA COLLECTION

As our study design is ambispective, data will have to be collected both retrospectively and prospectively.

As all clinical data used in this analysis is part of the routine management of the patients (as it was explained in **Section 7**) it is already registered in the medical history of the patients. We do not expect any information needed for the variables to not be registered in the system.

A responsible person will be appointed in each hospital to collect the information. It will be necessary to make a joint database research purposes so that each of them can access and enter the data from its own sample. There will be a data manager to assess the process and perform data quality control.

The data collection form to be used when uploading information to the database is presented in **Annex 9.**

STATISTIC ANALYSIS

1. DESCRPTIVE ANALYSIS

l These descriptives will be computed at baseline and at 24 months.

All the analyses will be stratified by the covariables. For that the quantitative co-variable “age” will be categorized in quartiles.

2. BIVARIATE INFERENCE

The analysis must be paired as the subjects are used as their own control.

The difference of proportions of clinicohematologic response, incidence of a thrombotic event, molecular response and discontinuation of treatment at baseline and at 24 months will be tested using the McNemar’s test (paired test equivalent to the χ^2).

In the case of the molecular response found on each patient at the time of 24 months from the start of treatment, a further stratification by the presence of high-risk mutations will be carried out.

Again, the analyses will be stratified by the covariables.

3. MULTIVARIABLE ANALYSIS

For the multivariable analysis, dependent variables “clinicohematologic response” and “molecular response” found on each patient at the time of 24 months from the start of treatment will be categorized as: overall clinicohematologic response/no response and molecular overall response/no response, respectively.

Once categorized we will have dichotomous variables. These variables, together with “incidence of a major thrombotic/bleeding event” and “discontinuation of treatment”, will be used as dependent variables in logistic regressions. The independent variable will be an indicator of being at baseline or at 24 months from the start of treatment.
In the regressions, all covariates will be included to control for confounding factors.

In order to accomplish the fifth objective, the logistic regression of the molecular response at 24 months from the start of the treatment, once categorized, will be stratified by the presence or absence of high-risk mutations.

The regression will be estimated using the conditional method, since the observations are paired.

WORK PLAN AND CHRONOGRAM

The research team will be formed by:

- The general coordinator (GC) will be an hematologist from ICO Girona (Dra. Anna Angona) as the coordinating center. She will participate in the recruitment of new patients for the study, patient follow-up, interpretation of the results of the statistical analysis and the creation of the article and its publication.
- One hematologist from each hospital will be designated as the person responsible for data collection. In the case of the ICO Girona this person may be the GC herself. It is recommended that the person in charge of the database is involved in patient follow-up.
- Hematologists who regularly follow these patients, as they will be involved in the follow-up of patients.
- A statistician who will make the statistical analysis of the results obtained.
- A data manager to assist hematologists in collecting the information and who will be responsible for data quality control.

WORK PLAN

Stage 0: Protocol design

This stage involves reviewing previous literature and designing the study protocol.
This stage has been carried out between October 2021 and January 2022.

Stage 1: Ethical evaluation

Once the protocol has been done, the Comitè Ètic d'Investigació Clínica (CEIC) will be contacted to propose the study and await acceptance.
We estimate that this phase will last 4 to 5 months.

Stage 2: Preparation and coordination

At this stage the GC will contact the hospitals to present the study and ask for their participation. Then, the heads of each center and other hematologists involved in the study as well as the statistician will be appointed. An online meeting will be organized to explain

the project and resolve any doubts that may arise. All participants, PI, statistician, data manager and hematologists in charge of follow-up and data collection must be present and understand the study plan.

Also in this phase, the joint online database will be created to facilitate the collection of information. It should be computerised and encoded for the preservation of patient privacy.

This phase will last 3 months.

Stage 3: Data collection and follow-up of patients

Once the team is defined, a stage will begin where several interventions must be carried out at the same time. Online catch-up meetings will be carried out every 6 months.

On the one hand, hematologists will continue to recruit those patients who meet the criteria into the study. They will do the necessary tests and follow the already established protocol.

On the other hand, the hematologist responsible for data collection will be able to start collecting the information from the medical records to incorporate them into the joint database. The data manager will be responsible for overseeing the inclusion of information and assisting the hematologists in the process.

Inclusion of patients into the study will continue for one year, and two more years will be required for the follow-up of these new participants. We will allow one more month to finish entering the latest collected information into the database.

This phase will therefore require a duration of 3 years, lasting until approximately October 2025.

Stage 4: Data analysis

In a first step, the statistician will receive the collected information in order to be able to analyze it. Then the results of the analysis will be given to the principal investigator.

