Thesis

Outcomes of Patients With Primary Myelofibrosis in Relation to Mutation Status

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Graz, 16 May 2025

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Graz, 16 May 2025 *Philipp Zaininger m.p.*

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Zusammenfassung

Hintergrund: Seit der Einführung von Next-Generation-Sequencing (NGS) als diagnostisches Instrument für hämatologische Neoplasien konnten verschiedene Mutationen als Risikofaktoren identifiziert werden. So auch bei myeloproliferativen Neoplasien (MPN) wie der primären Myelofibrose (PMF). Neben den klassischen driver-Mutationen in den Genen *JAK2*, *CALR* und *MPL* konnte gezeigt werden, dass einige non-driver Mutationen (nDM) die Prognose von PMF-PatientInnen negativ beeinflussen, indem sie sowohl das Gesamtüberleben (OS) als auch das ereignisfreie Überleben (EFS) verschlechtern. Obwohl die pathophysiologische Rolle solcher Mutationen bisher kaum definiert ist, wurden einige genetische Aberrationen in Hochrisiko-Genen (HMR) wie *ASXL1*, *SRSF2* und *IDH1/2* in neuere prognostische Scores aufgenommen. Ziel dieser Studie war es, den Einfluss sowie mögliche Zusammenhänge klinischer und genetischer Parameter in einer unselektierten Kohorte von PMF-PatientInnen, die an der Medizinischen Universität Graz diagnostiziert wurden, zu untersuchen und die Ergebnisse mit publizierten internationalen Daten zu vergleichen.

Methoden: In diese retrospektive Studie wurden 156 PatientInnen eingeschlossen, bei denen zwischen 2015 und 2022 an der Medizinischen Universität Graz eine PMF diagnostiziert wurde. Klinische und genetische Parameter wurden für alle PatientInnen aus der elektronischen Datenbank MEDOCS entnommen und mittels deskriptiver Statistik zusammengefasst. Überlebensraten wurden mittels Kaplan-Meier-Kurven dargestellt. Die statistische Signifikanz wurde durch Log-Rank-Tests und paarweise Vergleiche überprüft. Unabhängige Risikofaktoren für schlechtere Überlebensraten wurden mittels Cox-Regression identifiziert.

Ergebnisse: Im Vergleich zu publizierten Daten beobachteten wir verbesserte 5 - und 8 Jahres OS -, sowie EFS-Raten, was vermutlich auf einen höheren Anteil klinischer low - und intermediate-1 Risiko Patient:innen in unserer Kohorte zurückzuführen ist. Dennoch zeigte sich, dass PatientInnen mit einer *JAK2*-Mutation, neben etablierten klinischen Risikofaktoren wie höherem Alter und fortgeschrittenem Erkrankungsstadium, ein signifikant schlechteres OS und EFS

im Vergleich zu PatientInnen mit anderen driver-Mutationen aufwiesen. Auch die Präsenz von mindestens einer nDM verschlechterte das OS signifikant im Vergleich zu PatientInnen ohne eine solche Mutation. Dies führte weiters zu einem unabhängig erhöhten Risiko für ungünstige Ereignisse wie leukämische Transformation, Thromboembolien oder Tod. Eine genauere Analyse erlaubte jedoch, aufgrund der geringen Fallzahlen in den jeweiligen Subgruppen, keine Aussage über den Einfluss spezifischer Mutationen auf die Überlebensraten.

Schlussfolgerungen: Trotz der höheren Anzahl an PatientInnen mit Erkrankung im Frühstadium als erwartet, konnten wir sowohl das Vorliegen einer *JAK2* driver-Mutation als auch das Vorhandensein zusätzlicher nDM als unabhängige genetische Marker mit negativen Auswirkungen auf die Prognose, in einer unselektierten Kohorte von PMF-PatientInnen bestätigen. Weitere Studien mit größeren Kohorten sind jedoch notwendig, um den Einfluss spezifischer (non-)driver Mutationen auf den Krankheitsverlauf bei PMF besser zu verstehen.

Abstract

Background: Since the implementation of next generation sequencing (NGS) as a diagnostic tool for hematological malignancies, various mutations have been identified as risk factors, also in myeloproliferative neoplasms (MPN) like primary myelofibrosis (PMF). Apart from the classical disease driver mutations in the *JAK2, CALR* and *MPL* genes, some non-driver mutations (nDM) have been proven to affect the prognosis of PMF patients by impairing overall (OS) - and event-free survival (EFS) as well. Although the pathophysiological role of such mutations is hardly defined, some genetic aberrations in high molecular risk (HMR) genes, like *ASXL1, SRSF2* and *IDH1/2* have been included in more recent prognostic scores. The aim of this study was to determine the impact as well as possible correlations of clinical and genetic parameters in an unselected cohort of PMF patients diagnosed at the Medical University of Graz and to compare these results with data from published international studies.

Methods: This retrospective study included 156 patients who were diagnosed with PMF at the Medical University of Graz between 2015 and 2022. Clinical -, as well as genetic parameters were retrieved from the electronic database MEDOCS for all patients and summarized using descriptive statistics. Survival rates were visualized using Kaplan-Meier plots and statistical significance was assessed using log-rank tests and pairwise comparisons. Independent risk factors for decreased survival rates were identified through Cox regression analysis.

Results: When compared to published data we observed an improved 5-year and 8-year OS and EFS, probably related to an increased number of clinically low - and intermediate-1 risk patients in our cohort. Nevertheless, aside from well established clinical parameters such as age and advanced disease stage we found that patients carrying a *JAK2* mutation had a significantly worse OS and EFS compared to patients with other driver mutations. The presence of at least one nDM significantly impaired OS in comparison to patients without a nDM and resulted in an independently increased risk of experiencing an adverse event like leukemic transformation, thromboembolism or death. However, further analysis did

not allow for revealing a significant impact of distinct mutations on survival rates probably due to the small numbers of patients in these subgroups.

Conclusions: Although we observed a higher number of early stage disease patients in our cohort than expected, we could confirm the presence of a *JAK2* driver mutation as well as the presence of any additional nDM as independent negative prognostic genetic markers in an unselected cohort of patients with PMF. However, further studies analyzing larger cohorts are needed to clarify the impact of distinct (non-)driver mutations on the outcome of patients with PMF.

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Abbreviations

MPN	Myeloproliferative Neoplasm
WHO	World Health Organization
CML	Chronic Myeloid Leukemia
PV	Polycythemia Vera
ET	Essential Thrombocythemia
PMF	Primary Myelofibrosis
MF	Myelofibrosis
JAK	Janus Kinase
STAT	Signal Transducer and Activator of Transcription
JH1/2	Janus Homology Domain 1/2
CNL	Chronic Neutrophilic Leukemia
CEL	Chronic Eosinophilic Leukemia
NOS	Not Otherwise Specified
AML	Acute Myeloid Leukemia
SEER	Surveillance, Epidemiology and End Results
HSC	Hematopoietic Stem Cell
ATP	Adenosine Triphosphate
G-CSF	Granulocyte Colony - Stimulating Factor
PI3K/AKT	Phosphoinositide 3-Kinase / Protein Kinase B Pathway
MAPK	Mitogen-Activated Protein Kinase
SH	Src Homology
EPOR	Erythropoietin Receptor
MPL	Myeloproliferative Leukemia Virus Oncogene
CALR	Calreticulin
ER	Endoplasmic Reticulum
MHC	Major Histocompatibility Complex
IPSET	International Prognostic Score for Thrombosis in Essential Thrombocythemia
ASA	Acetylsalicylic Acid
HU	Hydroxyurea
IWG-MRT	International Working Group for Myeloproliferative

	Neoplasms Research and Treatment
ANAHYDRET	Anagrelide Hydroxyurea European Trial
PROUD-PV	Phase III Randomized Trial of Ropeginterferon Alpha-2b in Polycythemia Vera
CONTINUATION-PV	Follow Up Study to PROUD-PV
ECLAP	European Collaboration on Low-Dose Aspirin in Polycythemia Vera
EMH	Extramedullary Hematopoiesis
LDH	Lactate Dehydrogenase
TGF-β	Transforming Growth Factor Beta
VEGF	Vascular Endothelial Growth Factor
IL	Interleukin
MDS	Myelodysplastic Syndrome
IPSS	International Prognostic Scoring System
WBC	White Blood Cell Count
DIPSS	Dynamic International Prognostic Scoring System
MIPSS70	Mutation-Enhanced International prognostic Scoring System for Patients ≤ 70 Years
allo-HSCT	Allogenic Hematopoietic Stem Cell Transplantation
HMR	High Molecular Risk
VHR	Very High Risk
GIPSS	Genetically Inspired Prognostic Scoring System
MYSEC-PM	Myelofibrosis Secondary to Essential Thrombocythemia or Polycythemia Vera Prognostic Model
NCCN	National Comprehensive Cancer Network
MTSS	Molecular and Translational Science Section
TNF-alpha	Tumor Necrosis Factor Alpha
COMFORT-I/II	Controlled Myelofibrosis Study with Oral JAK Inhibitor Therapy
ELN	European LeukemiaNet
SIE	Società Italiana di Ematologia (Italian Society of Hematology)

ESA	Erythropoiesis-Stimulating Agent
TIPS	Transjugular Intrahepatic Portosystemic Shunt
MANIFEST	Clinical Trial for BET Inhibitor in Myelofibrosis
TRANSFORM	Clinical Trial for New Therapies for Myelofibrosis
REFINE	Clinical Trial for Novel JAK Inhibitors in Myelofibrosis
BCL	B-Cell Lymphoma
BET	Bromodomain and Extra-Terminal Domain
NGS	Next-Generation Sequencing
LKH	Landeskrankenhaus

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1 Introduction - Myeloproliferative Neoplasms

Myeloproliferative neoplasms (MPNs) is an umbrella term for a heterogenous group of hematological diseases, which are characterized by an overproduction of blood cells. In September 2017 the World Health Organisation (WHO) published a new edition of the classification system for tumors of the hematopoietic and lymphoid tissues, which also included a reform for MPNs, containing seven subcategories in total [1]. Although the WHO classification system got updated to its fifth edition in 2022, the MPN main types remain largely unchanged and are shown below [2]:

- Chronic myeloid leukemia (CML)
- Polycythemia vera (PV)
- Essential thrombocythemia (ET)
- Primary myelofibrosis (PMF)
    - prefibrotic PMF
    - overtly fibrotic PMF
- Chronic neutrophilic leukemia (CNL)
- Chronic eosinophilic leukemia (CEL)
- Juvenile myelomonocytic leukaemia
- Myeloproliferative neoplasm, not otherwise specified (NOS)

Classic Philadelphia chromosome-negative -, or *BCR-ABL* negative MPNs contain PV, ET and myelofibrosis (MF) [3]. Characteristics of these entities are overproduction of fully functional and terminally differentiated blood cells, causing various clinical complications, such as venous - and arterial thrombosis, hemorrhages and transformation to acute myeloid leukemia (AML) [4]. These subtypes of MPNs are often driven by one of three types of mutations, so called „driver mutations“, which can be detected in over 90% of *BCR-ABL* negative patients. [5] An example for *BCR-ABL* positive MPNs would be CML, which is caused by reciprocal translocation between chromosome 9 and 22, forming the fusion gene *BCR-ABL*, also known as „Philadelphia chromosome“. This

chromosomal abnormality in the hematopoetic stem - , and progenitor cells can be detected by various diagnostic procedures [6]. However, only classic *BCR-ABL* negative entities will be discussed in this diploma thesis. As far as etiology is concerned, epidemiological studies identified various factors, that are associated with a higher risk for developing MPNs. An overview of these risk factors is provided in Table 1-1 [7]. In all of these correlations, a family history of MPNs showed the strongest evidence for an elevated risk.

Factor	Significant Associations
Family history and ethic factors	Relatives with MPNs, (Ashkenazi) Jewish decent
Lifestyle	Smoking, high Body Mass Index
Environment and exposure	Ionising radiation, chemical exposure (Benzene, Petroleum, dark hair dye use for more than 10 years and ET)
Medical conditions	Crohn´s disease, giant cell arteritis
Profession	Poultry-, agricultural-, rural sector-, Petroleum refinery-workers, commercial pressmen, cooks and waiters, clerks, working with electrical devises;

Table 1-1: Factors associated with MPNs [7];

1.1 Epidemiology

Since the discovery of mutations in the *JAK2* gene in 2005, diagnostics have improved and registrations of MPNs increased. Many countries have analysed incidence rates, which have a wide variety, depending on given databases and publications. Overall, the estimated annual incidence rates are 0.84 per 100.000 for PV, 1.03 per 100.000 for ET and 0.57 per 100.000 for PMF. Due to the relatively low mortality rates of MPNs, especially in PV and ET, prevalence is much higher. While PV and PMF show no significant difference in annual incidence rates between males and females, ET occurs more often in males [7]. The SEER (Surveilance, Epidemiology, and End Results) Program offers a huge database with calculated incidence rates of 1.09 per 100.000 for PV, 0.96 per 100.000 for ET

and 0.31 per 100.000 for PMF in the United States. In Europe annual incidence rates ranged from 0.4 - 2.8 per 100.000 for PV, 0.38 - 1.7 per 100.000 for ET and 0.1 - 1 per 100.000 for PMF [7]. Although MPNs are most commonly diagnosed in the seventh decade of life (65 - 74 for PV, 64.3 - 73 for ET and 69 - 76 PMF), the number of diagnosed MPNs in young adults and adolescents is increasing, especially ET in females [8, 9].

1.2 Pathophysiology

Every entity of MPN has its origin in a single somatically mutated hematopoietic stem cell (HSC). From these mutated and clonally expanding HSCs virtually all myeloid cells can arise. Depending on the present mutations, the MPN phenotype can be accompanied by single- or multilineage hyperplasia [4]. Over the years many mutations have been discovered, that pose a role as disease progressors or also possible initiators. With the identification of the cardinal and mutually exclusive „driver“ mutations that occur in *JAK2, CALR or MPL*, substantial progress has been made in the understanding of the pathogenetic basis of the conditions [10]. As a result of these driver mutations, dysfunctional proteins lead to pathologic and persistent activation of the JAK-STAT-pathway [11]. In approximately 10% of patients no driver mutation can be found (triple negative), which is associated with worse prognosis in MF patients [3].

Apart from driver mutations, alterations in genes, functioning as epigenetic regulators like *TET2, DNMT3A, EZH2 and ASXL1* can play a role in disease initiation and in the case of *TET2* and *DNMT3A* may even precede the acquisition of the most common driver mutation *JAK2V617F* [4]. Furthermore these high molecular risk (HMR) mutations are associated with an inferior overall survival and carry a higher likelihood of leukemic transformation [3].

1.2.1 JAK2-STAT Signalling Pathway

The originally named „just another kinase“ or JAKs form a group of intracellular non-receptor tyrosine kinases, and got renamed to „Janus Kinases“ after their importance in the pathogenesis of various diseases such as MPNs, inflammatory and autoimmune diseases became more evident. „Janus“, the name of the double faced Roman god of gates was picked to hint at the two kinase domains JH1 and JH2. Whereas JH1 is the active and catalytic phosphotransferase domain, JH2 suppresses ligand-independent kinase activity by reducing the affinity for adenosine triphosphate (ATP) and its competitive inhibitors [12], and has a more regulatory function. This pseudoligand domain (JH2) interacts directly with JH1 and is also required for ligand-induced kinase activity [13]. Although there are four JAK proteins (JAK 1-3 and TYK2) involved in the signalling path of various cytokine receptor families [14], JAK2 plays the most important role in the pathogenesis of *BCR-ABL* negative MPNs, as the JAK2-STAT signalling pathway is essential for hematopoiesis. The physiological activation of JAK2 via hematopoetic cytokines such as erythropoietin, thrombopoietin and granulocyte colony stimulating factor (G-CSF), activate various trascriptions factors, including STAT1, STAT 3 and STAT5 and also Phosphoinositide 3-kinase/protein kinase B pathway (PI3K/AKT) - and mitogen-activated protein kinase (MAPK) signalling pathways to promote proliferation, differentiation and survival of myeloid progenitor cells [15]. This happens through phosphorylation at specific tyrosine residues of a receptor´s cytoplasmic domain, which is mediated by JAKs after a cytokine binds to its corresponding receptor. These receptor domains serve as docking stations for STATs, which are then phosphorylated by JAKs. Activated STATs translocate to the nucleus, to allow the transcription of various genes, encoding molecules that are important for cell-differentiation, -proliferation and -apoptosis [14].

Although the JAK2-STAT pathway plays an important role in pathogenesis of MPNs, it has become increasingly evident that factors outside of JAK-STAT signalling pose an issue as well. PI3K and MAPK signalling has shown to be active in MPNs, but also in AML and CML. Furthermore these pathways seem to remain active, even after administration of the JAK inhibitor ruxolitinib [16].

1.2.2 *JAK2* - Mutations

In 2005 a single point mutation, which leads to *JAK2V617F* was identified in 95% of PV-, and 50-60% of ET- or MF-patients [15]. This genetic anomaly causes the loss of the inhibiting function in the JH2 domain, resulting in a constitutively active *JAK2V617F* protein and therefore an overactivation of the subsequent JAK2-STAT pathway. Additionally the mutation could also be responsible for direct activation of the JH1 domain via SH2-JH2 linker [10], as these linker domains regulate activation by reducing ATP affinity [12]. Since *JAK2* is intimately associated with several receptors, such as those for erythropoietin (EPOR), thrombopoietin (MPL) and G-CSF (G-CSFR) [10], *JAK2*-mutations have a wide variety of clinical appearance and correlate with all three classic *BCR-ABL* negative MPNs, as they are the most prevalent mutations in PV, ET and PMF [17]. In addition to the *JAK2V617F* mutation, four different and less common mutations in *exon 12* of the *JAK2* gene were identified in patients, who tested negative for a *JAK2V617F* mutation, in 2007 [18]. It appears that *exon 12* mutations are strongly associated with PV or an erythrocytosis phenotype, as they cannot be found in *JAK2617F*-negative ET and only in patients with MF and AML, secondary to PV [19].