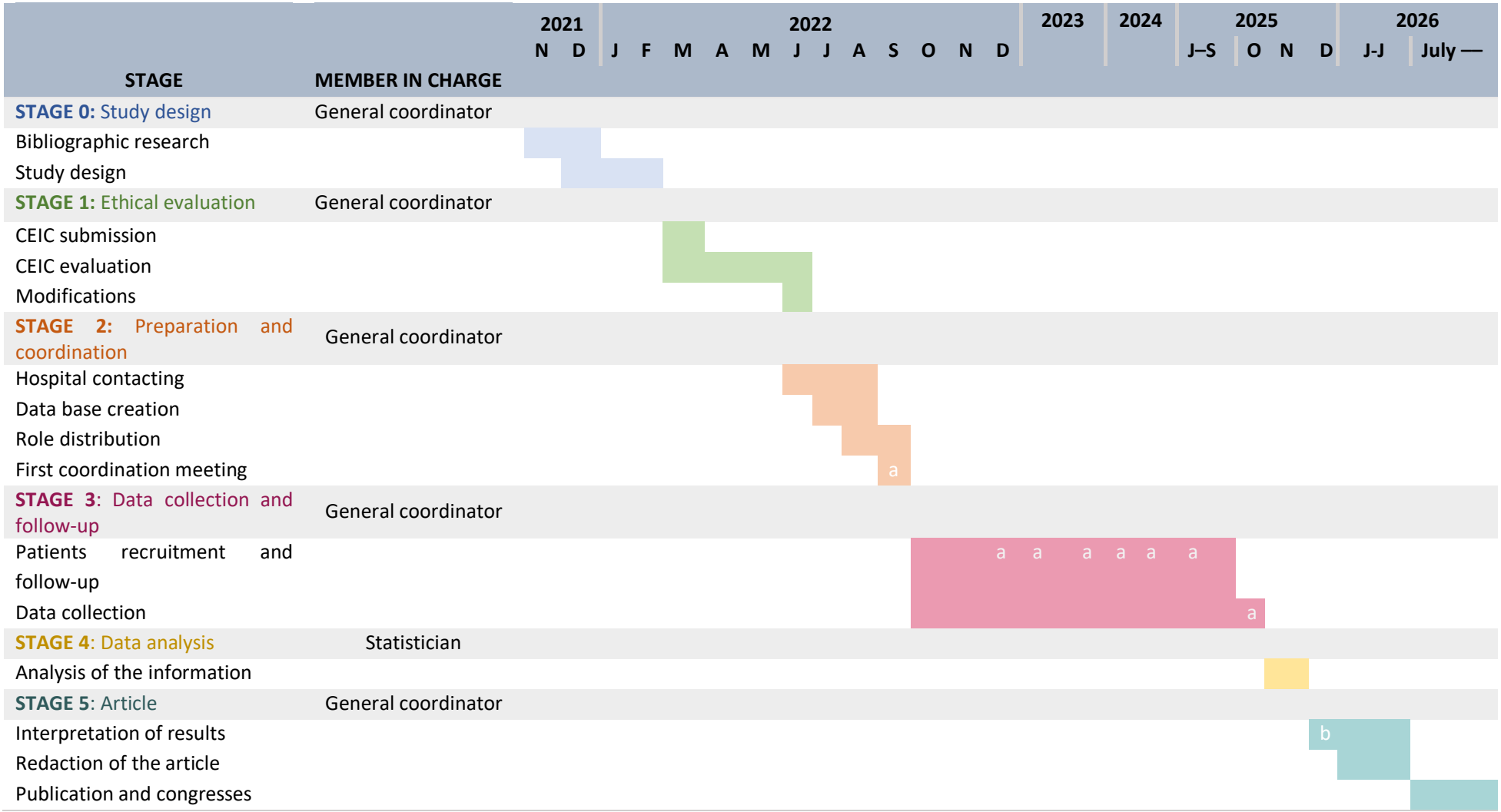
This stage will take 1 month.

Stage 5:Interpretation, redaction, publication and dissemination of results

The principal investigator will be given six months to interpretate the results, write up the study and submit it to the journals of choice. For the interpretation discussion, a meeting with all the head hematologists of each center will be carried out during the first or second month.

If possible, the results will be published and presented at a national or international congress during 2026.

CHRONOGRAM OF THE STUDY



[a] First and subsequent online catch-up meetings

[b] Presential meeting to discuss results

ETHICAL CONSIDERATIONS

This study protocol will be evaluated by the CEIC from Hospital Universitari Dr. Josep Trueta (HJT) And may be initiated only after receiving their approval. Any protocol modification and contributions from the CEIC will be included in the study later.

All patients included in the study must have been invited to read the information document and they must sign the informed consent if they agree to participate in it (See **Annex 3-6**). The information document must be understandable for the patients regarding vocabulary and language. It must contain detailed information about the study objectives, relevance and procedures, as well as the terms and conditions for entering the investigation. All the information related to cession, collection and processing of personal medical data, as well as their confidentiality and communication, will be explained in the document.

No compensation or financial expense will be obtained for the patients participation in the study. The patient's right to withdraw their consent at any time must be respected at any point of the study, and this will not cause any harm to their health care.

Each person of the research team and each hospital direction must sign a statement attesting to have read and approved the final protocol and agree with the national and international ethical aspects of the research.
According to the *"Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal"*, personal and clinical information of participants will be kept confidential at all times and will only be used for the purpose of the research. Personal information such as name, telephone number, address and other clinical history information will remain anonymous after their introduction on the database. Data will only be accessible for the responsible researchers of the project.

The study will be conducted according to the international ethics guidelines and laws established by the World Medical Association in the *Helsinki Declaration of Ethical Principles*

for Medical Research Involving Humans Subjects. Principles of biomedical and human research will be ethically respected in the study: non-maleficence, beneficence, patient autonomy and justice.

The investigators of the study will have to declare to not have any conflicts of interest.

STUDY LIMITATIONS

Study design

Designing an ambispective study, we have the advantage that time (and therefore cost) of execution is low since there is a part of the follow-up of patients that has already been done. However, we can find a limitation with the data collection bias since we have a retrospective part. To try to avoid this bias, we have made sure that the parameters needed to evaluate our dependent variables are those checked regularly on these patients and a structured collection of data can be carry out.

A quasi-experimental study has been designed where no other control group has been defined and neither is there a randomization of the subjects. Therefore, we expect confusion bias to take place. We have tried to minimalize the effects of a possible confounding bias by defining the plausible confounding factors described in the literature as covariables, with the use of multivariate logistic regression analysis.

On the other hand, this design allows the patient to be his own control and that way both groups are obviously comparable. A comparison with other historical groups and cohorts found on previous literature can be carried out.

Sample

Since we are using a non-probabilistic sampling this may affect the external validity of the study.

There is a risk that subjects are lost due to participation withdrawal or others such as low tolerance to treatment. For this purpose we have already accepted and made our calculations expecting a 10% subject loss. All withdrawals and situations where the patient's follow-up is not possible will be registered in the study.

Although we have calculated the sample and we think that the objective is achievable including hospitals belonging to the ICO and ICS (Institut Català de la Salut), a larger sample would be necessary to increase the statistical power of the study.

Investigator variability

As a multicenter study with one reference person per center, there will probably be some variability between investigators evaluations of the variables included in this study, especially in those that can be subjective, for example spleen size, the importance given to the patient's symptoms or the decision of treatment discontinuation.

Collaborating hospitals:

There is the possibility that some hospitals decide not to collaborate, which could lead to a difficulty of achieving the calculated sample size. However, the ICO centers have been contacted and they have already accepted to participate in the study, covering 50% of MPN patients in Catalunya.

Evaluation of variables

To assess the molecular response we can quantify the *JAKV617F* and *CALR* allele burden, but not the *MPL* since no technique is available or in use in our centers. This may be a limitation when studying one of the dependent variables since it won't be possible to use all our samples for it. However, *MPL* - mutated patients represent a very small part of our sample since the incidence is low in these diseases.

In our intervention some patients may receive a few weeks of treatment with HU previous to peg-IFNα-2a. Therefore, the response to treatment that we assess may be biased by the use of a previous drug. To minimize this bias, we have determined to exclude from the study (using the defined exclusion criteria) those patients who have received more than two months of HU treatment.

FEASIBILITY

We consider it feasible to carry out the study as there are no major obstacles to its implementation. Since no additional measures are needed in addition to those that patients have in their regular follow-up, the costs of the study are relatively low.

Patients

Patients do not have to undergo any additional intervention for participation in this study. They also do not have to actively participate in the study, as the information will be collected from their medical records.

We expect the duration of the study to be enough to obtain enough patients to cover the sample size proposed in the study. We do not expect hospitals to refuse to participate in the study since, as we have said, it does not involve the execution of new procedures in addition to those already established in the routine management of their patients.

Research team

Our research team will be composed of hematologists currently working in the included hospitals. They are experts in the field, so no pre-study training is required.

The only additional members that will be hired will be the statistician and the data manager.

Resources

The costs arising from the test, procedures and personnel required for the study are covered by the National Healthcare System. No extra material or health professionals will be required. The expenses expected are related to the publishing and congresses.

Data collection

We do not expect any missing data in the medical records, as this is included in the recommended follow-up protocol for these patients. In addition, we use the inclusion and exclusion criteria to ensure that all included patients have undergone the necessary tests at baseline.

STUDY BUDGET

As mentioned above, the costs of the procedures and the medical team in this study will be covered by the National Health System.

Statistician

The statistician will be given one month for data analysis. We estimate an approximate requirement of 140 hours to complete this work. The approximate salary for a statistician is between €30 and €40 per hour. Taking an average of €35/h the total cost will be around €4,900.

Data manager

The data manager will conduct data quality reviews at three points in the study: one at the beginning where he/she will assist hematologists in initiating data collection. The next between 2023 and 2024 to continue quality control. He will join one of the meetings in the period to intervene if necessary. Finally in the final period to finish the quality control before giving the data to the statistician.

We estimate 40 hours of work in each of the 3 moments of intervention.

Execution expenses

Printing the information sheets and the informed consent will be the only expenses in this section.

Travel expenses

A meeting of the team will be organized in the Hospital Universitari Josep Trueta to discuss analysis results and interpretation. It is estimated that one professional from at least nine hospitals will have to travel, and therefore we will count on €250 in travel, accommodation and per diem costs. We estimate a total cost of €2,250.

Publication expenses

One article will be written. We estimate that the revision, edition, formatting layout, graphic design, preparation of the digital metadata and translation will cost approximately 2.000€

Congresses expenses

Congress registration fees, as well as travel, accommodation and subsistence costs will be covered. Our aim is to present the results at the *"Congreso Nacional de la SEHH"* and at the European Hematology Association (EHA) Congress. We are estimating the budget on the assumption that we will be able to attend the congress in person. However, this could be done virtually due to the situation of the Sars-Cov-2 pandemic.