1.2.3 *MPL* - Mutations

In addition to *JAK2* alterations, there are less common mutations, that occour in the *myeloproliferative leukemia virus oncogene* (*MPL)*, which encodes for the thrombopoietin receptor (TPOR). The gain-of-function mutations *MPL W515L/K*, as well as *W515R/A/G*, and less commonly *S505*, occur in approximately 5-8% of MF -, and 1-4% of ET-patients and lead to conformation changes in the TPO-receptor, which then becomes active in the absence of TPO [3, 4]. Due to the physiological function of MPL, these mutations result in increased megakaryocyte proliferation, are characterized by isolated thrombocytosis and are exclusively found in patients with ET and MF [10].

1.2.4 *CALR* - Mutations

A rather suprising discovery was made with the identification of *Calreticulin* (*CALR*) mutations in 2013, as this gene neither encodes for cytokine receptors, nor participates in the JAK2-STAT signalling pathway [10]. Normally the endoplasmic-reticulum (ER)-resident chaperone CALR has more of a „housekeeping“ function, as it helps proteins to fold correctly withing in the ER. In addition CALR supports Ca(2+)-dependent processes, like adhesion and integrin signalling, as well as MHC-I antigen presentation. Furthermore *CALR* can be exposed on the surface of cancer cells, which promotes the uptake by phagocytes and supports the immune response to these dysfyunctional cells [20]. Over 50 *CALR*-mutations have been described in the majority of *JAK2* negative -, and *MPL* negative patients with ET (50-60%) and PMF (75%). They were all located on exon 9 and induce a +1 (-1+2) frameshift, which ultimately leads to a newly acquired positive electrostatic charge within the C-terminus of the protein [4, 10]. Negative charges, which are recquired for Ca(2+) binding are therefore lost to a certain extent. Most of the *CALR* mutations were classified as type-1 or type-2, whereas type-1 results from a 52bp-deletion and type-2 from a 5bp-insertion, both in exon 9. In type-1 almost all Ca(2+) binding sites have been lost, while type-2 mutations are closer to the wild-type sequence, keeping about 50% of negative charges [4]. This might explain a recognizable distinction in the phenotype between patients and murine models with type-1 or -2 *CALR* mutants [16]. Just like *MPL*-mutations, those calreticulin alterations are exclusively associated with ET or MF, suggesting that these mutations somehow result in a constitutive activation of MPL. Although the exact mechanisms have not yet been fully elucidated, it seems that the extracellular domain of MPL and the aberrant positive charge on the C-terminal, along with the N-terminal of CALR are required for the activating complexation [10]. It is unclear at which cellular compartment of MPL the receptor is activated, but there is evidence showing, that mutant-CALR chaperones traffic to the cell surface, along with MPL, in which case the activation could happen anywhere from the ER to the cell surface [4]. Furthermore it is believed that loss-of-function mutations in *CALR* compromise homeostatic

mechanism within healthy cells and also impair natural- and therapy induced immunosurveillance and therefore fosters oncogenesis [20].

1.3 Essential Thrombocythemia

1.3.1 Clinical Features

Patients with ET show a platelet count of ≥ 450.000 per cubic millimeter and symptoms have a wide varity when it comes to severity [21]. It is possible that patients are asymptomatic but most commonly they experience headache, dizziness, visual changes, paresthesia, fatique and easy bruising [22]. When patients present with persistent thrombocytosis, myeloid cancer like ET should be considered as a cause, if they additionally show any of the following symptoms: vasomotoric symptoms (e.g. migraine, pruritus), constitutional symptoms like night sweats, fever, weight loss or fatique. Also splenomegaly (less commonly hepatomegaly), thrombosis at multiple -, or unusual sites (e.g. hepatic -, portal -, or splenic veins) are considered to be treacherous signs for ET. More severe, but fairly common manifestations include thromboembolic complications, with an estimated prevalence ranging from 16.6 to 25.5% [21-23]. Arterial events like strokes (10.7%) and acute coronary symptomes (6.1%), and venous complications like deep vein (3.4%), splanchnic (1.4%), pulmonary (0.9%), and cerebral (0.7%) thrombosis are the most common [23]. With an estimated prevalence of 7.3%, hemorrhagic complications in ET pose a challenge as well, whereas cutaneous or mucosal and/or gastrointestinal bleedings are included in most cases [23].

1.3.2 Diagnosis

Table 1-2 provides an overview of the diagnostic criteria for ET, based on the current 2022 WHO classification [24]. To diagnose ET all four major criteria, or the first three major criteria and one minor criterion are required [25]:

Major criteria	Minor criterion
1. Sustained platelet count ≥ 450 x 10^9L	1. Other clonal marker present;
2. Bone marrow shows mainly megakaryocyte proliferation with increased numbers of enlarged, mature cells and hyperlobulated nuclei; No significant left-shift of neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers;	2. No evidence of reactive thrombocytosis;
3. Not meeting WHO criteria for other myeloid neoplasms;	
4. *JAK2 / CALR / MPL* mutated	

Table 1-2: 2022 WHO diagnostic criteria for ET [25];

Although these criteria apply in most cases, the distinction between ET, PV and PMF, based on bone marrow morphology only might have some drawbacks, owning to their mimicry to transform into each other. An example for this issue would be the differentiation between ET and pre-PMF patients, as these diseases have different characteristics, when it comes to clinical behavior, besides their morphological features [1]. Thus, a bone marrow biopsy is necessary to enable an accurate diagnosis. But since driver mutations can be found in peripheral blood samples of most ET patients (only 10 to 20% are triple negative), the right diagnosis can be suspected even before a biopsy, if the clinical presentation is appropriate [21].

1.3.3 Risk Factors and Stratification

Several risk factors affect the overall survival of ET patients, including advanced age, leukocytosis and thrombosis history. Age has a very high relevance, as patients older than 70 years have an median survival of 8.1 years, whereas patients younger than 40 years have a median survival of 34.7 years [21, 26]. As mentioned, thrombotic events are frequent in patients with ET and substantially raise mortality. Therefore the International Prognostic Score for Thrombosis in Essential Thrombocythemia (IPSET) was developed in 2012, revised in 2015 and has been validated in multiple studies. Unlike the conventional model, which had only two categories (low - and high risk), the revised IPSET introduced four risk

types, including very low-, low-, intermediate- and high risk. To allow accurate risk stratification, the revised IPSET is built around three aspects, including age ≤/>60 years, history of thrombotic events and *JAK2V617F* mutation, as shown in table 1-3 [27].

Risk	Clinical Attributes	Molecular Variables
Very low	Age ≤60 years, no prior thrombosis	*JAK2* wild type
Low	Age ≤60 years, no prior thrombosis	*JAK2* positive
Intermediate	Age >60 years, no prior thrombosis	*JAK2* wild type
High	Age >60 years or prior thrombosis, regardless of other factors	*JAK2* positive

Table 1-3: 2015 revised IPSET for risk assessment in ET [27];

1.3.4 Therapy

Austrian Guidelines for ET Therapy

Unfortunately, controlled trials have shown that current drug options do not increase survival or prevent leukemic -/fibrotic transformation, which is why the primary goal of treatment is to prevent thrombohemorrhagic complications [26]. Austrian guidelines suggest a watch and wait approach when dealing with very low risk classified patients, in the absence of cardiovascular risk factors. Although the usage of platelent reducing treatment is recommended in all patients with ET, it is important to mention that low-dose acetylsalicylic acid (ASA) might even increase the risk of bleeding in patients with a low-risk disease. Particular caution is advised in patients with a platelet count of >1000 G/L, a history of bleeding, or a *CALR* mutation, as these parameters are associated with an acquired von Willebrand´s disease and a higher bleeding tendency. Patients with an intermediate risk disease should also be treated with low dose ASA, if their platelet count is below 1 million/ µL [28]. Although recommended for many patients, it is unsure if low dose ASA actually results in a sufficient 24 hour thromboxane A2 suppression, if given only once per day, due to accelerated platelet turnover in ET [21]. This is why a twice

per day application of ASA seems reasonable for patients with aspirin resistant symptoms, or *JAK2*-mutated patients with cardiovascular risk factors [26].
When it comes to cytoreductive treatment, patients with an intermediate risk, but a platelet count between 1-1.5 million/µL and all high risk patients qualify for substances like anagrelide, interferon or hydroxyurea (HU). According to Austrian guidelines, anagrelide with an initial dose of 0.5-1mg/d and a maximum dose of 5mg/d is the first line therapy for such patients [28]. The superiority of HU over anagrelide was long debated. In 2013 the ANAHYDRET study was able to evaluate the differences of the two substances [29]. Due to the more severe long term effects of HU, like potentially increased risk of secondary leukemia, higher hematotoxicity and mucocutaneous lesions, anagrelide was established as the first line therapy in Austria. If a patient shows intolerance or therapy resistance against anagrelide, interferone alpha can be used as back up medication and has been successfully administered as an off-label drug for ET, PV and PMF [28]. Although the rarity of large, prospective studies has hindered the approval for interferones as a labeled treatment option for ET, the PROUD-PV study found promising results regarding hematological and splenic responses in PV patients and superior results in contrast to HU in the long run, as responses improved over time [30].

1.3.5 Complications and Prognosis

An important step when it comes to prognostic estimation is the clear differentiation of ET and prefibrotic MF, as they share clinical-, pathological-, and molecular features but have differing clinical outcomes (10-year survival is 89% in ET and 76% in prefibrotic MF) [21]. This is due to an increased risk of leukemic transformation (0.7% and 5.8%) and a higher rate of fibrotic progression (0.8% and 12.3%) in prefibrotic MF. With a rate of 2-3% at 10 years and 5% of 15 years, ET rarely evolves into AML, when compared to other MPNs. Presumedly anemia, platelets count <1 million per µL, advanced age, leukocytosis, reduced bone marrow cellularity and also reticulin grading (if more than 0) are accompanied by an increased risk of leukemic transformation [31]. Although the risk of such a blast phase is relatively small in ET, the associated outcome is dismal. This complicates prognostic estimations, since present prognostic scores are incapable of predicting

the risk of AML accurately [32]. ET more frequently undergoes myelofibrotic transformation, than it develops into AML. The cumulative risk for post-ET myelofibrosis is estimated at 3.9% at 10 years and 9.3% at 15 years [33].

1.4 Polycythemia Vera

1.4.1 Clinical Features

Although the count of all cells of the myeloid lineage can be increased, PV is mostly characterized by exuberant red cell production. In many cases the disease is incidentally found, when blood tests display high hemoglobin and hematocrit levels [34]. Just like ET, PV often presents with mild and unspecific symptoms like fatigue, headache, dizziness, epigastric discomfort, weight loss, et cetera. Frequent physical findings include splenomegaly, hepatomegaly, plethora and hypertension, but PV also develops more pressing manifestations due to its tendency to thromboembolic events. Figures of the International Working Group of Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) database showed that PV patients already experienced arterial (16%)-, or venous (7.4%) thrombosis, before or at the time of diagnosis, whereby splanchnic - and portal vein - thrombosis are most commonly found [35]. Another characteristic feature of PV is aquagenic pruritus, which affects approximately 40% of PV patients and gets triggered when the skin gets in contact with water [36]. A study found in 2013 that the occurrence of pruritus in PV correlates with a lower incidence of arterial thrombosis, suggesting that the symptom might be a favorable risk factor for survival [37].

1.4.2 Diagnosis

With the 2016 revision of diagnostic criteria for MPNs by the WHO, the threshold for PV detection got lowered. A major reason for these changes was the introduction of a new entity called „masked PV“, as patients with this condition have worse survival rates, possibly due to delayed recognition of the disease and lower treatment intensity [38]. Sensitivity for PV got increased by lowering the

threshold for hemoglobin and hematocrit levels to 16.5 g/dL and 49% for men and 16 g/dL and 48% for women. Furthermore bone marrow biopsy has been added to the major criteria, to allow a better distinction between PV and ET and also to determine the grade of bone marrow fibrosis upon diagnosis [38]. In the new 2022 WHO classification the criteria have not been changed, except for the fact that increased red mass cell as determined by 51Cr-labeling of red cells has been removed as a diagnostic criterion, since it has become uncommon in routine clinical practice [39]. All 3 major -, or the first 2 major and the minor criterion are required to diagnose PV (table 1-4).

Major criteria	I. Hb >16.5 g/dL or Hct >49% (men), Hb >16.0 g/dL or Hct >48% (women); II. bone marrow biopsy shows hypercellularity for age with trilineage growth (panmyelosis), including prominent erythroid-, granulocytic- and megakaryocyc proliferation with pleomorphic, mature megakaryocytes (difference in sizes); III. *JAK2V617F* or *JAK2 Exon 12* mutation detected;
Minor criteria	Subnormal serum EPO level

Table 1-4: 2022 WHO PV diagnosis criteria [39];

1.4.3 Risk Stratification

Since thrombotic complications are the main factor for increased morbidity and mortality when it comes to PV, risk stratification is based on cardiovascular hazards. Currently patients are stratified into either low-risk, or high-risk groups, based on their age and previous thrombosis, as shown in table 1-5 [38].

Low risk	High risk
Age <60 years	Age >60 years
No prior thrombosis	and/or prior thrombosis

Table 1-5: Risk assessment for thrombosis in PV patients [38];

Although not formally included into the risk stratifications score, circumstances like hypertension, smoking habits and leukocytosis increase the risk of cardiovascular

events and should also be taken into consideration [38]. Furthermore the addition of an intermediate risk-category is being discussed, for younger patients with leukocytosis and/or cardiovascular risk factors, in the absence of previous thrombotic events [40].

1.4.4 Therapy

Just like in ET, low dose aspirin plays a major role in PV therapy to prevent thrombotic complications. The multicenter study ECLAP (European Collaboration on Low-Dose Aspirin in Polycythemia Vera) showed, that an ASA intake of 70-100 mg/d reduces the accumulated risk of non-fatal myocardial infarction, stroke, pulmonary embolism, major venous thrombosis and death from other cardiovascular causes by 60%. Patients with a higher risk of arterial thrombosis or who show resistancy against once per day intake might benefit from twice daily intake of ASA [34]. Aside from ASA, scheduled phlebotomy, with a targeted Hct of <45% is an important cornerstone for PV treatment, as its effectiveness against thrombotic complications is supported by various studies. Considering that the risk of cardiovascular death and major thrombosis is significantly lower with a Hct target of <45%, rather than 45-50%, careful management is advised [41, 42]. Both low dose ASA and phlebotomy find use in all PV patients, regardless of risk stratification, although high risk patients should receive additional cytoreductive drugs, like HU and ropeginterferon alfa-2b. Despite the fact that HU initially is a well tolerated and manageable drug, 11% of patients become resistant and 13% develop intolerance against the drug, over time [34]. The phase III clinical trial PROUD-PV study and its extension study CONTINUATION-PV found promising resultscompared HU to ropeginterferon alfa 2b in a randomized fashion. While PROUD-PV initially showed that ropeginterferon alfa-2b was inferior to HU in terms of haematological response and spleen size reduction at 12 months, as only 21% of ropeginterferon alfa-2b patients reached the primary endpoint of complete haematological resoponse and normal spleen size, versus 28% of HU treated patients.

Suprisingly, after 36 months, 53% of patients in the ropeginterferon alfa-2b group met the primary endpoint, versus only 38% of patients in the HU group, in the

CONTINUATION-PV trial [30]. These results lead to the regulatory approval of ropeginterferon alfa-2b for PV-patients in the European Union. Furthermore, 54.3% of patients receiving ropeginterferon alfa-2b reached a *JAK2 V617F* allele burden of <10% after 5 years of treatment [43]. Improved allele burden results in a decreasing risk for disease progression into secondary myelofibrosis, which is consistent with the results of a retrospective study that compared the MF free-survival rates of patients treated with interferon versus HU or phlebotomy, over a median follow-up of 10 years [44]. These findings corroborate the possible disease-modyfing ability of ropeginterferon alfa-2b and might even suggest the potential of treatment discontinuation in patients achieving a molecular response [43].

1.4.5 Complications and Prognosis

When it comes to overall survival in PV, age remains to be the most important predictor. In a study at the Mayo Clinic, patients with ages ≤40 years, ages 41-60 years and ages >60 years had corresponding median survivals of 37, 22 and 10 years [42]. In 2013 a study found that age-independent risk factors with adverse prognostic value include leukocytosis, venous thrombosis, leukoerythroblastic blood smear and abnormal karyotype. Furthermore, pruritus correlated with a better survival [37]. Similar to ET, transformations to AML or secondary MF pose complications with potentially fatal outcomes. In the mentioned international study, the risk for PV to transform into AML was 2.3% at 10 years, 5.5% at 15 years and 7.9% at 20 years. Factors fostering leukemic transformation include older age, abnormal karyotype, leukocytosis ≥ 15 x 10^9/L and the use of pipobroman and P32/chlorarmbucil, as well as *TP53* and *RUNX1* mutations [37]. Evolution to a blast phase has a dismal outcome and shortens the median survival to <6 months [33].

For post-PV MF, the range of reported transformations are 4.9-6% at 10 years and 6-14% at 15 years. Patients with higher age, leukocytosis, reticulin fibrosis, splenomegaly and *JAK2V617F* mutations are at a higher risk for post-PV MF. If myelofibrotic transformation occurs, the median survival in drastically shortened to 5.7 years [33].

1.5 Primary Myelofibrosis

1.5.1 Clinical Features

Primary myelofibrosis (PMF) is characterized by a hyperproliferation of myeloid cells, ultimately resulting in bone marrow fibrosis, osteosclerosis and also pathologic angiogenesis. Assumedly the stromal changes in the bone marrow are induced by aberrant cytokine production of clonal cells, leading to immune reactions, which trigger reactive disposition of stromal reticulin and collagen fibers [45, 46]. A higher degree of collagen fibrosis correlates with a more severe clinical course of PMF and a lower rate of treatment response [45]. Since approximately 25% of patients are asymptomatic when PMF is diagnosed, the disease is often an incidental finding, usually on the basis of suspicious blood tests or splenomegaly [47].

Just like PV and ET, PMF can manifest with constitutional symptoms like fatigue, night sweats and fever, as well as other symptoms like hepatosplenomegaly, bone pain, splenic infarcts, pruritus and thrombohemorrhargic complications. Anemia poses a major problem as PMF progresses, as more than 60% of patients suffer from hemoglobin concentrations of less than 10g/dL. The main causes for anemia in PMF are ineffective erythropoiesis and hepatosplenic extramedullary hematopoiesis (EMH), which also promote organomegaly, resulting in early blood cell sequestration. Furthermore ascites and variceal bleeding are fostered by hepatomegaly and portal hypertension [46, 47]. Other laboratory findings include leukocytosis or leukopenia, thrombocytosis or thrombocytopenia, as well as high lactate dehydrogenase (LDH) levels. Patients with PMF also show elevated levels of cytokines like TGF-β, VEGF, IL-1/2/6 [47].

The variability of driver - and non-driver mutations have an impact on the clinical phenotype of PMF, as certain mutation types can be associated with different symptom manifestations and disease progressions. Patients with older age, higher Hb levels, leukocytosis and lower platelet counts are more likely to show *JAK2V617F* mutations, in contrast to younger patients with higher platelet counts and anemia, who are more likely to have *CALR* mutations, and also show a lower incidence of leukocytosis [48].

1.5.2 Diagnosis

The diagnosis of PMF is based on the revised 2016 and recently published 2022 WHO-criteria, which distinquish between overt PMF and prefibrotic PMF, as shown in table 1-6. This distinction was first introduced with the revised 2016 WHO classification of myeloid neoplasms and is kept in the current 2022 version [49]. In both, overt - and prefibrotic PMF, meeting all 3 major criteria and at least one minor criterion is necessary. Additionally, the diagnosis of prePMF requires confirmation in two consecutive determinations [47].

Entity	Major Criteria	Minor Criteria
Overt PMF	I. Megakaryocyte proliferation and atypia accompani by either reticulin or collagen fibrosis grade 2 or 3, both; II. Not meeting WHO criteria for *BCR-ABL* positive MF CML, PV, ET MDS or other MPNs; III. Detection of driver mutations, or other clonal marke like *ASXL1*, *EZH2*, *TET2*, *IDH1/2, SRSF2, SF3B1*, absence of reactive bone marrow fibrosis;	I. Increased serum LDH lev II. Anemia (not associated with concominant disease III. Palpable splenomegaly IV. Leukoerythroblastosis V. Leukocytosis ≥11 x 10^9/l
Pre PMF	I. Megakaryocytic proliferation and atypia, without reticulin fibrosis < grade 1, accompanied by increas age-adjusted bone marrow cellularity, granulocytic proliferation and often decreased erythropoiesis; II. Not meeting WHO criteria for *BCR-ABL* positive MF CML, PV, ET MDS or other myeloid neoplasms; III. Detection of driver mutations, or other clonal marke like *ASXL1, EZH2, TET2, IDH1/2, SRSF2, SF3B1*, absence of minor reactive (grade 1) bone marrow fibrosis;	I. Increased serum LDH lev II. Anemia (not associated with concominant disease III. Palpable splenomegaly IV. Leukocytosis ≥11 x 10^9/l

Table 1-6: WHO criteria for overt -, and prefibrotic PMF [1];

To ensure a save diagnosis, careful examination of all parameters is advised, as other myeloid neoplasms like PV, ET and CML share features with PMF and are sometimes hard to differentiate. Even if post - PV or post - ET MF show substantial bone marrow fibrosis, the correct distinction between PMF and secondary MF is prognostically relevant [46]. A separate list of criteria for post-PV and post-ET MF is provided by the WHO, which requires meeting both major criteria and 2 out of 4 minor criteria, illustrated in table 1-7 [47].

Major Criteria	Minor Criteria
I. WHO conform, documented diagnosis of PV/ET; II. Bone marrow fibrosis grade 2-3 to European definition;	I. Palpable splenomegaly ≥5 cm or new occurence of palpable splenomegaly; II. New systemic symptoms like unexplained fever >37.5 ºC, weight loss >10% in 6 months and night sweats; III. Anemia or decreased Hb ≥2g/dL from baseline; IV. Only ET: increased serum LDH level

Table 1-7: WHO criteria for post-PV and post-ET MF [47];

1.5.3 Risk Stratification and Prognostic Models

IPSS

The first of contemporary prognostic model for PMF was developed in 2009 by the IWG-MRT and is called the International Prognostic Scoring System (IPSS).

It groups patients into low-, intermediate-1-, intermediate-2- and high risk, using five independent factors, associated with inferior survival. Table 1-8 provides the risk factors included in the IPSS, as well as median survival rates of each category [46].

Attributes and Points	Risk Groups and Median Survival
Age >65 years (1)	0 = low, 11.3 years
Hb <10g/dL (1)	1 = intermediate 1, 7.9 years
White blood cells (WBC) >25 x 10^9/L (1)	2 = intermediate 2, 4.0 years
Circulating blasts ≥1% (1)	≥3 high, 2.3 years
Constitutional symptoms (1)	

Table 1-8: IPSS with corresponding median survival rates [46];

DIPSS and DIPSS+

The IPSS only applies at the time of diagnosis, since all variables were acquired then. This downside led to the development of the Dynamic IPSS (DIPSS) and DIPSS+ (table 1-9), which can be used at any point in time over the course of the disease. The DIPSS utilizes the same risk variables as the IPSS, with the addition that an Hb of <10g/dL got assigned two adverse points, as anemia has a greater impact on survival, compared to other risk factors [50]. With the implementation of further unfavourable variables (the need for red cell transfusion, unfavorable karyotype and thrombocytopenia), that impair overall survival of PMF patients, the DIPSS+ was developed. Within the DIPSS+, the need for red cell transfusion, platelets <100x10^9/L, DIPSS intermediate-1 risk and an adverse karyotype (defined as complex karyotype or single or two aberrations including +8, -7/7q, I(17q), -5/5q, 12p-, inv(3) and 11q23 rearrangement) are assigned one adverse point respectively, whereas DIPSS intermediate-2 risk gets 2 -, and high risk gets 3 points [51].

Prognostic Score	Attributes	Risk Groups and Median Survival
DIPSS	• Age >65 years (1) • Hb <10g/dL (2) • WBC >25 x 10^9/L (1) • Circulating blasts ≥1% (1) • Constitutional symptoms (1)	0 = low, not reached 1-2 = intermediate 1, 14.2 years 3-4 = intermediate 2, 4.0 years 5-6 = high, 1.5 years
DIPSS+	- DIPSS low (0) - DIPSS intermediate 1 (1) - DIPSS intermediate 2 (2) - DIPSS high (3) - Unfavorable karyotype (1) - Red cell transfusion need (1) - Platelets <100 x 10^9/L (1)	0 = low, 15.4 years 1 = intermediate 1, 6.5 years 2-3 = intermediate 2, 2.9 years 4-6 = high, 1.3 years

Table 1-9: DIPSS and DIPSS+ with corresponding median survival rates [51];

MIPSS70 and MIPSS70+

Over 80% of PMF patients carry non-driver mutations, of which some show evidence to impair overall survival and leukemia free survival. These HMR aberrations include gene mutations in *ASXL1, SRSF2, EZH2* and *IDH1/2*. Recognition of the negative prognostic impact of HMR mutations and the absence of type-1-like *CALR* mutations, led to the development of the Mutation-Enhanced IPSS and MIPSS+ (table 1-10). One of the main goals of these scores was to provide a better decision tool for selection of patients for allogeneic hematopoietic stem cell transplantation (allo-HSCT), as this curative treatment option carries a high rate of therapy-related complications and mortality, but can cure MF. The MIPSS70 and MIPSS70+ only apply to patients younger than 70 years, due to the age restriction regarding transplantation. With the inclusion of newer genetic findings and sex-adjusted Hb levels, the MIPSS70+ was updated to the MIPSS70+ v2.0, which can be used to predict outcomes of allo-HSCT (table 1-10) [52, 53].

Prognostic Score	Attributes	Risk Groups and Median Survival
MIPSS70+	• Hb <10g/dL (1) • Circulating blasts ≥2% (1) • Constitutional symptoms (1) • 1 HMR mutation (1) • ≥2 HMR mutations (2) • Type-1-/ type-1-like *CALR* mutation absent (2) • Unfavorable karyotype (3)	0-2 = low, 20 years 3 = intermediate, 6.3 years 4-6 = high, 3.9 years ≥7 = very high, 1.7 years
MIPSS70+ v2.0	• Very high risk (VHR) karyotype (4) • Unfavorable karyotype (3) • ≥2 HMR mutations (3) • 1 HMR mutation (2) • Type-1- / type-1-like *CALR* mutation absent (2) • Severe anemia (Hb <8g/dL in men, <9 g/dL in women) (2) • Moderate anemia (Hb <9-10.9g/dL in men, <8-9.9g/dL in women) (1) • Circulating blasts ≥2% (1) • Constitutional symptoms (2)	0 = very low, not reached 1-2 = low, 16.4 years 3-4 = intermediate, 7.7 years 5-8 = high, 4.1 years ≥9 = very high, 1.8 years

Table 1-10: MIPSS70+ and MIPSS70+ v2.0 with corresoponding median survival rates [52, 53];

GIPSS

The Genetically Inspired Prognostic Scoring System for PMF (GIPSS) represents an approach to categorize the prognosis of PMF patients purely on their genetic changes, based on the hypothesis that clinical factors are surrogates for known or unknown genetic aberrations. It was created as a facile tool, to complement the more complex MIPSS70+ and classifies patients into low -, intermediate-1 and 2 - and high risk groups (table 1-11) [54].

Attributes	**Risk Groups and Median Survival**
- VHR karyotype (2) - Unfavorable karyotype (1) - Type-1-/ type-1-like *CALR* mutation absent (1) - *ASXL1* (1) - *SRSF2* (1) - *U2AF1Q157* (1)	0 = low, 26.4 years 1 = intermediate 1, 8.0 years 2 = intermediate 2, 4.2 years ≥3 = high, 2.0 years

Table 1-11: GIPSS with corresponding median survival rates [54];

Post-ET and Post-PV MF

Given the importance of distinguishing PMF and MF as a result of PV or ET, a separate prognostic score, called Myelofibrosis Secondary to PV and ET-Prognostic Model (MYSEC-PM) was designed. A study confirmed the superior accuracy of MYSEC-PM, in comparison to the DIPSS, when it comes to survival prediction of post-PV/ET MF patients. However, it also points out performance issues, especially when used on patients undergoing allo-HSCT [55].

Myelofibrosis Transplant Scoring System (MTSS)

To get a better understanding of the prognosis after allo-HSCT, the MTSS was developed and first published in 2019 [56]. In the related study 360 patients with PMF or post PV/ET myelofibrosis, who underwent allo-HSCT were evaluated to determine factors increasing the risk of nonrelapse mortality. The study concluded in a four-tiered scoring system, predicting the outcome of patients undergoing allo-HSCT (table 1-12) [50, 56].

Attributes	Risk Groups and 5-Year Survival
- Age ≥ 57 years (1) - WBC > 25 x 10^9/L (1) - Platelets < 150 x 10^9/L (1) - *ASXL1* mutation (1) - Karnofsky Performance Status <90% (1) - HLA-mismatched unrelated donor (2) - Non *CALR/MPL* mutated disease	- 0-2 = low risk (90%) - 3-4 = intermediate risk (77%) - 5 = high risk (50%) - 6-9 = very high risk (34%)

Table 1-12: MTSS with corresponding 5-year survival rates in parentheses [50];

Current Practical Use of Prognostic Models

At the time of writing, the IPSS, DIPSS and DIPSS+ are most commonly used, when it comes to PMF risk stratification. Although, in 2022 the National Comprehensive Cancer Network (NCCN) implemented the MIPSS70+ and MIPSS70+ v2.0 as a recommendation for risk stratification concerning PMF patients. If molecular testing is not available, DIPSS and DIPSS+ present adequate options for timing and selection of treatment. With regard to the GIPSS, the NCCN recognizes the scoring system and its further validation, but has not yet included the score into current guidelines. Patients with post-PV/ET MF should be stratified with the MYSEC-PM, as stated by the NCCN guidelines [3].

Emerging prognostic models, like the MPN Personalized Risk Calculator and the Myelofibrosis Transplant Scoring System (MTSS) have yet to be further validated, as they are relatively new [50].

1.5.4 Therapy

Allogeneic Stem Cell Transplantation

When it comes to curative treatment, allo-HSCT represents the only option for patients with MF. As mentioned previously, transplantation entails a high rate of therapy-related morbidity and mortality, occuring in at least 50% of patients with MF. Therefore, careful selection of patients for allo-HSCT is of utmost importance. For example, asymptomatic patients with MIPSS70+ v2.0 „low“ or „very low“ risk disease have worse survival rates if treated with transplantation, when compared to conventional therapy options. Consequently allo-HSCT is only recommended for patients with MIPSS70+ v2.0 „high“ or „very high“ -, or IPSS/DIPSS „intermediate

2“ or „high“ risk disease and a good performance status. [46, 47]. Another important factor regarding transplantation is age. Studies have shown, that PMF patients older than 55 years have poorer survival, and patients with age >60 years, appear to gain almost no survival benefit for allo-HSCT at all. Given that the median age of MF patients is >60 years, most are not considered as transplantation candidates [57]. If patients do not qualify for allo-HSCT, they might be eligible for participating in clinical trials, testing novel drugs [58].

Therapy for Very Low -, Low - and Intermediate Risk Patients

Based on the MIPSS70+ v2.0 score, very low-, and low-risk patients should only be observed, if they have no treatment requiring symptoms. The same applies to patients with a low risk GIPSS -, MIPSS70 - and IPSS/DIPSS score. MIPSS70+ v2.0 intermediate risk patients require treatment and should preferably be considered for clinical trials. If they do not qualify to participate in such, conventional therapy, based on individual symptoms is recommended [47, 58].

Therapy with JAK - Inhibitors

When it comes to targeted therapies for PMF, JAK inhibitors, notably ruxolitinib play an important role. The mentioned drug is an oral JAK1 and JAK2 inibitor, which leads to a downregulation of constitutional JAK-STAT activation, within the scope of PMF, but also post-PV or post-ET myelofibrosis [47]. Even though ruxolitinib inhibits JAK1 and JAK2, it is not exclusively indicated for patients carrying a *JAK2* mutation and also shows symptom improvements in PMF patients with a *JAK2*-wild-type disease, since *CALR* and *MPL* mutations also result in an upregulation of the JAK-STAT pathway [47]. In addition, ruxolitinib intake results in a plasma level reduction of proinflammatory cytokines like IL-6 and TNF-alpha and inflammation markers like C-reactive protein [59]. In November 2011, ruxolitinib was approved by the FDA for MF, on basis of the results of two phase 3 trials, the COMFORT-I and COMFORT-II studies. In these clinical trials, the primary endpoint of ≥35% reduction of spleen volume was reached by 41.9% of patients after 24 weeks in the COMFORT-I -, and by 53.4% of patients after 48 weeks in the COMFORT-II study, compared to 0.7% and 0% of patients in the placebo - and best available treatment group [59, 60]. Furthermore, a 5-year analysis reinforced

the efficacy of ruxolitinib, as 88% of patients maintained improvements in spleen volume [61]. Additionally, stabilization of bone marrow fibrosis was observed in 48% of patients and long-term data suggested, that an extended intake of ruxolitinib can result in a *JAK2V617F* allele burden reduction [47]. Even though ruxolitinib plays a major role in symptom relief and spleen size reduction, JAK-inhibition therapy has not shown to induce remission, reverse bone marrow fibrosis or prolong overall survival significantly [62]. It is strongly recommended that patients classified as IPSS intermediate-2 risk or high risk should be treated with ruxolitinib. Patients suffering from symptomatic or severe splenomegaly (≥15cm below costal margin), but classify as IPSS intermediate-1 should also be considered for treatment, according to evidence based criterea by the European LeukemiaNet (ELN) and the Italian Association for Hematology (SIE) [63]. Furthermore, the ELN-SIE association issued additional recommendations for the usage of ruxolitinib. These were based on the disease-specific MPN-score and in regards to symptoms like refractory severe pruritus, unintentional weight loss, unexplained fever and elevated risk for thromboembolic events, regardless of the DIPSS score [63].