TYPE OF COST	UNIT COST	HOURS/UNITS	TOTAL
PERSONNEL EXPENSES			
Statistics analysis	35€/h	140 hours	4,900€
Data manager	30€/h	120 hours	3,600€
MATERIALS			
Printing: Information sheet and informed consent	0,05 cents/page	291	14.40€
PUBLICATION EXPENSES AND CONGRESSES			
Article publication	2000€	1	2000€
National Congress			
Inscription fee	400€	1	400€
Travelling and accomodation	200€/day	3	600€
EHA Congress			
Inscription fee	440€	1	440€
Travelling and accomodation	200€/day	3	600€
			TOTAL: 12,554.4€

CLINICAL AND HEALTHCARE IMPACT

Although PV and ET are rare entities, they are among the most frequent myeloid pathologies in our environment. Although the survival times of these patients are not yet comparable to those of the normal population, long survival times have been achieved, especially in younger patients.

Given these long survival times, we must try to access treatments that minimize long-term progression and unwanted effects as much as possible. With this study we will be able to see if peg-IFNα-2a is a good treatment option in our setting and supports the evidence already reported in the scientific literature.

In addition, it appears that peg-IFNα-2a allows the option of discontinuing treatment while maintaining both the clinical and molecular response, which would allow patients to pause treatment, improving their quality of life and avoiding unwanted effects.

Furthermore, it is hoped that our results will support the idea that peg-IFNα-2a is a good option for older patients (up to 70 years of age) as has been seen in previous studies and in the daily clinical practice of hematologists in Catalunya.

The continuous creation of scientific knowledge that allows us to better understand these entities will help us to offer our patients a treatment that is better adapted to their profile and needs, improving their satisfaction, quality of life and control of the disease.

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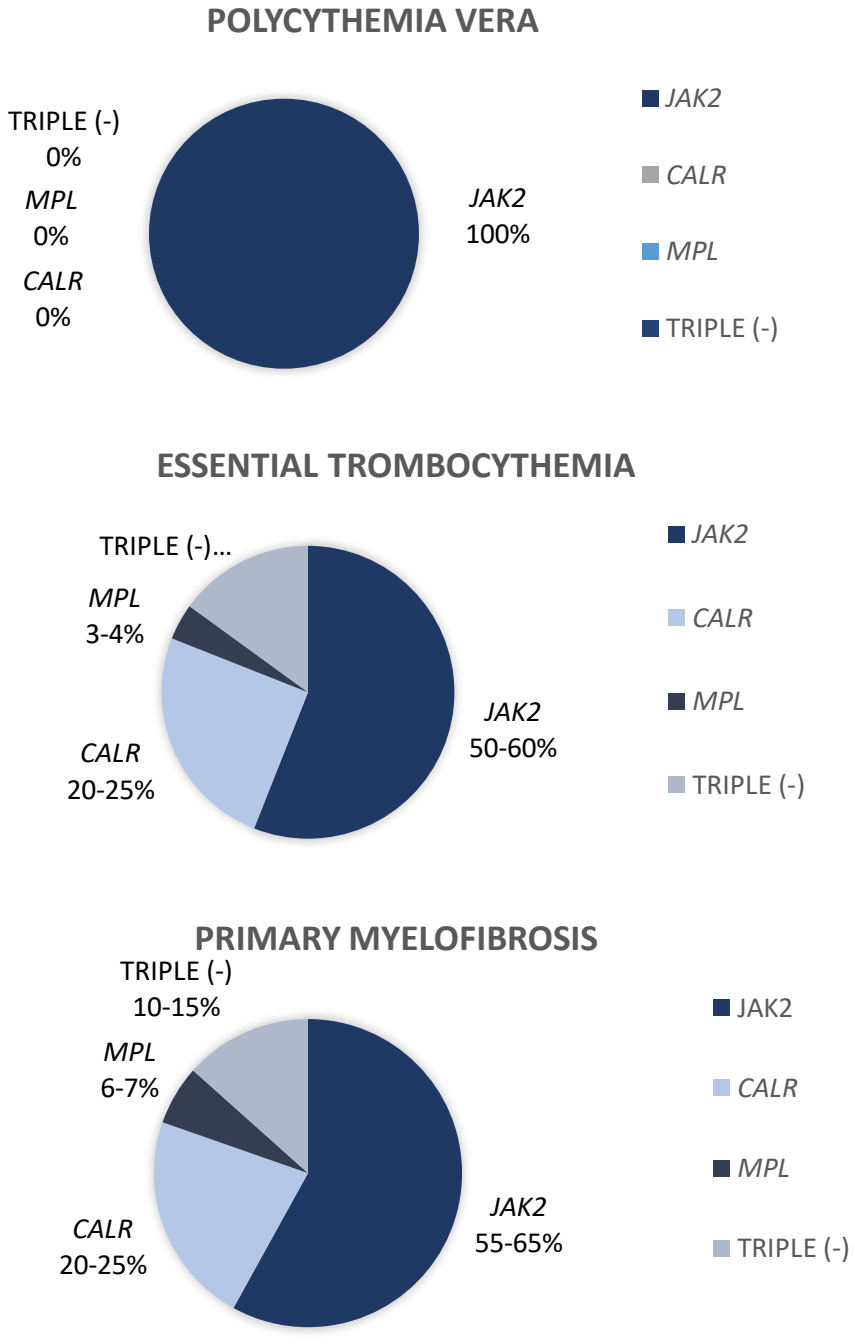
ANNEXES