Alternatives to Ruxolitinib

Patients carrying non-driver mutations like *ASXL1, EZH2 or RAS* are predisposed for a poor response when it comes to ruxolitinib [64]. Even though around 50% of MF-patients respond well initially, after 3 years about half of the patients have to stop ruxolitinib intake, due to lack of response, cytopenia or disease progression [64]. If patients do not experience symptom improvement or satisfatory spleen size reduction after titration of dosage within 6 months, treatment alternatives are needed [47]. In this case, other JAK inhibitors like fedratinib, momelotinib and pacritinib pose valid options and have similiar results as ruxolitinib, regarding splenomegaly. However, fedratinib has a higher non-haematological toxicity, mainly in the gastrointestinal tract. Pacritinib and momelotinib also have increased risk of gastrointestinal side effects, but seem to reduce the need for red blood cell tranfusions in anaemic patients [64]. Momelotinib has recently been approved by the European Medicines Agency and is available for treatment of MF patients in Austria.

Conventional Therapy Based on Symptoms

Anemia:

Recommendations for PMF associated anemia therapy include androgens (testosterone enanthate, fluoxymesterone), prednisone, danazol and thalidomide ± prednisone or lenalidomide ± prednisone. For patients with serum erythropoietin less than 500 mU/mL, therapy with erythropoietin stimulating agents (ESAs), like epoetin alfa and darbepoetin alfa is appropriate. Nevertheless, it is advised to refrain from the use of ESAs in transfusion-dependent patients, as they bring no clinical benefit and could exacerbate splenomegaly. Recent data suggests that the JAK inhibitors momelotinib and pacritinib induce erythropoietic activity.

Momelotinib´s ability to improve anaemia in MF patients has also been documented in phase 2 - and phase 3 studies [62]. In any case, the therapy choice should be based on overall toxicity profile and expected impact of side effects on the individual patient [3, 40, 46].

Splenomegaly:

If conventional treatment fails to reduce spleen size, splenectomy and splenic irradiation pose alternatives to medication. A report has shown, that over 75% of 314 patients who underwent splenectomy experienced benefits like transfusion-independence and resolution of severe thrombocytopenia, with an impact that lasted over the median of one year [62]. Although splenic irradiation is also an option for JAK inhibitor refractory patients, its need is becoming less certain and it can induce severe and protracted pancytopenia [62].

Others:

Patients with MF-associated non-hepatosplenic EMH, pulmonary hypertension and extremity pain benefit from involved-field radiation treatment. Regarding portal hypertension, transjugular intrahepatic portosystemic shunt (TIPS) poses a treatment option. Although its therapeutic value is yet to be determinated by systematic studies, case reports verify the efficacy of TIPS in the context of MF [46].

Next Generation Therapeutics

Since current conventional treatments only reduce symptom burden but do not significantly impact disease progression in the majority of patients, new therapy options are currently being explored in randomized phase III-studies. Investigated agents target mechanisms like apoptosis, epigenetic modulation, bone marrow microenvironment and signal transduction pathways [65].

Recent trials like the MANIFEST -, TRANSFORM -, REFINE- and other studies have found promising - and potentially disease-modifying results in the combination of ruxolitinib and BCL2/BCL-xL or BET inhibitors. Therefore, expectations are high for significant improvements of MF-therapy in the future [64].

1.6 Prognostic Impact of Driver - and Non-Driver Mutations

The mutational status of MPNs remains to be one of the most important prognostic factors. However, the impact of driver - and non-driver mutations on overall survival of MPN patients depends strongly on the given entity.

ET

Driver mutations in ET patients carry prognostic relevance regarding risk of thrombosis, which is higher in *JAK2* - and *MPL* mutated patients. As for myelofibrotic progression, *MPL* mutated patients have a decreased chance for MF free survival. In *JAK2V617F* mutated cases, there is a lower risk for post ET MF. However, driver mutation status has no impact on overall -, or leukemia free survival [17]. A targeted deep sequencing study found various non-driver mutations (*SH2B3, SF3B1, U2AF1, TP53, IDH2, EZH2*) in 53% of inspected patients with ET. In this particular study, patients with such adverse mutations had a median survival of 9 years, whereas those without non-driver mutations reached 22 years [66].

PV

When it comes to PV, abnormal karyotypes have an adverse effect on overall-, leukemia free-, and MF free survival, but not on thrombosis free survival, as a study by the Mayo Clinic has confirmed [67]. Almost all PV patients (97%) carry a *JAK2V617F* mutation, whereas *CALR*- and *MPL* alterations are not found. *JAK2* mutations in other exons, including exon 12, occur in the remaining 3% of PV cases, although clinical outcomes regarding *JAK2V617F* -, and *JAK2 exon 12* - mutations have not shown significant differences. Apart from *JAK2*, a non-driver mutation prevalence of 57%, involving 27 genes was identified in the previously mentioned deep sequencing study [66]. *ASXL1, SRSF2* and *IDH2* were identified as adverse mutations and occured with a prevalence of 15%. Patients with such adverse mutations had an inferior median survival (7.7 years) as compared to patients without (16.9 years) [66].

PMF

Regarding driver mutations in MF, patients carrying type-1-/type-1-like *CALR* mutations show superior survival rates, whereas „triple-negative“ patients have the worst outcomes. Furthermore, various combinations of driver - and non-driver mutations display different prognostic profiles. For example, patients with *ASXL1* mutated and *CALR* wild type karyotype have a significantly worse prognosis, than patients with *CALR* mutation, in combination with *ASXL1* wild type. Due to the unfavorable prognostic effect of mutations in *ASXL1, EZH2, SRSF2* and *IDH1/2*, these DNA alterations are classified as HMR mutations and are reported in 27.0% of pre-PMF -, and 44.4% of overt PMF patients, respectively [48].

2 Patients and Methods

2.1 Goals and Methods

The aim of this study was to analyse the impact and possible correlations of genetic - and clinical parameters on overall - and event free survival in this unselected cohort of PMF patients diagnosed at the Medical University of Graz. Due to the large pool of MPN patients available and the necessity to collect a number of parameters for each patient, this study focused on PMF patients exclusively. To determine the prognostic impact of the mutation status in this cohort it was important to identify both driver - and non-driver mutations.

The primary endpoint for overall survival analysis was death of any cause. Regarding event free survival, thromboembolism, AML transformation and death of any cause was considered as an adverse event. Furthermore multivariant analyses with a focus on patients carrying non-driver - and HMR mutations were conducted to determine independend risk factors, impacting the outcome.

The collected statistical data were then compared to internationally published data to potentially find differences, especially regarding patients whose non-driver - and HMR mutation status was known at the time of conducting this study.

This study was approved by the institutional review board of the Medical University of Graz (EK-Votum 30-145 ex 17/18).

The following AI tool was used for the sole purpose of linguistic optimization:

- Chat GPT (Version GPT-4o)
- OpenAI, L.L.C.
- Date 27 February 2023 - 27 March 2025
- https://www.chatgpt.com

All academic content was developed independently.

2.2 Patients

This retrospective study initially included n=249 patients, who were diagnosed with primary myelofibrosis between 12th of March 2015 and 11th of August 2022 by bone marrow biopsy at the Diagnostic and Research Institute of Pathology at the

Medical University of Graz. Data were extracted from the electronic database MEDOCS until the 7th of June 2024.

For each patient the following parameters were collected:

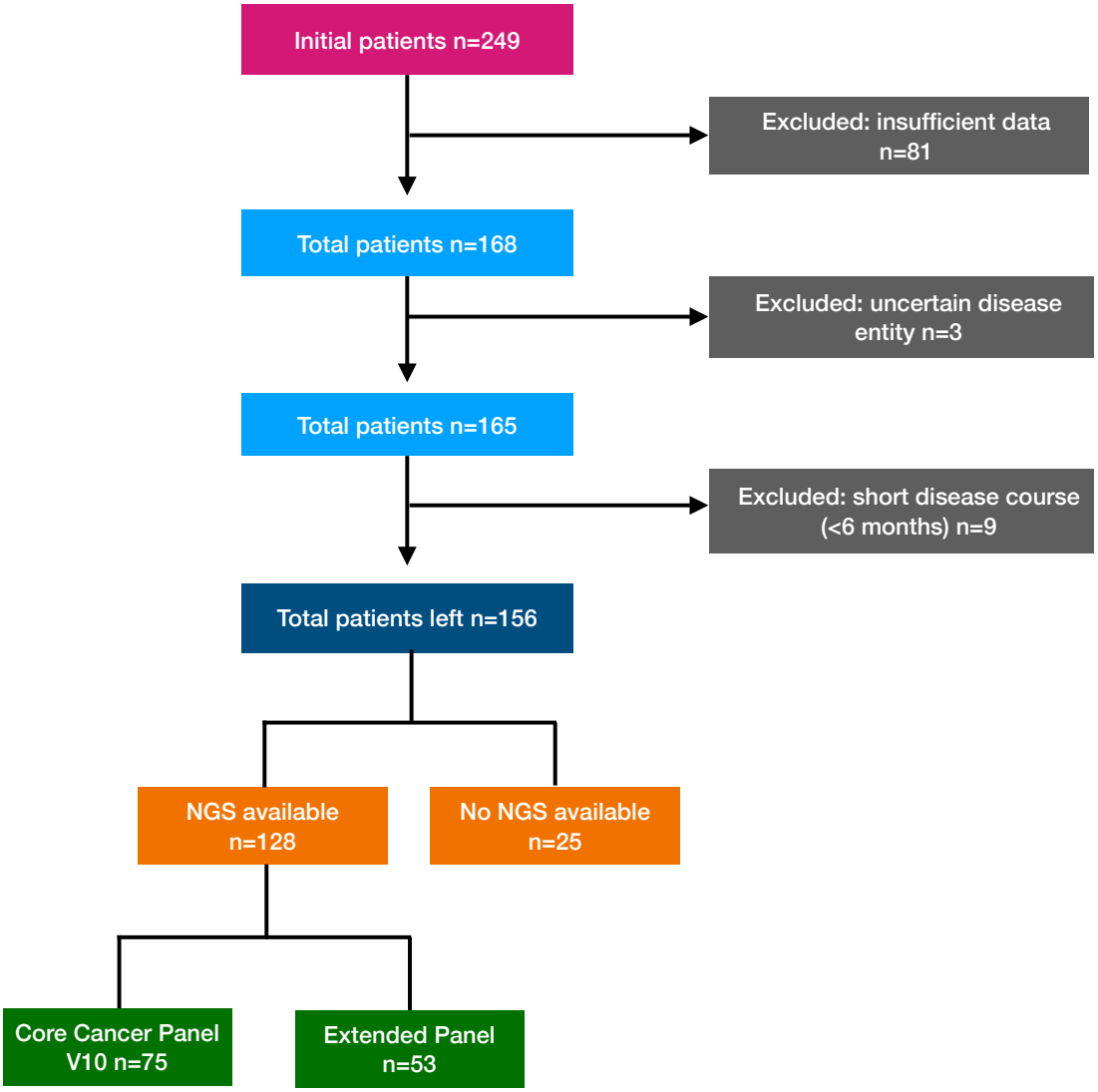
- Age/date of birth
- Gender
- Date of diagnosis
- Date of death
- Dates of adverse events (thromboembolism, AML transformation, death)
- Presence of constitutional symptoms
- Laboratory parameters at the time of diagnosis and at the time of last follow up (white blood cell counts, hemoglobin levels and peripheral blasts counts)
- Grading of bone marrow fibrosis
- Next generation sequencing (NGS) panel used, as well as driver and non-driver mutation status
- Therapeutic modalities

For each patient the DIPSS score was calculated at the time of diagnosis, as well as at the time of last follow up or death. Location of treatment varied between Landeskrankenhaus (LKH) Graz, Krankenhaus der Barmherzigen Brüder Graz, LKH Oststeiermark Standort Fürstenfeld, LKH Hochsteiermark Standort Leoben and LKH Murtal.

2.3 Exclusion Criteria

In order to execute statistical analysis with prognostic scores such as the IPSS and DIPSS, the availability of certain clinical parameters was necessary. Therefore, 81 patients for whom clinical data such as white blood cell count, hemoglobin level, presence of constitutional symptoms, and the presence of peripheral blasts was missing, were excluded from the study. Furthermore, 3 patients were excluded due to uncertainty regarding the presence of secondary myelofibrosis, resulting from ET or PV, instead of PMF. Nine patients were

excluded because of short documentation courses with less than 6 months, due to sudden death or loss to follow up. This left n=156 patients from the original 249 patients diagnosed with PMF. Flowchart 2-1 shows a visualisation of the exclusion process.



Flowchart 2-1: exclusion process
Eighty-one patients were excluded due to unavailability of data such as WBC, Hb levels or presence of consitutional symptoms.
Due to uncertainty of entity, 3 patients were excluded.
Nine patients were excluded because of short observation periods (<6 months).

2.4 Molecular Analysis

NGS was performed in most patients (n=128) to evaluate mutation status, using either Core Cancer panel V10 or Ion Torrent Ampliseq panel for myeloid neoplasia (MNP2019v2). Although both panels are able to identify driver mutations in the genes *JAK2, MPL* and *CALR*, the Core Cancer V10 panel has its limitations when it comes to finding non-driver- or high-molecular-risk mutations.
The lists below provide an overview of all genetic regions analysed in each NGS panel:

Core Cancer Panel V10:
KRAS (G12, G13, A59-Q61, K117, A146),
NRAS (G12, G13, A59-Q61, K117, A146),
HRAS (G12, G13, A59-Q61, K117, A146),
BRAF (Exon 11 und D594-K601),
KIT (Exons 8,9,11,13,17,18,20),
PDGFRA (Exons 12, 14, 18),
JAK2 (*V617* in exon 14 and all of exon 12),
MYD88 (L265), *MPL* (W515), *CALR* (exon 9), *CSF3R* (T618)

MNP2019v2:
This panel analyses the complete coding regions of the genes
ANKRD26, BCOR, CEBPA, DDX41, DNMT3a, ELANE, ETV6, GATA2, HAX1, NF1, PHF6, SF3B2, SFRP1, SRP72, STAG2, TP53, ZRSR2 as well as mutational hotspots in the genes *NPM1, ASXL1, BRAF, CALR, CBL, CSF3R, CXCR4, ETNK1, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NRAS, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, STAT3, STAT5B, TET2, U2AF1 and WT1*.

NGS was performed by the the Diagnostics and Research Institute of Pathology and Human Genetics at the Medical University of Graz.

2.5 Statistical Analysis

All data were collected anonymously and processed in IBM SPSS ® Statistics version 29. Descriptive statistical analysis was conducted for the entire cohort and specific subgroups. Overall survival rates, as well as event free survival rates were visualized using Kaplan-Meier survival curves. Statistical significance was assessed using log-rank tests and pairwise comparisons. Additionally, independent risk factors for poorer overall survival were identified through Cox regression analysis.

3 Results

3.1 Cohort Demographics and Disease Specific Characteristics

A total of 156 patients with PMF were included in the analysis. Among these, 37 patients had overt-PMF with a fibrosis grade of 2-3. One hundred and nineteen were pre-fibrotic with a fibrosis grade of 0-1. Overall, more men than women were affected, with 91 male and 65 female patients. The age at disease onset ranged from 23 to 85 years, with a median age of 65 years. At the time of diagnosis, 36,5% of the patients were over 70 years old. The most common mutation was found in the *JAK2* gene, present in 59,6% of the total cohort, followed by *CALR* mutations in 25,6% and *MPL* mutations in 4,5% of patients. In seven patients, no driver mutation was detected in the genetic analysis, while the mutation status was not evaluated in two individuals. In two patients, both a *CALR* and an *MPL* mutation were detected simultaneously through NGS, with the *CALR* mutation showing a significantly higher allele burden. Results from NGS done with the MNP2019v2 panel were available for 34% of the patients, allowing identification of the full spectrum of additional non-driver mutations. A core panel aimed at identifying driver mutations was performed in 48,1% of patients. In 17,9% of the cases, no NGS was conducted. At the last follow-up, 82% of the total cohort had survived the disease, while 18% had passed away.

Table 3-1 shows the distribution of demographic and disease-specific characteristics in the patient cohort. The corresponding percentages of each number can be found in parentheses. In the row concerning DIPSS, a quantitative overview of the scoring distribution at the time of diagnosis and at the last follow-up is shown.