Annex 1. GROUP OF CIRCLE CHARTS SHOWING THE PROPORTIONS OF DRIVER MUTATIONS IN EACH ENTITY



Annex 2. LIST OF 79 MUTATIONS ON THE HAEMATOLOGICAL DISEASE PANEL

Haematological NGS panel								
ARID5B	*ASXL1*	*ASXL2*	*ATRX*	*BCOR*	*BCORL1*	*BLNK*	*BRAF*	*CALR*
CBL	*CDKN2A*	*CDKN2B*	*CEBPA*	*CHIC2*	*CREBBP*	*CRLF2*	*CSF3R*	*CSNK1A1*
CUX1	*DDX3X*	*DDX41*	*DNMT3A*	*EP300*	*ETNK1*	*ETV6*	*EZH2*	*FBXW7*
FLT3	*GATA1*	*GATA2 (e intron 4)*	*GATA 3*	*HAVCR2*	*IDH1*	*IDH2*	*IKZF1*	*IL7R*
JAK1	*JAK2*	*JAK3*	*KIT*	*KMT2A*	*KMT2C*	*KRAS*	*MPL*	*NF1*
NF2	*NFE2*	*NOTCH1*	*NPM1*	*NR3C1*	*NRAS*	*P2RY8*	*PAX5*	*PHF6*
PIGA	*PPM1D*	*PTEN*	*PTK2B*	*PTPN11*	*RAD21*	*RB1*	*RUNX1*	*SETBP1*
SF3B1	*SH2B3*	*SMC1A*	*SMC3*	*SRP72*	*SRSF2*	*STAG1*	*STAG2*	*STAT5B*
TET2	*TP53*	*TPMT*	*TYK2*	*U2AF1*	*WT1*	*ZRSR2*		

Annex 3. INFORMATION SHEET FOR PATIENTS DIAGNOSED WITH ET SPANISH VERSION

HOJA DE INFORMACIÓN AL PACIENTE CON TROMBOCITEMIA ESENCIAL

Buenos días,

Este documento tiene la finalidad de aportar toda la información relativa al estudio

"Pegylated Interferon as a first line treatment in polycythemia vera and essential thrombocythemia patients: a quasi-experimental study"

La trombocitemia esencial es una enfermedad que consiste en un aumento del número de plaquetas en la sangre y como consecuencia de ella existe un riesgo incrementado de trombosis y hemorragia. Usted es candidato/a a recibir uno de los tratamientos propuestos para dicha enfermedad, llamado interferón pegilado (peg-IFNα-2a).

Le invitamos a participar en este estudio en el que se pretende evaluar a los pacientes tratados con este mismo fármaco.

Generalidades del estudio

Este estudio será coordinado por el centro ICO de Girona y se invita a participar a pacientes hematológicos con diagnóstico de Trombocitemia Esencial o Policitemia Vera que vayan a recibir tratamiento con peg-IFNα-2a de hospitales de toda Cataluña.

Objetivos y finalidad del estudio

En el estudio se propone la valoración de la eficacia de este fármaco mediante el estudio comparativo del estado de los pacientes antes de recibirlo y tras dos años de tratamiento. También se valorarán los efectos indeseados que el fármaco pueda producir en éstos.

Beneficios que se derivarán de este estudio

La intención de este estudio es generar conocimiento y no se espera que usted obtenga un beneficio directo por participar. No obstante puede ayudar a futuros pacientes con su mismo diagnóstico.

Riesgos que se derivan de la participación en este estudio

Su participación en este estudio no conlleva la realización de ninguna práctica distinta a la habitual en su seguimiento y por lo tanto no supondrá un riesgo para su persona.

Este estudio ha sido aprobado por el Comité Ético de Investigación Clínica del Hospital Universitari Dr. Josep Trueta

Su participación en el estudio no implica ninguna prueba adicional a las que se le realizan en su consulta de forma habitual. Con la participación en este estudio usted permite a su médico/a registrar algunos datos existentes en su historia clínica en un formulario diseñado para este fin. Dentro de esta información no será incluido ningún dato que le identifique directamente o que potencialmente pudiera llegar a identificarle en un futuro. Toda la información será tratada con total reserva y solamente estará analizada por los investigadores de este estudio con la única finalidad comentada anteriormente.

Debe saber que su participación es totalmente voluntaria y podrá retirar su consentimiento cuando así lo desee sin tener que dar ninguna explicación al equipo médico o de investigación. En ningún caso su tratamiento o procedimientos a los que deba ser sometido serán diferentes a los de otros pacientes que no participen en el estudio. En caso de rechazar su inclusión en el estudio, o si toma la decisión de retirar su consentimiento, la calidad de su seguimiento y atención de su enfermedad no se verán perjudicados.

En cualquier caso se cumplirá lo establecido en la legislación vigente sobre la protección de datos de carácter personal (Ley Orgánica 15/1999 de 13 de diciembre de Protección de Datos de Carácter Personal), lo cual significa, entre otras garantías, que sus datos personales no pueden ser desvelados a terceros, ni su identidad puede aparecer en ninguna publicación de los resultados del estudio.

Para poder incluirlo/a en el registro usted deberá danos su autorización mediante la firma de la hoja de consentimiento informado que su médico/a le ofrecerá si desea participar.

Annex 4. INFORMATION SHEET FOR PATIENTS DIAGNOSED WITH PV SPANISH VERSION

HOJA DE INFORMACIÓN AL PACIENTE CON POLICITEMIA VERA

Buenos días,

Este documento tiene la finalidad de aportar toda la información relativa al estudio

"Pegylated Interferon as a first line treatment in polycythemia vera and essential thrombocythemia patients: a quasi-experimental study"

La policitemia vera es una enfermedad que consiste en un aumento del número de glóbulos rojos en la sangre y como consecuencia de ella existe un riesgo incrementado de trombosis y hemorragia. Usted es candidato/a a recibir uno de los tratamientos propuestos para dicha enfermedad, llamado interferón pegilado (peg-IFNα-2a).

Le invitamos a participar en este estudio en el que se pretende evaluar a los pacientes tratados con este mismo fármaco.

Generalidades del estudio

Este estudio será coordinado por el centro ICO de Girona y se invita a participar a pacientes hematológicos con diagnóstico de Trombocitemia Esencial o Policitemia Vera que vayan a recibir tratamiento con peg-IFNα-2a de hospitales de toda Cataluña.

Objetivos y finalidad del estudio

En el estudio se propone la valoración de la eficacia de este fármaco mediante el estudio comparativo del estado de los pacientes antes de recibirlo y tras dos años de tratamiento. También se valorarán los efectos indeseados que el fármaco pueda producir en éstos.

Beneficios que se derivarán de este estudio

La intención de este estudio es generar conocimiento y no se espera que usted obtenga un beneficio directo por participar. No obstante puede ayudar a futuros pacientes con su mismo diagnóstico.

Riesgos que se derivan de la participación en este estudio

Su participación en este estudio no conlleva la realización de ninguna práctica distinta a la habitual en su seguimiento y por lo tanto no supondrá un riesgo para su persona.

Este estudio ha sido aprobado por el Comité Ético de Investigación Clínica del Hospital Universitari Dr. Josep Trueta.

Su participación en el estudio no implica ninguna prueba adicional a las que se le realizan en su consulta de forma habitual. Con la participación en este estudio usted permite a su médico/a registrar algunos datos existentes en su historia clínica en un formulario diseñado para este fin. Dentro de esta información no será incluido ningún dato que le identifique directamente o que potencialmente pudiera llegar a identificarle en un futuro. Toda la información será tratada con total reserva y solamente estará analizada por los investigadores de este estudio con la única finalidad comentada anteriormente.

Debe saber que su participación es totalmente voluntaria y podrá retirar su consentimiento cuando así lo desee sin tener que dar ninguna explicación al equipo médico o de investigación. En ningún caso su tratamiento o procedimientos a los que deba ser sometido serán diferentes a los de otros pacientes que no participen en el estudio. En caso de rechazar su inclusión en el estudio, o si toma la decisión de retirar su consentimiento, la calidad de su seguimiento y atención de su enfermedad no se verán perjudicados.

En cualquier caso se cumplirá lo establecido en la legislación vigente sobre la protección de datos de carácter personal (Ley Orgánica 15/1999 de 13 de diciembre de Protección de Datos de Carácter Personal), lo cual significa, entre otras garantías, que sus datos personales no pueden ser desvelados a terceros, ni su identidad puede aparecer en ninguna publicación de los resultados del estudio.

Para poder incluirlo/a en el registro usted deberá darnos su autorización mediante la firma de la hoja de consentimiento informado que su médico/a le ofrecerá si desea participar.

Annex 5. INFORMED CONSENT; SPANISH VERSION

CONSENTIMIENTO INFORMADO

"Pegylated Interferon as a first line treatment in polycythemia vera and essential thrombocythemia patients: a quasi-experimental study"

Yo (nombre y apellidos) _____

con DNI/NIE _____ y fecha de nacimiento ____ / _____ / _____, declaro que:

- He sido debidamente informado por el Dr./Dra. _____
- He recibido y leído la hoja de información para el paciente sobre el estudio.
- He podido hacer todas las preguntas necesarias sobre el estudio.
- He recibido suficiente información sobre el estudio.
- He sido informado de las finalidades del estudio.
- Entiendo que se respetará la confidencialidad de mis datos.
- Entiendo que mi participación es voluntaria y no repercute en mis cuidados médicos.
- Entiendo que puedo revocar mi consentimiento de participación al estudio sin necesidad de justificarme.

Presto libremente mi conformidad para que mis datos sean utilizados en el estudio.

Firma del/la participante **Firma del/la investigador/a**

En _____, a _____ de _____ de _____ .