		TOTAL (%)	OVERT PMF, Grade 2-3 (%)	PRE-FIBROTIC PMF, Grade 0-1 (%)
		156	37	119
GENDER	**female**	65 (41,7)	15 (40,5)	50 (42,0)
	male	91 (58,3)	22 (59,5)	69 (58,0)
AGE AT DIAGNOSIS	**median**	65	69	65
	minimum	23	28	23
	maximum	85	85	85
	< 70 years old	99 (63,5)	19 (51,4)	80 (67,2)
	≥ 70 years old	57 (36,5)	18 (48,6)	39 (32,8)
DRIVER MUTATION STATUS	***JAK2***	93 (59,6)	25 (67,6)	68 (57,1)
	CALR	40 (25,6)	6 (16,2)	34 (28,6)
	MPL	12 (7,7)	3 (8,1)	9 (7,6)
	Triple neg.	7 (4,5)	2 (5,4)	5 (4,2)
	Unknown	2 (1,3)	0 (0)	2 (1,7)
	CALR + MPL	2 (1,3)	1 (2,7)	1 (0,8)
DIPSS at diagnosis; at last follow up	**low risk**	66 ; 42 (42,3 ; 26,9)	5 ; 5 (13,5 ; 13,5)	61 ; 37 (51,3 ; 31,1)
	int 1	75 ; 71 (48,1 ; 45,5)	20 ; 8 (54,1 ; 21,6)	55 ; 63 (46,2 ; 52,9)
	int 2	12 ; 32 (7,7 ; 20,5)	9 ; 15 (24,3 ; 40,5)	3 ; 17 (2,5 ; 14,3)
	high risk	3 ; 11 (1,9 ; 7,1)	3 ; 9 (8,1 ; 24,3)	0 ; 2 (0 ; 1,7)
NGS PANEL	**core panel**	75 (48,1)	10 (27)	65 (54,6)
	extended panel	53 (34)	21 (56,8)	32 (26,9)
	no NGS	28 (17,9)	6 (16,2)	22 (18,5)
PATIENT STATUS	**alive**	128 (82)	24 (64,8)	104 (87,4)
	died	28 (18)	13 (35,2)	15 (12,6)

Table 3-1: Distribution of demographic and disease-specific characteristics

Figure 3-1 provides an overview of the number of patients in each DIPSS category at the time of diagnosis versus at the time of last follow up.

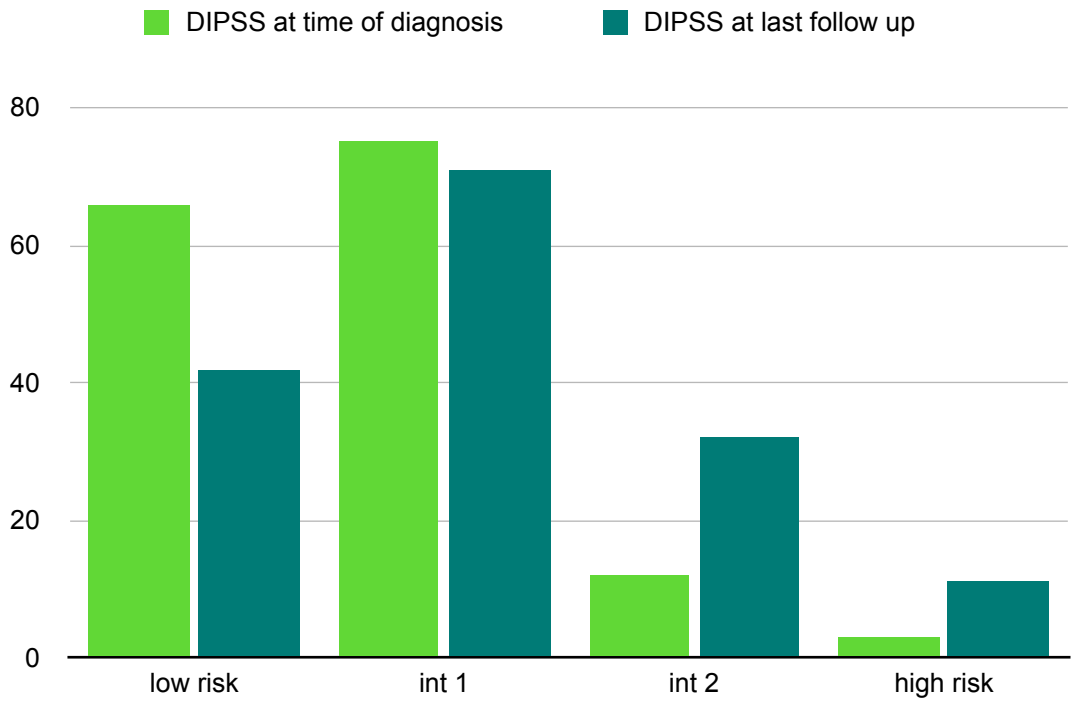


Figure 3-1: bar chart of DIPSS distribution during the time of diagnosis and last follow up;

3.2 Survival

3.2.1 Overall -, Event Free - and Progression Free Survival

Overall Survival

As shown in figure 3-2, the 5-year survival rate of the overall cohort was 80,7%, and the 8-year survival rate was 63,3%. Due to the short period of follow-up data, it was impossible to determine a 10 year survival rate.

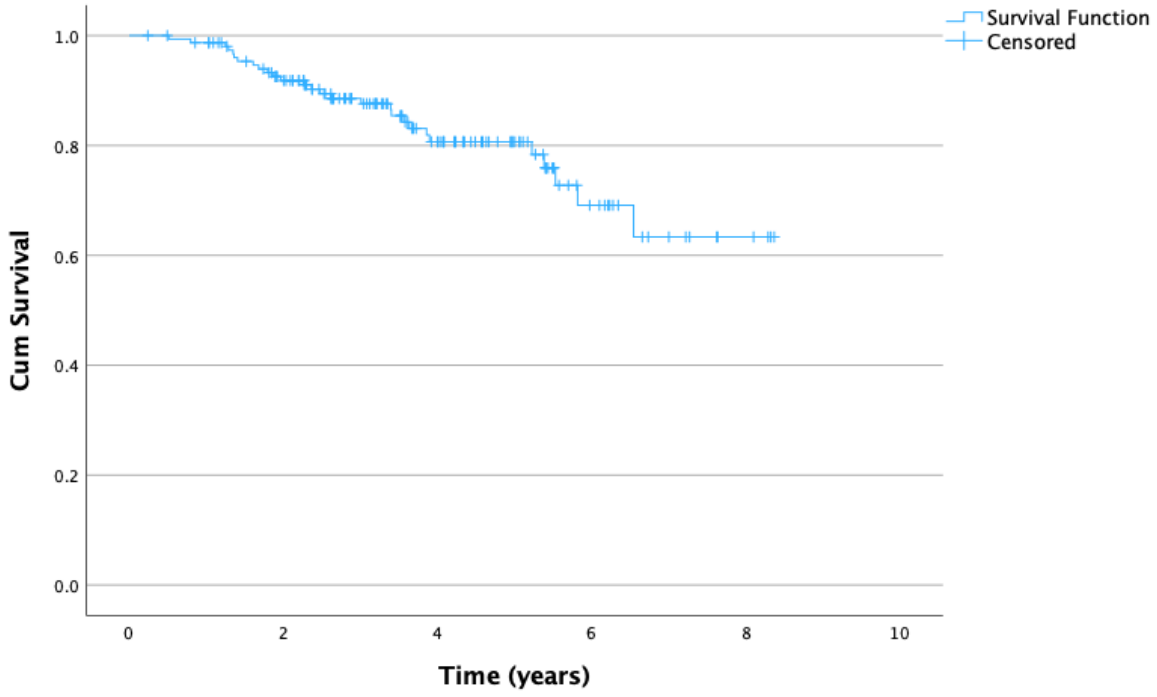


Figure 3-2: Kaplan Meier curve of overall survival of the cohort;
5 year survival was 80,7% and 8 year survival 63,3%;

Event Free Survival

In this study an adverse event was defined as a thromboembolic event, AML transformation, or death. Regarding event-free survival, figure 3-3 shows that after 5 years, 71,2% of the overall cohort had not experienced an adverse event, while 38,8% had. After 8 years, 52,8% had experienced such an event.

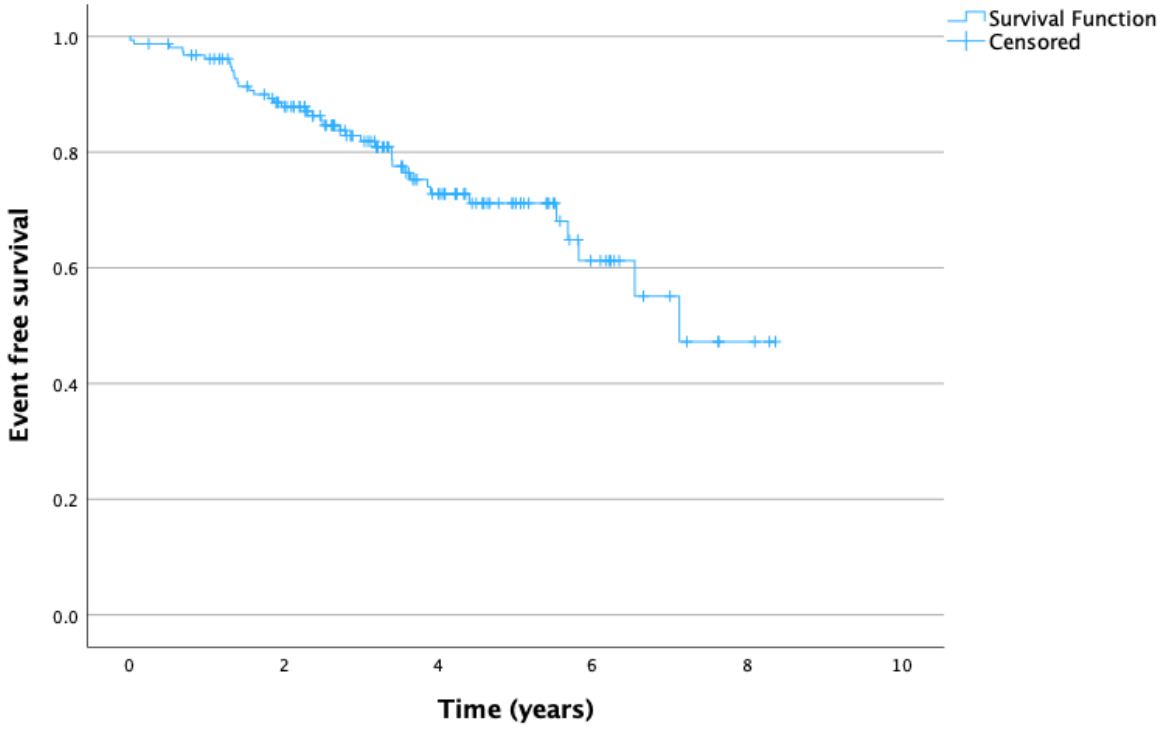


Figure 3-3 Kaplan Meier curve of event free survival of the cohort; 38,8% of patients experienced an adverse event (thrombembolic event, AML transformation or death) after 5 years and 52,8% after 8 years;

Progression Free Survival

Progression free survival was fairly similar to the overall survival of the cohort. After 5 years 78,3% of the patients showed no progression and 60,6% after 8 years, while progression was defined as AML transformation or death (figure 3-4).

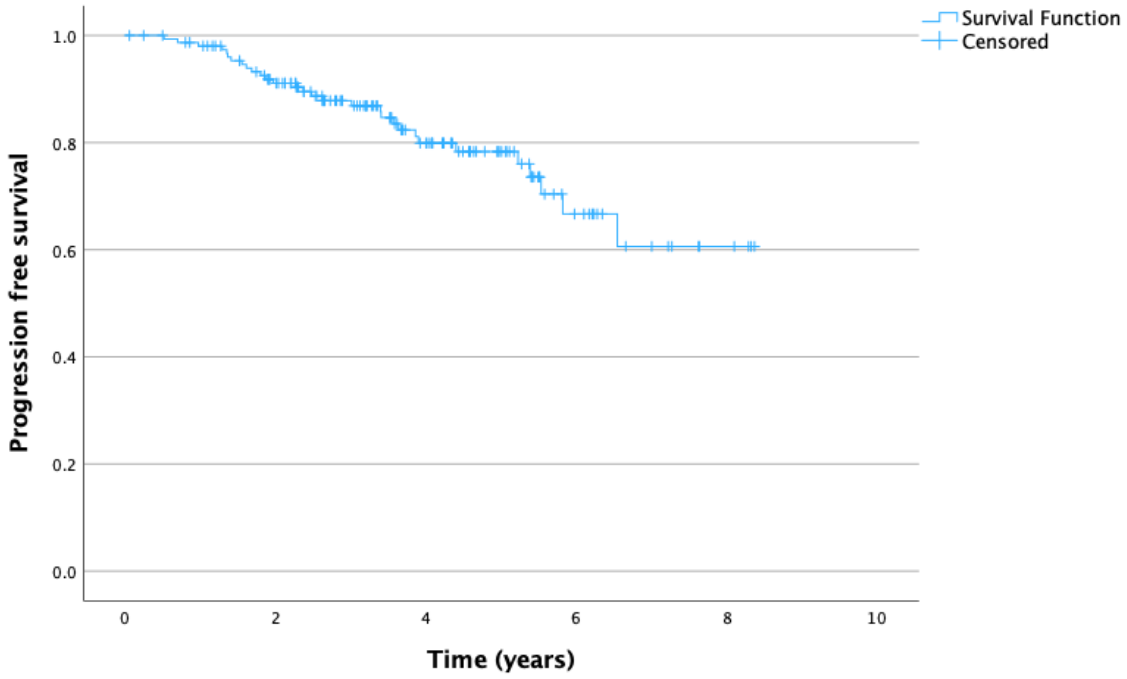


Figure 3-4: Kaplan Meier curve of progression free survival; 21,7% and 39,4% of patients died or suffered from AML transformation after 5 - and 8 years;

3.2.2 Overt-PMF Versus Pre-PMF

Patients diagnosed with prefibrotic PMF, or those with a fibrosis grade of 0 or 1, showed a 5-year survival rate of 84,9% and an 8-year survival rate of 72,6%. In cases of overt PMF, with a fibrosis grade of 2 or 3, patients showed a 5-year survival rate of 68,5% and a 7-year survival rate of 47%, which was the point of last censoring in this group. A p-value of p = 0,017 indicated that there was a statistically significant difference between the two groups (figure 3-5).

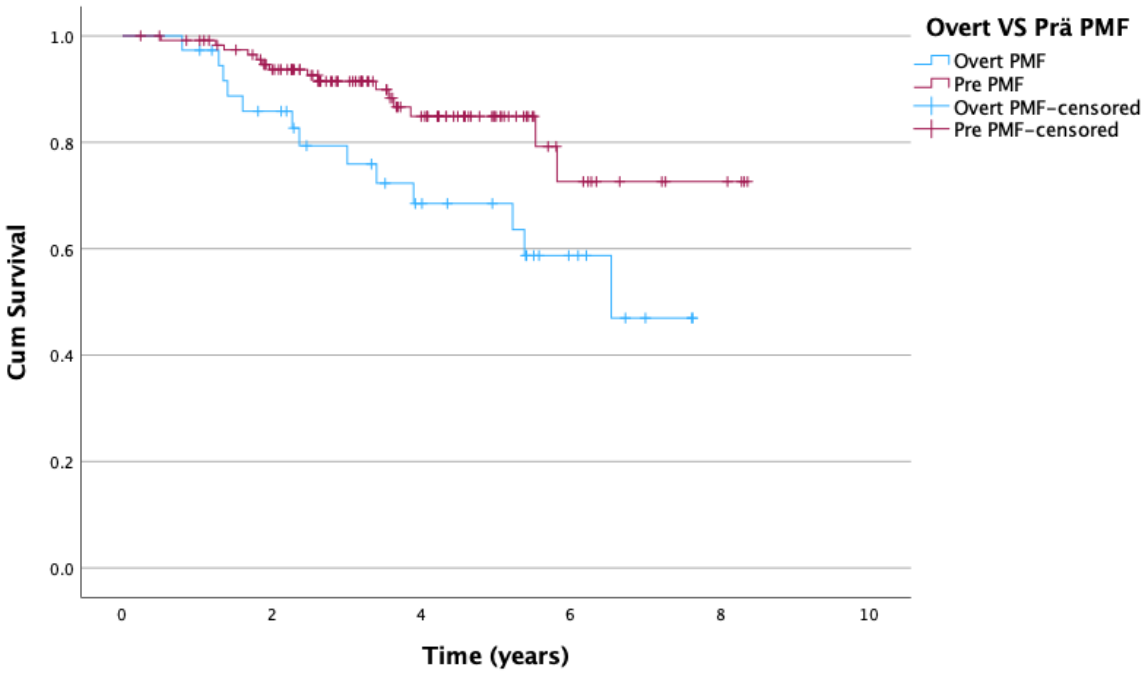


Figure 3-5: Kaplan Meier curve of overall survival rates regarding overt PMF VS Pre-PMF;
A statistically significant difference was found between the two groups (p = 0,017);

3.2.3 Effect of Gender on Overall Survival

In regards to gender, there was no statistically significant difference between female - and male PMF patients (p = 0,646) in terms of overall survival. However, in comparison to males, female patients had a better 5-year survival rate of 84,8%, but a worse survival rate after 8 years with 52%, without statistical significance. Male survival rates were 78% after 5 years and 73,1% after 8 years (figure 3-6).

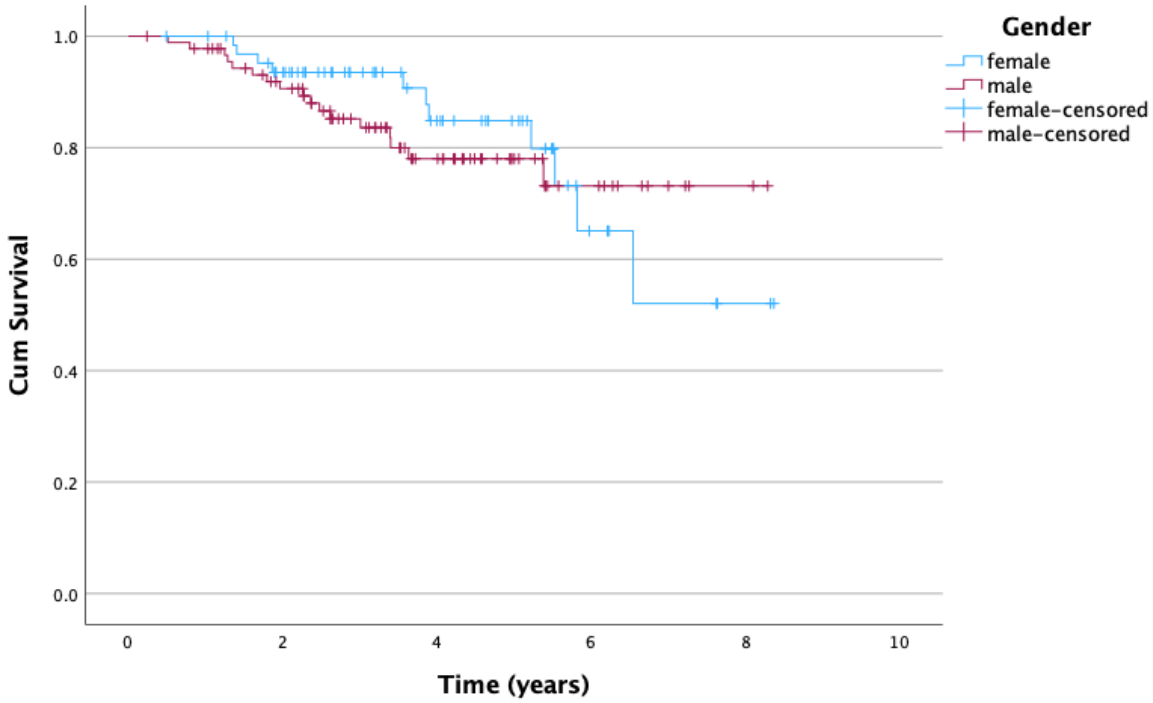


Figure 3-6: Kaplan Meier survival curve of female - and male survival rates; There was no statistically significant difference (p = 0,646);

3.2.4 Age at the Time of Diagnosis

Splitting the cohort into two sperate age groups showed, that patients who were less than 70 years old at the time of diagnosis had a significantly better survival rate than patients who were 70 years or older ($p < 0,001$). After 5 years 92,5% of PMF patients under 70 years old were still alive and 80,4% lived after 8 years. Patients with the age of 70 years or older had survival rates of 60,4% after 5 years and only 32,2% after 8 years (figure 3-7).

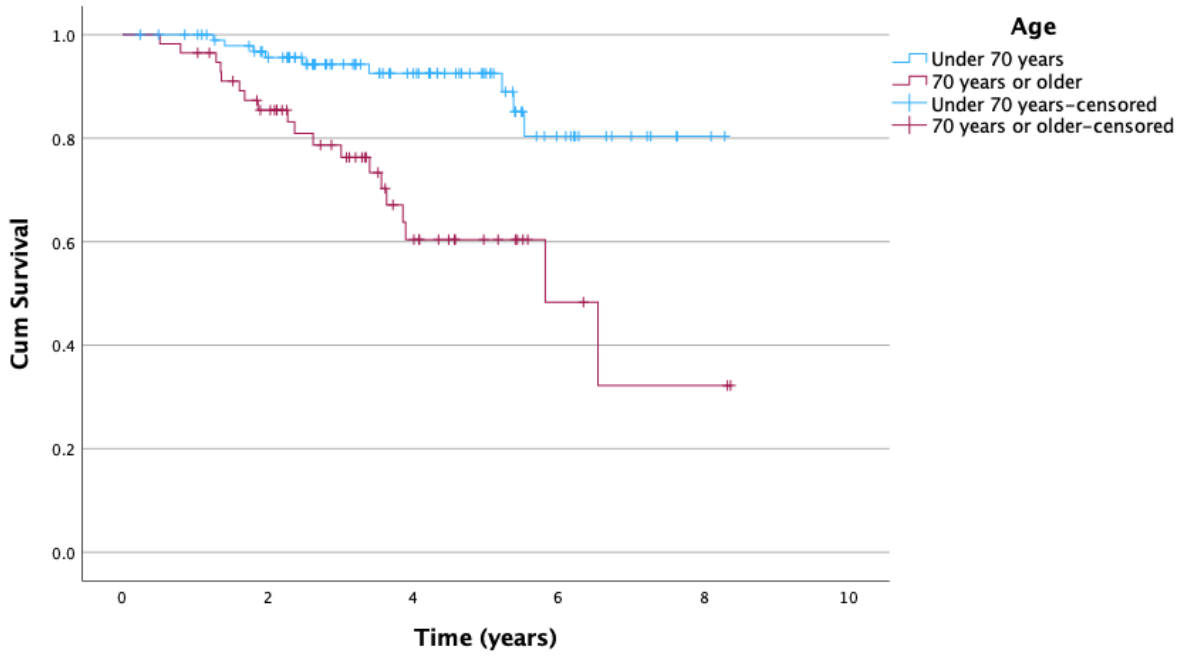


Figure 3-7: Kaplan Meier curve of survival rates of age groups <70 years versus ≥70 years; There was a stastically significant difference ($p < 0,001$);

3.2.5 DIPSS at the Time of Diagnosis

Figure 3-8 shows an overview of the survival rates depending on the DIPSS at the time of diagnosis. As expected, low risk - and intermediate risk 1 patients had the best chance of survival with 93,5% and 80% after 5 years and 85% and 61,1% after 8 years. In the Kaplan-Meier curve for the intermediate 2 risk group, the survival probability drops to 0% after approximately 5,4 years. However, this might be misleading as 5 out of 12 patients were still alive during the last follow up but were all censored after 2,8 years, due to a shorter observation period. In this particular cohort, 5 year survival was 0% in the intermediate 2 risk group, but should be interpreted with caution, considering the size of the group. The survival rate at the time the last living patient was censored (2,8 years) was 63,5%. Three people were classified as DIPSS high risk patients at the time of diagnosis. Only one patient was still alive at the last follow up resulting in a survival rate of 33,3% at 2,1 years.

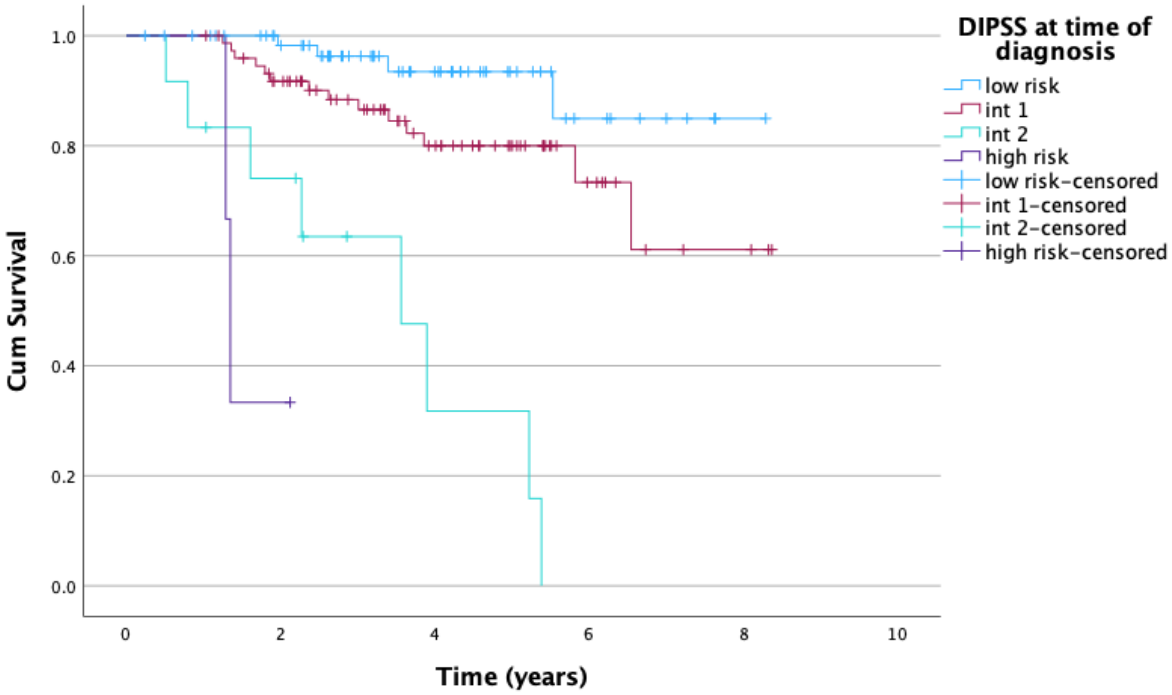


Figure 3-8: Kaplan Meier curve of survival rates in regards to DIPSS risk groups; As expected low - and int 1 risk patients had the best survival rates; The results of the int 2 group should be interpreted with caution due to group size and short observation periods; 5 of 12 patients in the int 2 group were still alive at last follow up; One patient was alive at the last follow up in the high risk group;

An interesting fact is that the DIPSS of the surviving high risk patient changed to intermediate 1 risk (age > 65 years) at the time of last follow up, after participating in the TRANSFORM-2 study, receiving ruxolitinib and navitoclax.

When summarizing the low - and intermediate 1 risk groups together and comparing it to the intermediate 2 risk - plus high risk group, there is a statistically significant difference ($p < 0{,}001$) in the overall survival rates. Although it is important to mention, that the low/int1 risk group is significantly bigger with n = 141 patients, than the int2/high risk group, which only contains n = 15 patients. As mentioned in the previous survival analysis, the short observation time in the intermediate 2 risk group results in a misleading curve, that drops to 0% after approximately 5,4 years, which is also reflected in this analysis. Even though the curve of the int2/high risk group implies that all patients died after 5,3 years, 6 patients were still alive during the time of last follow up. When looking at the time the last patient was censored, the survival rate in this group was 60% at 2,8 years. Patients in the low/int1 risk group had survival rates of 86% after 5 years and 71% after 8 years (figure 3-9).

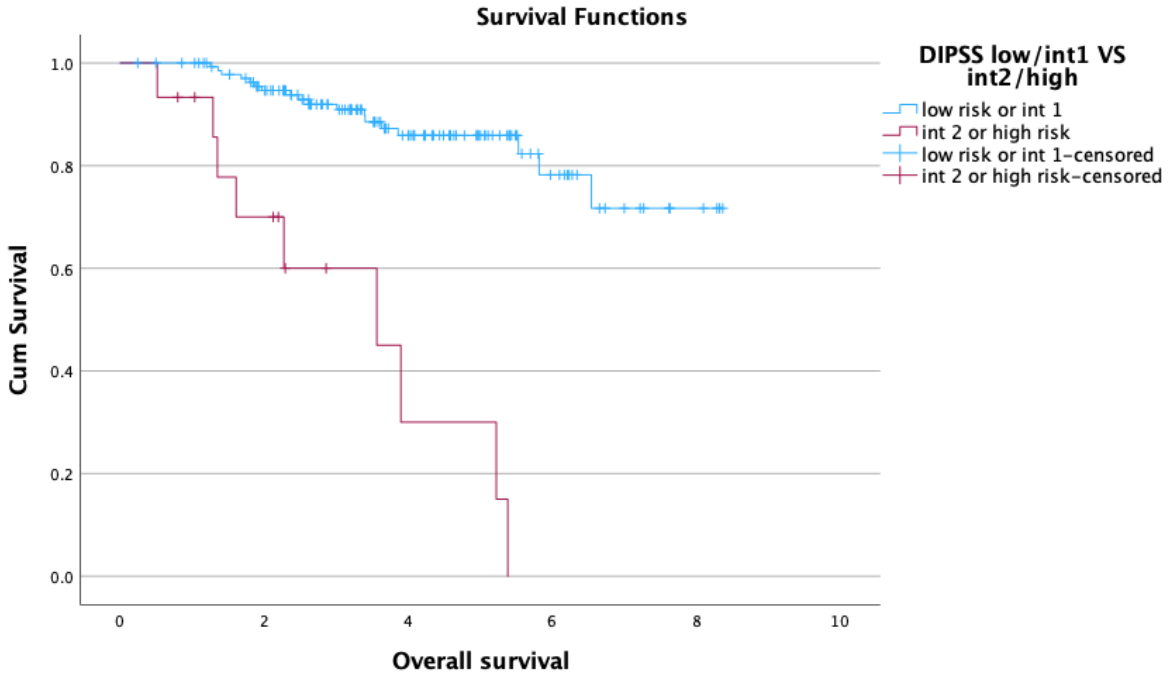


Figure 3-9: Kaplan Meier curve of survival rates, comparing low/int1 risk VS int2/ high risk groups; A statistically significant difference was found ($p < 0{,}001$); Group sizes varied greatly and observation periods were short in the int2/high risk group, which impaired the analysis;

3.2.6 First Line Treatment

In regards to first line treatment, patients had relatively similar 5 year survival rates when treated with hydroxyurea (77,5%), ruxolitinib (72,3%), or were in the observation group (83%) (figure 3-10). Better survival in the observation group might be explained by the high rate of low - and intermediate 1 risk DIPSS scoring at the time of diagnosis (93,5%). Patients treated with thromboreductin or peginterferon alpha 2b had a 5 year survival rate of 100%, although it is also worth mentioning that all of these patients had a DIPSS of low - or intermediate 1 risk and approximately 87% of these patients were younger than 70 years old. The curves of thromboreductin and peginterferon alpha 2b are overlapping in figure 3.11. In pairwise comparisons of the log rank test, all p values were p > 0,05, indicating that there was no statistical significance in group comparisons.
Six patients were excluded from this analysis, due to unknown treatment status or cytoreductive drug combinations and clinical trials, leaving a total of n = 150 patients for this analysis.

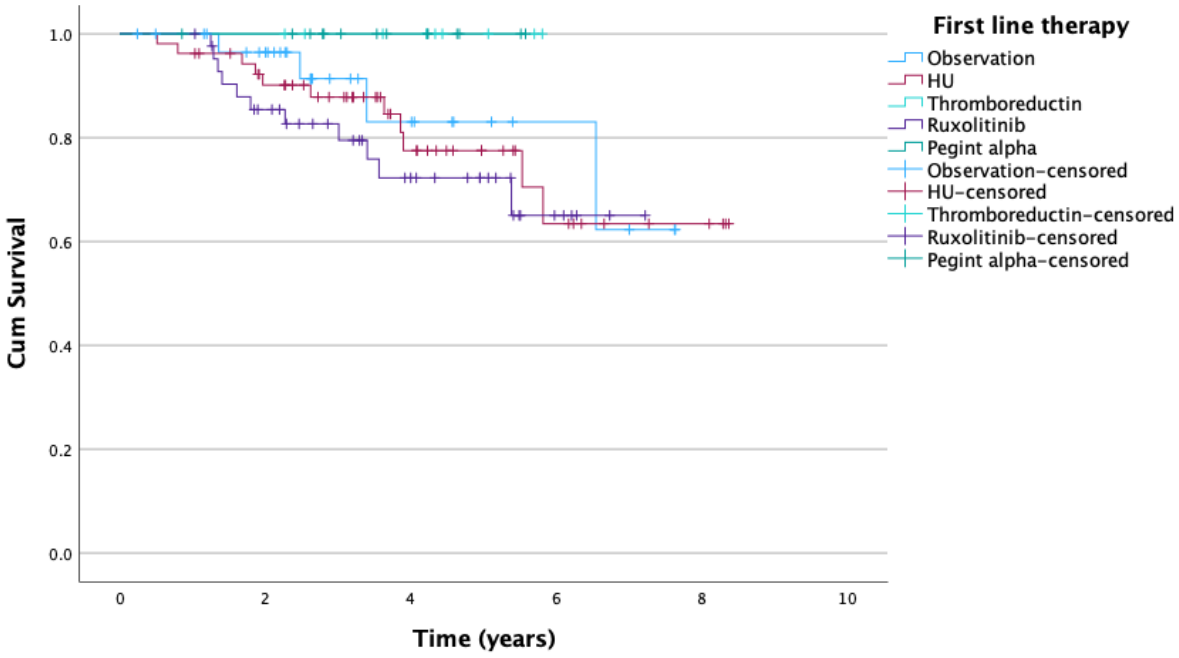


Figure 3-10: Kaplan Meier curve of survival rates depending on the first line treatment; Patients receiving thromboreductin or peginterferon alpha 2b or were in the observation group had the best survival rates, but also the most patients with low - or int1 DIPSS and were also mostly younger than 70 years; The corresponding curves are overlapping; The analysis did not show statistic significance in pairwise comparisons of the log rank test (p > 0,05);

3.2.7 Cox-Regression Analysis

A Cox proportional hazards regression was conducted to examine the impact of various prognostic factors on the hazard of dying. Age at diagnosis was significantly associated with an increased risk (HR = 1.071, p = 0.014), with each additional year increasing the risk of dying by 7.1% in this cohort. Gender showed a hazard ratio of 0.504, suggesting a protective effect for male patients, though this was not statistically significant (p = 0.108). The DIPSS at the time of diagnosis was a significant predictor of mortality (overall p = <0,001) in this cohort. In comparison to the reference group „DIPSS low risk“, patients in the DIPSS intermediate 1 risk group showed a 2,9 times higher hazard of dying (HR = 2,926; p = 0,058). Patients in the intermediate 2 risk group had a significant hazard ratio of 16,919 (p = <0,001) and DIPSS high risk patients demonstrated a substantial 60-fold higher hazard of dying when compared to low risk patients (HR = 60,088; p = <0,001). However, these findings should be interpreted with caution, due to the strong variability in group sizes, which is apparent in the widening of the confidence intervals in higher risk groups. Lastly, the comparison between overt and pre-fibrotic PMF revealed a slightly increased risk of dying in overt PMF patients of this cohort, although there was no statistically significant difference (HR = 1.088, p = 0.869). Table 3-2 provides an overview of these findings.