Annex 6. INFORMATION SHEET FOR PATIENTS DIAGNOSED WITH ET CATALAN VERSION

FULL D'INFORMACIÓ AL PACIENT AMB TROMBOCITÈMIA ESSENCIAL

Benvingut/benvolguda pacient,

Aquest document té la finalitat d'aportar total la informació rellevant relativa al l'estudi *"Pegylated Interferon as a first line treatment in polycythemia vera and essential thrombocythemia patients: a quasi-experimental study"*

La trombocitèmia essencial és una malaltia que consisteix en un augment del nombre de plaquetes a la sang i com a conseqüència d'aquesta existeix un risc augmentat de trombosi i hemorràgia. Vostè és candidat/a a rebre un dels tractaments proposats per a aquesta malaltia, anomenat interferó pegilat (peg-IFNα-2a).

Us convidem a participar en aquest estudi en què es pretén avaluar els pacients tractats amb aquest mateix fàrmac.

Generalitats de l'estudi

Aquest estudi serà coordinat pel centre ICO de Girona i es convida a participar a pacients hematològics amb diagnòstic de Trombocitèmia Essencial o Policitèmia Vera que rebin tractament amb peg-IFNα-2a d'hospitals de tot Catalunya.

Objectius i finalitat de l'estudi

A l'estudi es proposa la valoració de l'eficàcia d'aquest fàrmac mitjançant l'estudi comparatiu de l'estat dels pacients abans de rebre'l i després de dos anys de tractament. També es valoraran els efectes adversos que el fàrmac pugui produir-hi.

Beneficis que derivaran d'aquest estudi

La intenció d'aquest estudi és generar coneixement i no s'espera que obtingueu un benefici directe per participar. No obstant això, pot ajudar futurs pacients amb el mateix diagnòstic que vostè.

Riscos que es deriven de la participació en aquest estudi

La seva participació en aquest estudi no comporta la realització de cap pràctica diferent de l'habitual en el seguiment i, per tant, no suposarà un risc per a la seva persona.

Aquest estudi ha estat aprovat pel Comitè Ètic de Recerca Clínica de l'Hospital Universitari Dr. Josep Trueta.

La seva participació a l'estudi no implica cap prova addicional a les que es fan a la seva consulta de forma habitual. Amb la participació en aquest estudi vostè permet al metge/essa registrar algunes dades existents en la seva història clínica en un formulari dissenyat per a aquest fi. Dins d'aquesta informació no serà inclosa cap dada que us identifiqui directament o que potencialment pogués arribar a identificar-vos en un futur. Tota la informació serà tractada amb reserva total i només estarà analitzada pels investigadors d'aquest estudi amb l'única finalitat comentada anteriorment.

Heu de saber que la vostra participació és totalment voluntària i podreu retirar el vostre consentiment quan així ho desitgeu sense haver de donar cap explicació a l'equip mèdic o de recerca. En cap cas el seu tractament o els seus procediments a què hagi de ser sotmès seran diferents dels d'altres pacients que no participin a l'estudi. En cas de rebutjar la seva inclusió a l'estudi, o si pren la decisió de retirar el seu consentiment, la qualitat del seguiment i l'atenció de la malaltia no es veuran perjudicats.

En qualsevol cas es complirà allò establert a la legislació vigent sobre la protecció de dades de caràcter personal (Llei Orgànica 15/1999 de 13 de desembre de Protecció de Dades de Caràcter Personal), la qual cosa significa, entre altres garanties, que les seves dades personals no poden ser revelats a tercers, ni la seva identitat pot aparèixer a cap publicació dels resultats de l'estudi.

Per poder incloure'l al registre vostè haurà de donar-nos la seva autorització mitjançant la signatura del full de consentiment informat que el seu metge o metgessa us oferirà si voleu participar.

Annex 7. INFORMATION SHEET FOR PATIENTS DIAGNOSED WITH ET CATALAN VERSION

FULL D'INFORMACIÓ AL PACIENT AMB POLICITÈMIA VERA

Benvingut/benvolguda pacient,

Aquest document té la finalitat d'aportar total la informació rellevant relativa al l'estudi *"Pegylated Interferon as a first line treatment in polycythemia vera and essential thrombocythemia patients: a quasi-experimental study"*

La policitemia vera és una malaltia que consisteix en un augment del nombre de glòbuls vermells a la sang i com a conseqüència d'aquesta existeix un risc incrementat de trombosi i hemorràgia. Vostè és candidat/a a rebre un dels tractaments proposats per a aquesta malaltia, anomenat interferó pegilat (peg-IFNα-2a).

Us convidem a participar en aquest estudi en què es pretén avaluar els pacients tractats amb aquest mateix fàrmac.

Generalitats de l'estudi

Aquest estudi serà coordinat pel centro ICO de Girona i es convida a participar a pacients hematològics amb diagnòstic de Trombocitèmia Essencial o Policitèmia Vera que rebin tractament amb peg-IFNα-2a d'hospitals de tot Catalunya.

Objectius i finalitat de l'estudi

A l'estudi es proposa la valoració de l'eficàcia d'aquest fàrmac mitjançant l'estudi comparatiu de l'estat dels pacients abans de rebre'l i després de dos anys de tractament. També es valoraran els efectes indesitjats que el fàrmac pugui produir-hi.

Beneficis que derivaran d'aquest estudi

La intenció d'aquest estudi és generar coneixement i no s'espera que obtingueu un benefici directe per participar. No obstant això, pot ajudar futurs pacients amb el mateix diagnòstic que vostè.