VARIABLES	HR	p-value	95% CI lower	95% CI upper
Age At Diagnosis	1,071	0,014	1,014	1,132
Male VS Female	0,504	0,108	0,219	1,161
Overt- VS Pre-PMF	1,088	0,869	0,401	2,947
DIPSS int 1	2,926	0,058	0,962	8,897
DIPSS int 2	16,919	<0,001	4,855	58,957
DIPSS high risk	60,088	<0,001	9,822	367,584

Table 3-2: Cox regression for hazard ratio analysis of the parameters age, gender, PMF classification, and DIPSS score in comparison to DIPSS low risk patients;

3.3 Mutation Specific Analysis

3.3.1 Distribution of Driver Mutations

As mentioned in the chapter on "Descriptive Statistics" the most common driver mutation was found in the *JAK2* gene (59,6%), followed by mutations in the *CALR* gene (25,6%), with *MPL* mutations ranking third (7,7%). In 4,5% of patients, no driver mutation was detected and in 1,3% of cases, the driver mutation status was unknown. In another 1,3% of patients simultaneous *CALR* and *MPL* mutations were present. Figure 3-11 provides an overview of the distribution of driver mutations within this cohort. These mutation frequencies are consistent with international data [3, 4, 10, 15].

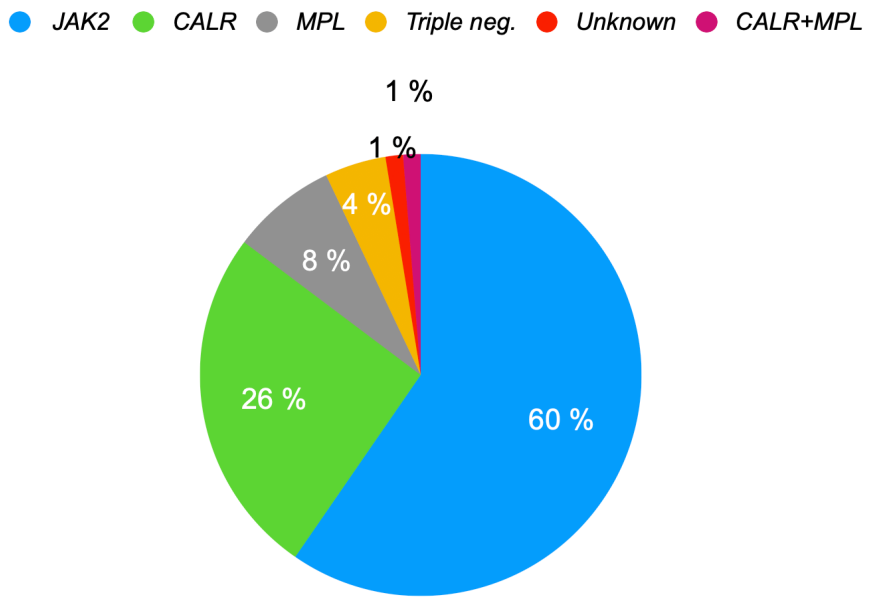


Figure 3-11: pie chart of driver mutation distribution in the cohort;
Percentages are rounded;

3.3.2 Driver Mutations and Their Impact on Overall Survival

For this analysis patients with unknown mutation status and patients with simultaneous *CALR* + *MPL* mutations were excluded from the analysis, leaving n = 152 patients. Worst 5 year and 8 year survival rates were found among *JAK2*

mutated patients with 75,7% and 51,6%. Regarding patients carrying a *CALR* mutation, 90,4% were still alive after 5 years, and 72,3% of patients after 8 years. *MPL* mutated patients had a 5-year survival rate of 81,8% which remained the same until the point of last censoring (5,7 years). Triple negative patients maintained a 5-year survival rate of 85,7%, also unchanged at the time of last censoring (7,6 years). Surprisingly, triple negative patients had the best chance of survival after 7,6 years, which is unusual, since this mutation status is usually associated with a worse prognosis [3, 48].

Figure 3-12 provides an overview of the overall survival rates in regards to the underlying driver mutation status of the cohort. It is important to point out that this should be interpreted with caution, due to the small group size. The p-value of the *JAK2* - versus the *CALR*-mutated group was p = 0,028, indicating a statistically significant difference between the two survival rates. Other p values were >0,05 in pairwise comparisons of the log rank test.

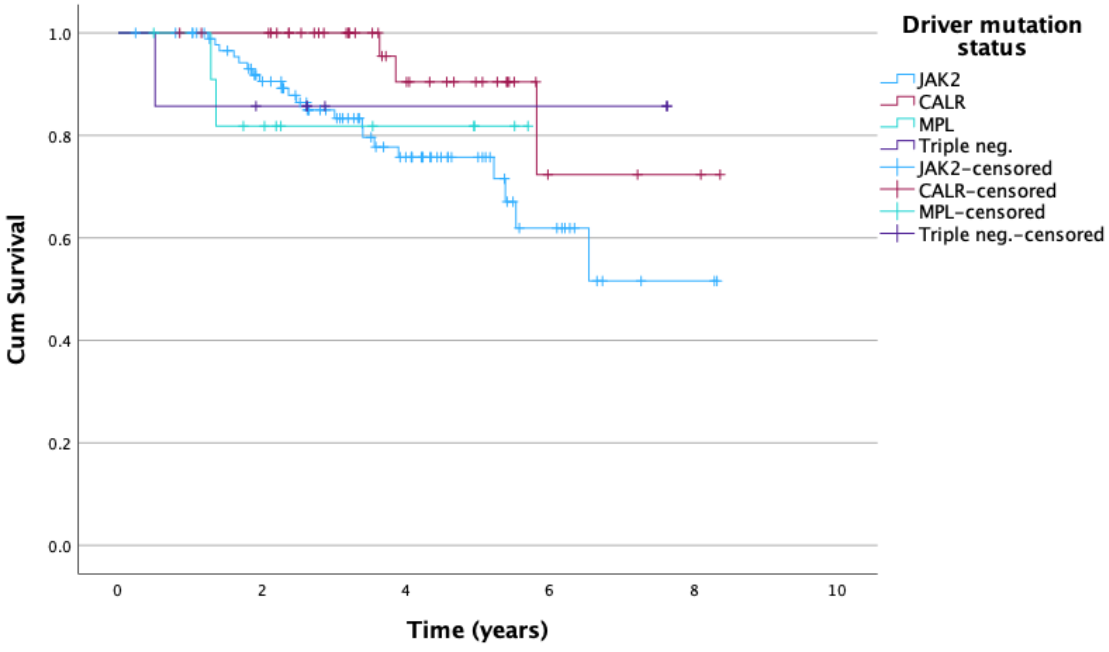


Figure 3-12: Kaplan Meier curve of driver mutation related overall survial rates; Patients carrying a *JAK2* mutation had the worst 5 - and 8 - year survival rates; The survival rates of triple negative patients was the highest after 7,6%, although this should be interpreted with caution due to the small group size. The p value of *JAK2* versus *CALR* mutated patients was p = 0,028;

3.3.3 Driver Mutations and Their Impact on Event Free Survival

65% of patients carrying a *JAK2* mutation -, 82,5% of *CALR* mutated patients -, 54,5% of *MPL* mutated patients - and 85,7% of triple negative patients were event-free after 5 years. After 8 years only 20,8% of patients with a *JAK2* mutation - and 70,7% of *CALR* mutated patients have not experienced an adverse event, although censoring should be taken into consideration when interpreting these results. In pairwise comparisons of the log rank test, statistic significance was found in the difference between the groups of *JAK2* - versus *CALR* mutated patients (p = 0,025) and *CALR* - versus *MPL* mutated patients (p = 0,027; figure 3-13;).

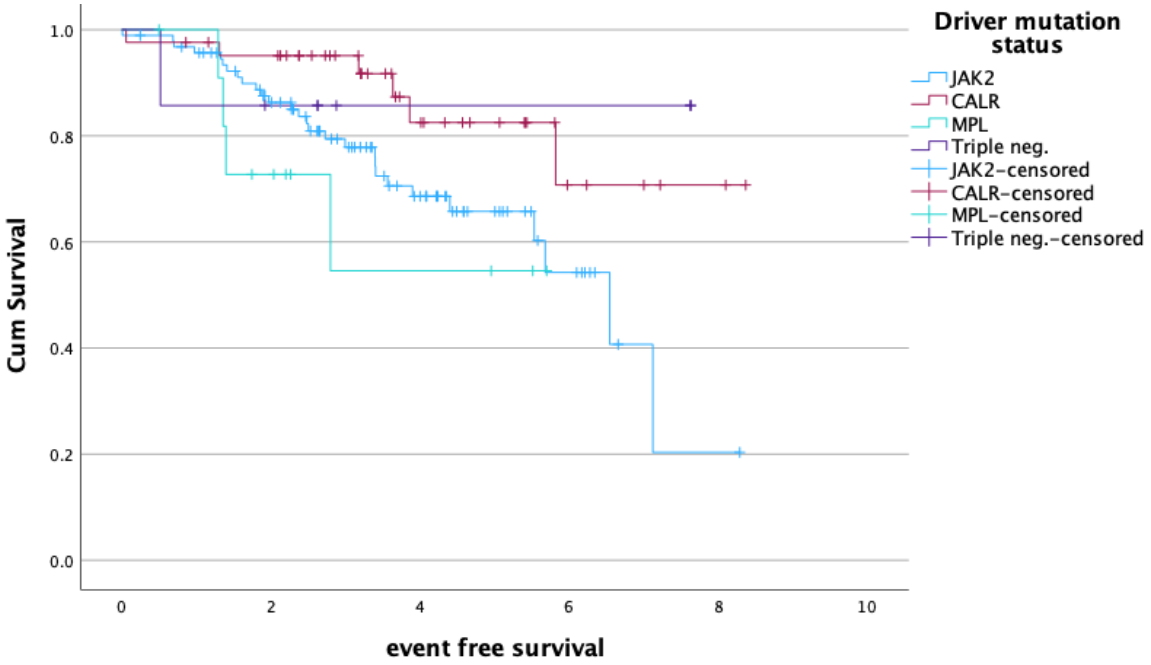


Figure 3-13: Kaplan Meier curve of driver mutation specific event free survival; In group comparisons of *JAK2* vs *CALR* (p = 0,025) and *CALR* vs *MPL* (p = 0,027) p values indicated statistic significance;

3.3.4 Distribution of Non-Driver Mutations

In this analysis we looked at 53 patients, whose bone marrow or blood cells were sequenced with the MNP2019v2 panel, also including non- driver mutations. Twenty-five of these patients had no non-driver mutation, while 8 patients were carrying 1 mutation, 11 patients had 2 mutations, 5 patients had 3 non driver mutations, 2 patients were carrying 5 mutations and in 1 patient each the NGS panel found 4 and 6 non driver mutations. Thirty-eight of these 53 patients did not carry a HMR mutation, while 11 patients did have 1 HMR mutation and 2 simultaneous HMR mutations were found in 4 patients. In total, 12 *ASXL1* -, 4 *SRSF2* - and 3 *U2AF1* HMR-mutations were found.

Figure 3-14 provides an overview of the quantitative distribution of non-driver - and HMR-mutations.

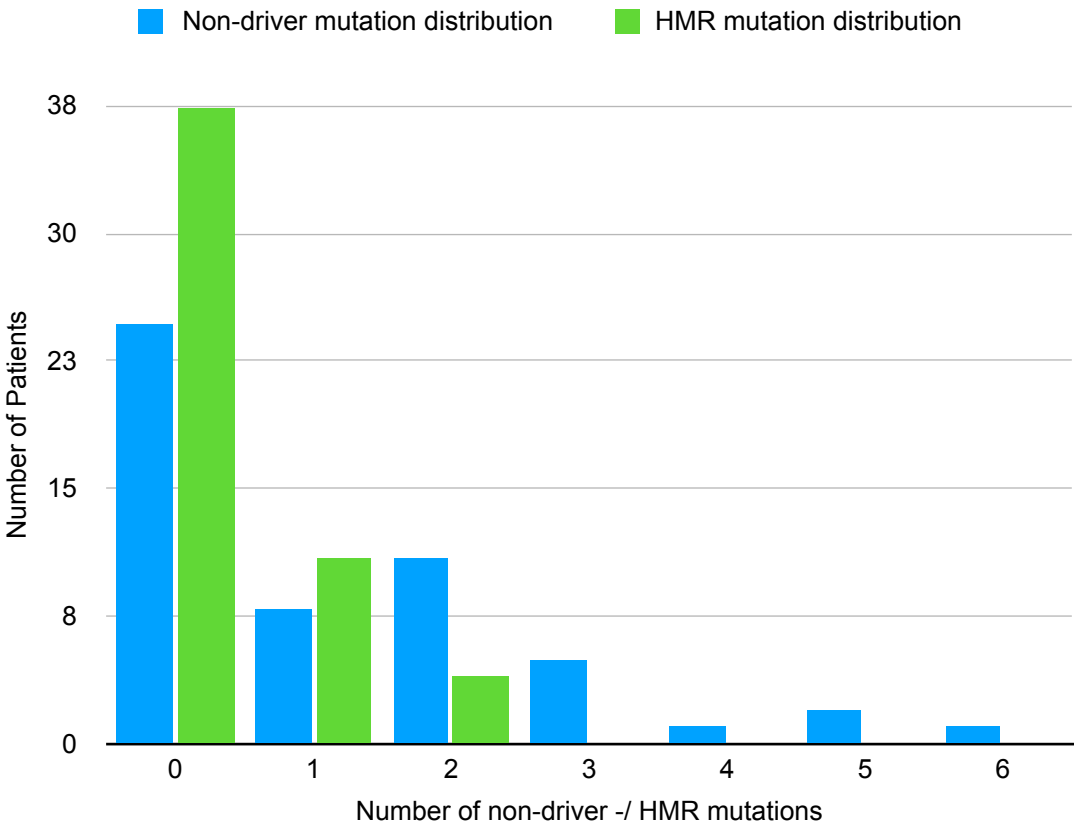


Figure 3-14: quantitative distribution of non-driver - and HMR-mutations in patients who received MNP2019v2 NGS panel;

3.3.5 Impact of Non-Driver Mutations on Overall Survival

When comparing the overall survival rates of patients carrying any number of non-driver mutations to patients where no such mutation was found in the MNP2019v2 NGS panel, a statistically significant difference was found (p = 0,004). Patients without any non-driver mutations had a 5-year surivival rate of 100% versus 70% in the non-driver mutation positive group. This 100% survival rate remained until the point of last censoring (7,6 years), while survival rate in the non driver mutation positive group dropped to 46,7% after 7,3 years (figure 3-15).

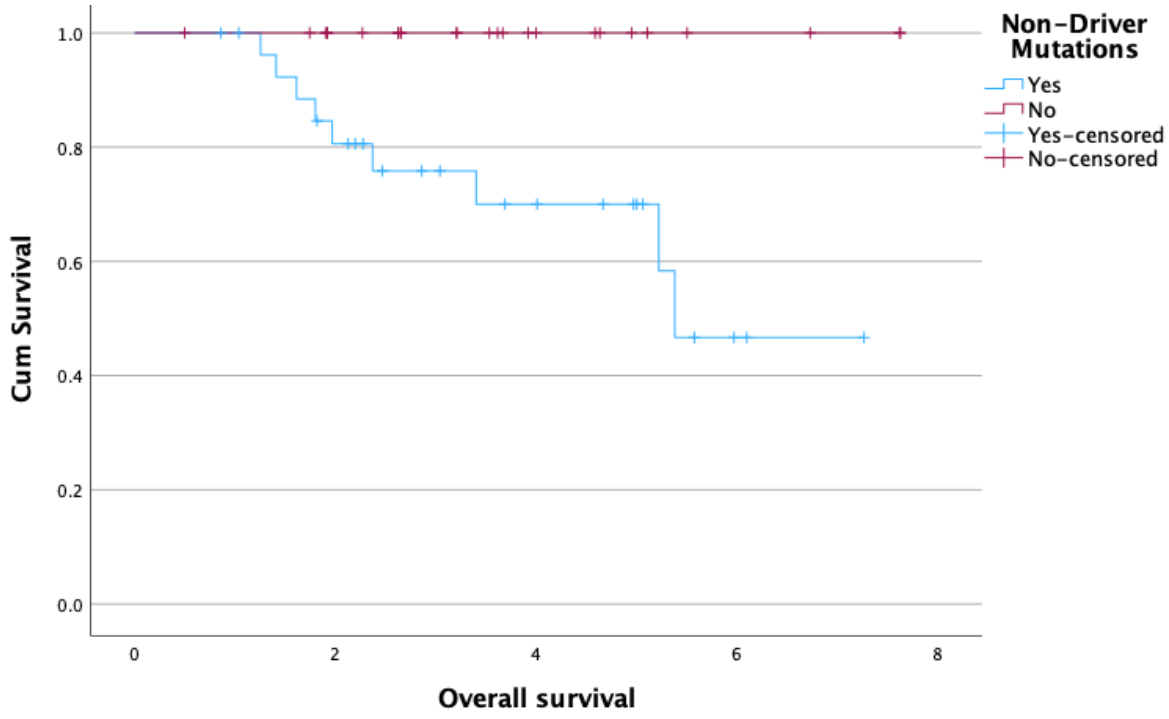


Figure 3-15: Kaplan Meier curve of overall survival based on detection of non-driver mutations;
Patients without any such mutations had a survival rate of 100% after 7,6 years;
The 5- and 7,3-year survival of non-driver mutated patients was 70% and 46,7%;
A statistically significant difference was found (p=0,004);

3.3.6 Impact of HMR Mutations within the Non-Driver Mutations Group

When comparing patients carrying at least one non-HMR - non-driver mutation (n = 13) with patients carrying at least one HMR mutation (n = 15), there was no statistically significant difference found (p = 0,283). Although non-driver mutated patients had better survival rates of approximately 76% at the time of last censoring (5,6 years), when compared to the survival rate of 38,8% in the HMR group at the same observation time (figure 3-16). Only patients carrying at least one non-driver mutation were included in this analysis (n = 28).

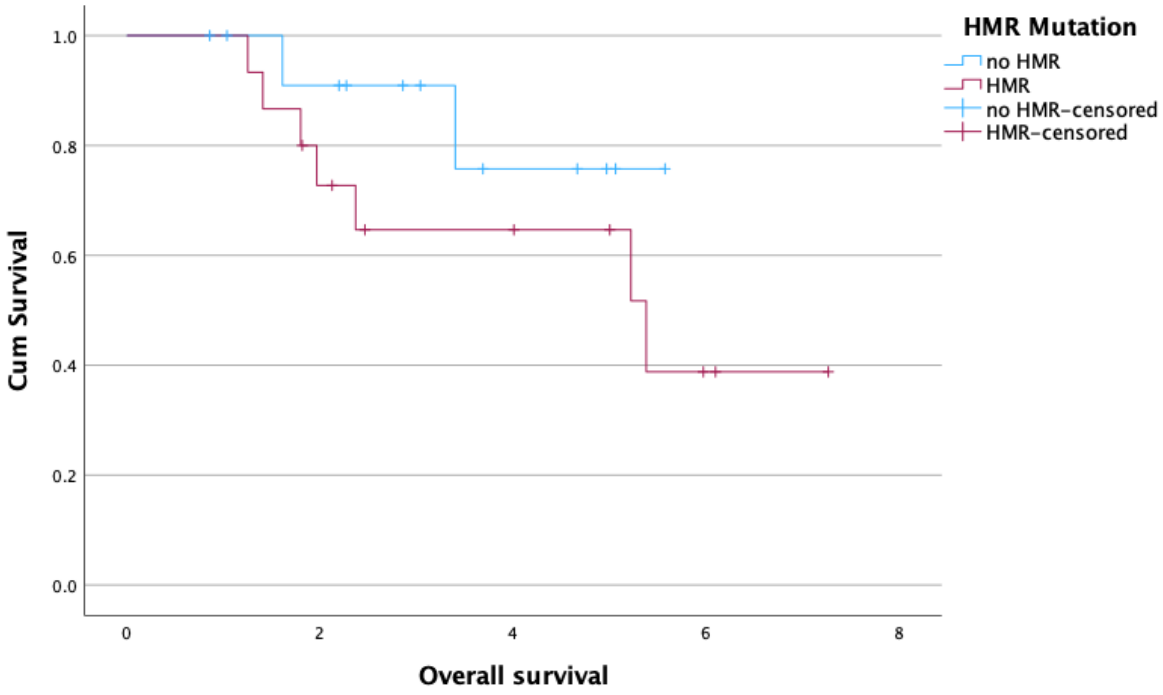


Figure 3-16: Kaplan Meier curve of overall survival rates of patients carrying at least one non-HMR non-driver mutation VS patients carrying at least one HMR mutation;
The curve suggests a difference that is not negigble, even though there was no statistically significant difference found (p = 0,283); This is most likely due to shorter observation periods in the non-HMR group;

3.3.7 Cox-Regression Analysis of the MNP2019v2 Panel Group

To further evaluate risk factors in this group, a cox regression was conducted (table 3-3). In this analysis the presence of non-driver mutations (HR = 3,876) and a DIPSS of int 2 (HR = 1,684) were the factors that carried the highest risks of dying, even though no statistical significance was found. In regards to age, the hazard of dying increased by 1,2% with each additional year in this cohort (HR = 1,012).

Due to the small group size, these results should be interpreted with caution, as a lower risk of dying in PMF patients is usually not associated with a high risk DIPSS (HR = 0) or overt PMF (HR = 0,408).

VARIABLES	HR	p-value	95% CI lower	95% CI upper
Age At Diagnosis	1,012	0,568	0,939	1,122
nDM VS no nDM	3,876	0,177	0,509	38,886
Overt- VS Pre-PMF	0,408	0,637	0,283	7,854
DIPSS int 1	0,469	0,275	0,342	43,572
DIPSS int 2	1,684	0,116	0,607	94,497
DIPSS high risk	0	0,992	0	.

Table 3-3: Cox regression for hazard ratio analysis of the parameters age, presence of nDM, fibrotic grading, and DIPSS score in comparison to DIPSS low risk patients;
The biggest risk factors were the presence of non-driver mutations and a DIPSS of int 2; There was no statistical significance found; Due to the small group size, these results should be interpreted with caution;

3.3.8 Non-Driver Mutations and Their Impact on Event-Free-Survival

The risk of experiencing an adverse event like thromboembolism, AML transformation or death was also higher in patients carrying a non-driver mutation, as seen in figure 3-17. After 5 years 17,9% - and 48,9% of the patients without - and with non-driver mutations had an event (p = 0,004; figure 3-17;).

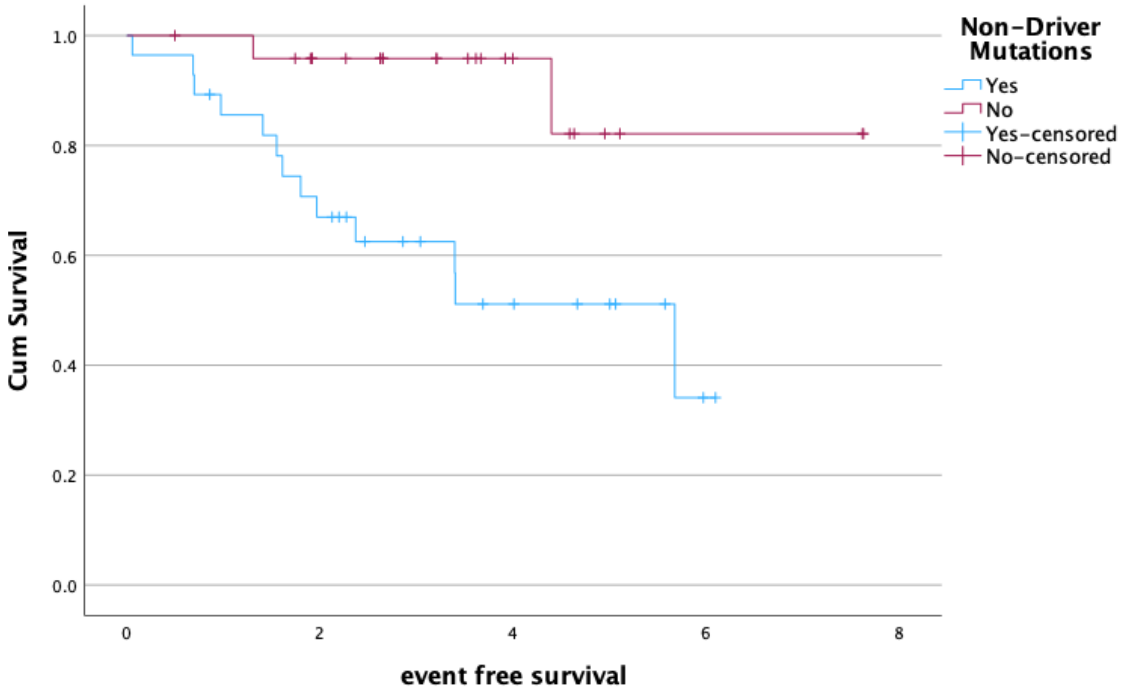


Figure 3-17: Kaplan Meier curve of event free survival of patients with - and without non driver mutations;
(p = 0,004);

3.3.9 Non Driver Mutations: Hazard Ratio of Adverse Events

To further evaluate the risk of experiencing an adverse event in non-driver mutated patients (nDM), in comparison to patients who did not carry a non-driver mutation (no nDM), a cox regression analysis was conducted. The results showed that non-driver mutated patients in this cohort carried a 6,55 times higher risk for having an event (p = 0,013; table 3-4;).

VARIABLE	HR	p - value	95% CI lower	95% CI upper
nDM VS no nDM	6,549	0,013	1,476	29,063

Table 3-4: Cox regression regarding the risk of experiencing an adverse event in patients carrying a nDM mutation versus patients without a nDM;

4 Discussion

In this study we reported the impact of driver (DM) - and non-driver mutations (nDM) as well as other risk factors on survival rates in patients diagnosed with myelofibrosis at the Medical University of Graz between 2015 and 2022. In comparison to published literature, the distribution of driver mutations aligned well with other cohorts, except for an underrepresentation of triple negative patients in our cohort (4,5% versus expected 8-10%) [1]. With 59,6%, *JAK2* was the most frequent driver mutation, followed by *CALR* (25,6%) and *MPL* (7,7%). Interestingly, two patients had two simultaneous driver mutations (*CALR* + *MPL*), with mutated *CALR* being the dominant mutation in terms of allele burden in both cases. Published similar cases suggest that such double driver mutations do not necessarily correlate with a more severe clinical course in MPNs, although its clinical impact is still unclear due to the rarity of coexisting driver mutations [7, 8]. Both of our patients were under 70 years of age (28 and 51), classified as DIPSS low risk and were event free at the time of the last follow up. In our entire cohort, patients carrying a *JAK2* mutation had the worst 8-year overall survival rate (51,6%), followed by *CALR* mutated patients (72,3%), with a statistically significant difference ($p = 0,028$). Furthermore, patients carrying a *JAK2* mutation had the highest rate of adverse events after 8 years. These results are fully coherent with published data [9, 10]. However, a limitation of this study is the lack of further subcategorization of the underlying *CALR* mutations, as *CALR* type-1 mutations have significantly better survival rates, compared to type 2 *CALR* mutations. This is also reflected by the fact that the absence of the *CALR* type-1 driver mutation is included as an adverse factor in prognostic scores such as the MIPSS70+ and the GIPSS [10, 11]. At the time of last censoring, triple negative patients showed a superior survival rate (85,7% at 7,6 years) in our cohort, when compared to the *JAK2, CALR* and *MPL* groups. This is unusual since triple negativity is usually associated with worse outcomes [11-13]. An explanation for this discrepancy might be our small group size of $n = 7$ patients with triple negativity and the fact that these patients were predominantly younger than 70 years and had a DIPSS of low - or int-1 risk.

Looking at the distribution of HMR mutations in our cohort, a comparison of exact percentages with published literature is difficult, since only 19 HMR mutations were found in our analyzed subgroup (n=52). However, it is worth mentioning that *ASXL1* was the most frequent HMR mutation in this cohort, followed by *SRSF2* and *U2AF1*, similar to known data [2]. When it comes to the impact of nDM on outcomes in our cohort, patients carrying at least one nDM, including HMR mutations, had significantly worse overall survival rates, compared to patients without any nDM (46,7% versus 100% after 7,3 years, p = 0,004). Accordingly, a Cox regression analysis for hazard ratios, within the MNP2019v2 panel group showed a 3,876 times elevated risk of dying for patients with any nDM. Further comparison between patients with a non-HMR nDM versus patients with a HMR mutation showed a trend towards a superior survival in the non-HMR group, even though there was no statistically significant difference found (76% versus 38,8% at 5,6 years, p = 0,283). The lack of statistical significance in this analysis can be explained by the rather small sample sizes and short observation periods in our cohort. Nevertheless, these results fit well with published data [16, 68]. Thus, when looking at figure 3-16 the Kaplan Meier overall survival curves might suggest an even bigger difference, which is hidden by shorter observation periods in the no-HMR group. Apart from overall survival, patients with any nDM also had a significantly higher risk of experiencing an adverse event like death, thromboembolism or leukemic transformation (HR = 6,549, p = 0,013). Even though the median survival of the HMR group was longer than expected (5,3 years), the observed shorter overall survival time and the higher risk of adverse events in this subgroup allign with published data [14]. However, as already outlined, only a limited number (n = 53) of patients was available for conducting statistical tests on the role of non-driver - and HMR mutations in our cohort.

Apart from the mutation status, other well established prognostic factors were found to be associated with significantly worse survival rates in our cohort, including age ≥70 years, overt PMF and a int-2 - or high risk DIPSS. Interestingly, despite literature suggesting poorer outcomes for males, our study found a trend towards better 8 - year survival in men [3]. An explanation for this discrepancy could not be found.

Looking at the overall patient composition we noted an underrepresentation of patients with overt PMF (23,7%) in our cohort. In other published cohorts overt PMF frequently accounted for more than 50% of patients. Another difference was observed regarding the DIPSS risk distribution at the time of diagnosis. In our cohort, low risk - and int-1 risk patients were overrepresented, while there were fewer int-2 - and high risk patients. However, the slightly higher proportion of male PMF patients as well as the median age of disease onset at 65 years are absolutely in line with the existing literature [11, 15, 16].

When it comes to survival rates, we noted much better survival rates of this cohort as compared to other studies [4, 5]. However, these observed higher survival rates (80,7% and 63,3% after 5 - and 8 years, respectively) can be well explained by the overrepresentation of DIPSS low - and int-1 risk patients in our cohort. Furthermore, most of our patients were diagnosed at a time, when JAK inhibitors have already been available for therapy of MF. Accordingly, a recent study reported a significant increase of survival rates over the last decade as therapeutic modalities have constantly improved [6]. As already mentioned, there were also less overtly fibrotic PMF patients in this cohort, which might have positively affected overall survival as well. A more detailed look on the outcomes associated with the grading of fibrosis revealed an expected worse survival in patients with an overt PMF in our cohort, which also reached statistical significance ($p = 0,017$). This fits well with findings reported in other cohorts [17, 18]. Interestingly, there are no established clinical prognostic scoring systems that directly take the grade of bone marrow fibrosis into account as a parameter, although some studies suggest a more accurate prognostic evaluation if done so [17, 19]. A possible reason for this could be the difficulty to standardize grading of fibrosis and other findings based on bone marrow morphology. A well established example for such a challenge is the difficult differentiation of pre-PMF and ET [20]. However, a study has suggested increasing reliability of fibrotic grading and low variability amongst hematopathologists [21].

A parameter that is also taken into account when it comes to prognostic scores like the IPSS and DIPSS+ is age. In good accordance with published studies patients in this cohort at the age of 70 years or older had a significantly worse overall survival, compared to patients younger than 70 years (80,4% versus 32,2% after 8

years, p <0,001) [69]. This impact was also seen in the Cox regression, where younger age was a significant protecting factor (HR = 0,071, p = 0,014). Survival rates depending on the DIPSS at the time of diagnosis were as expected, with low risk patients having the best survival rates, followed by int-1 risk patients at second place, while int-2 - and high risk patients had the poorest survival rates. Patients with a higher DIPSS also showed a higher risk of dying in the cox regression performed, compared to patients with a low risk DIPSS (HR = 2,926 for int-1, HR = 16,919 for int-2, HR = 60,088 for high risk). Although this reflects the reliability of the DIPSS, the small group sizes and short observation periods, especially in the int-2 - and high risk groups, may limit the general significance of our data. These limitations were also apparent when looking at the corresponding Kaplan-Meier curves, where the int-2 - and high risk group drop to zero, even though 6 patients were still alive at the last follow up. When it comes to gender differences, male patients had a better 8-year survival rate than female patients. Although not statistically significant, it is somewhat surprising, since a study suggests that male sex is associated with poorer outcomes [3]. In our study, a hazard ratio of HR = 0,504 even suggested a protective effect for male patients, even though this was also not statistically significant (p = 0,108).

Looking at survival rates depending on the first line therapy, it is important to note, that even though patients treated with thromboreductin or peginterferon alpha 2b had a 5 year survival rate of 100%, these groups consisted predominantly of patients younger than 70 years and all of the patients had a DIPSS of low - or int-1 risk. The 5 year survival rates of other groups were relatively similar (hydroxyurea = 77,5%, ruxolitinib = 72,3%, observation group = 83%), with no statistically significant difference between the groups (p > 0,05). When looking at the corresponding graph (figure 3-10) the survival rates of these groups became almost identical at around 6,5 years. Considering that the analyzed treatment subgoups were highly variable in terms of DIPSS, age and clinical disease severity a conclusion on the impact of any treatment modality on survival cannot be drawn from our cohort.

Summarized, in this study we reported the impact of molecular and clinical data on the outcome of patients with newly diagnosed PMF at our institution. Although our cohort had less patients with overt fibrosis and thus superior survival rates when

compared to published studies, we confirmed that driver mutation status, age, as well as IPSS and DIPSS status pose important risk factors regarding the outcome of PMF patients. Furthermore, the additional presence of non-driver mutations including HMR mutations negatively affected overall -, as well as event-free survival. However, due to the rather low number of patients in subgroups no significant effect of distinct nDM as well as of distinct treatment modalities could be found. For this purpose, larger and probably multicentric cohorts need to be analyzed in the future.

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