Riscos que es deriven de la participació en aquest estudi

La seva participació en aquest estudi no comporta la realització de cap pràctica diferent de l'habitual en el seguiment i, per tant, no suposarà un risc per a la seva persona.

Aquest estudi ha estat aprovat pel Comitè Ètic de Recerca Clínica de l'Hospital Universitari Dr. Josep Trueta.

La seva participació a l'estudi no implica cap prova addicional a les que es fan a la seva consulta de forma habitual. Amb la participació en aquest estudi vostè permet al metge/essa registrar algunes dades existents en la seva història clínica en un formulari dissenyat per a aquest fi. Dins d'aquesta informació no serà inclosa cap dada que us identifiqui directament o que potencialment pogués arribar a identificar-vos en un futur. Tota la informació serà tractada amb reserva total i només estarà analitzada pels investigadors d'aquest estudi amb l'única finalitat comentada anteriorment.

Heu de saber que la vostra participació és totalment voluntària i podreu retirar el vostre consentiment quan així ho desitgeu sense haver de donar cap explicació a l'equip mèdic o de recerca. En cap cas el seu tractament o els seus procediments a què hagi de ser sotmès seran diferents dels d'altres pacients que no participin a l'estudi. En cas de rebutjar la seva inclusió a l'estudi, o si pren la decisió de retirar el seu consentiment, la qualitat del seguiment i l'atenció de la malaltia no es veuran perjudicats.

En qualsevol cas es complirà allò establert a la legislació vigent sobre la protecció de dades de caràcter personal (Llei Orgànica 15/1999 de 13 de desembre de Protecció de Dades de Caràcter Personal), la qual cosa significa, entre altres garanties, que les seves dades personals no poden ser revelats a tercers, ni la seva identitat pot aparèixer a cap publicació dels resultats de l'estudi.

Per poder incloure'l al registre vostè haurà de donar-nos la seva autorització mitjançant la signatura del full de consentiment informat que el seu metge o metgessa us oferirà si voleu participar.

Annex 8. INFORMED CONSENT; SPANISH VERSION

CONSENTIMENT INFORMAT

"Pegylated Interferon as a first line treatment in polycythemia vera and essential thrombocythemia patients: a quasi-experimental study"

Jo (nom i cognoms) _____

amb DNI/NIE _____ i data de naixement ____ / _____ / _____, declaro que:

- He estat correctament informat/da pel el Dr./Dra. _____
- He rebut i llegit el full d'informació per al pacient sobre l'estudi.
- He rebut suficient informació sobre l'estudi.
- He estat informat de les implicacions i finalitats de l'estudi.
- Entenc que es respectarà la confidencialitat de les meves dades,
- Entenc que la meva participació és voluntària i no repercuteix en les meves cures mèdiques.
- Entenc que puc revocar el meu consentiment de participació a l'estudi sense necessitat de justificar-me..

Presto lliurement la meva conformitat perquè les meves dades siguin utilitzades a l'estudi.

Signatura del/la participant **Signatura de l'/la investigador/a**

A _____,a _____ de _____ de _____ .

Annex 9. DATA COLLECTION FORM

Number of the patient: _____

Study moment:

- ☐ Baseline
- ☐ 24 months from baseline

Age: ____ (years)

Sex:

- ☐ Male
- ☐ Female

Diagnose

- ☐ PV
- ☐ ET

High-risk mutations (≥1) [*TET2, ASXL1, DNMT3A, IDH1/2, EZH1*]

- ☐ Yes
- ☐ No

* This section must be filled only at baseline

Smoker

- ☐ Yes
- ☐ No

Diabetic

- ☐ Yes
- ☐ No

Socioeconomic level

- ☐ Class I
- ☐ Class II
- ☐ Class III
- ☐ Class IV
- ☐ Class V

Hypertension

- ☐ Yes
- ☐ No

Hypercholesterolemia

- ☐ Yes
- ☐ No

BLOOD TEST RESULTS

Hematocrit _____ (%)

Platelet count _____ (x 10^9/L)

WBC count _____ (x 10^9/L)

***JAK2V617F* allele burden** _____ (%)

***CALR* allele burden** _____ (%)

SYMPTOMATOLOGY / PHYSICAL EXAMINATION

Microvascular symptoms

☐ Yes
☐ No

Headache

☐ Yes
☐ No

Pruritus

☐ Yes
☐ No

Splenomegaly

☐ Yes
☐ No

TREATMENT

Phlebotomy need

☐ Yes
☐ No

THROMBOTIC/BLEEDING EVENTS

Incidence of a major thrombotic or bleeding event ☐ Yes ☐ No

* During the first 24 months from the start of the treatment. This section must be filled only at "24 months" study moment.

TOLERANCE

Discontinuation

☐ Yes
☐ No

Time from the start of the treatment ☐ (months)

Dosage ☐ (µg/week)

Discontinuation motive:

☐ Autoimmunity markers
☐ Liver damage
☐ Flu-like symptoms
☐ Cutaneous toxicity
☐ Gastrointestinal symptoms
☐ Thyroid disorder
☐ Mood disturbance
☐ Peripheral neuropathy
☐ Others

Please, make sure all this information is correctly gathered.

SUBMIT