

2021

# Analysis of essential thrombocythemia and its treatment

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**ANALYSIS OF ESSENTIAL THROMBOCYTHEMIA AND ITS TREATMENT**

by

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B.S., Baylor University, 2019

Submitted in partial fulfillment of the  
requirements for the degree of  
Master of Science

2021

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## ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my primary reader, Dr. Walsh, whose enthusiasm in teaching pharmacology inspired me to take on this endeavor with her aid. Her kindness and vigilance in every read thru and edit guided me to creating the best possible version of my thesis. Her vast knowledge of pharmacology and willingness to share in the wealth of knowledge she has acquired is truly astounding. Thank you for all your help Dr. Walsh.

I would also like to express how grateful I am for the aid of my secondary reader, Dr. Layne, whose advice and aid as my advisor guided me through the process and undertaking of this project. I cannot express how grateful I am for the promptness and thorough answers to my many questions. Thank you for all your help Dr. Layne.

Next, I would like to thank all of the library staff and Dr. Flynn for their aid in teaching us how to find sources and utilize the research tools needed for constructing our thesis. Thank you all for putting together a wonderful and informative class for the GMS department.

Finally, I would like to thank my biggest supporters for my thesis; my family and my fiancé. They endured my many readings out loud, supplied me with countless cups of coffee, and moral support. I cannot express how thankful I am for all of their love and support. Truly thank you for all your help.

# **ANALYSIS OF ESSENTIAL THROMBOCYTHEMIA AND ITS TREATMENT**

**CADE ALAN COWART**

## **ABSTRACT**

Essential thrombocythemia (ET) is a rare myeloproliferative neoplasm affecting 43.7 out of every 100,000 people in the United States. The disease is characterized by abnormally high platelet counts, mutational abnormalities in Janus Kinase 2 (JAK2)/Calreticulin (CALR)/myeloproliferative leukemia virus oncogene (MPL), and increased megakaryocyte production and differentiation. The average age of onset for patients with ET is between 65-70 years, but recent studies have demonstrated a downward trend in the age of diagnosis. Mechanistically, ET mutations cause the dimerization of JAK and upregulation of the JAK-STAT pathways. Common treatment approaches seek to use cytoreduction and platelet inhibition to lower the risk of a thrombotic event. Hydroxyurea and low-dose aspirin have been the gold-standard of treatment for ET patients. This thesis sought to compare the current available therapy with second-line treatments and investigational treatments. Anagrelide is a key second-line treatment for ET that is used in the event of intolerance to hydroxyurea. It acts through cytoreductive mechanisms which result in a decreased platelet count. Major bleeding is a severe adverse event associated with anagrelide. Interferons are another second-line defense in the treatment of ET despite a lack of FDA approval for this indication. Interferons act directly to reduce platelet counts and, unlike other drug classes, mount an immunological response against the JAK2 stem cells to reduce the allelic burden. An immunological approach to ET may be key to the

sustained treatment of the disorder without a daily dosing regimen. Despite the promise of interferons, severe adverse effects limit the adherence of many patients to this class of drugs. JAK inhibitors are an investigational drug class that acts directly through the JAK-STAT pathway. JAK inhibitors have shown little efficacy in the treatment of ET and may be better suited for treatment in combination therapies. Telomerase inhibitors are one such investigational drug class that may pair well with JAK inhibitors for the treatment of ET. All of these drug classes were compared to hydroxyurea with respect to their pharmacokinetics, pharmacodynamics, and patient evaluation. Hydroxyurea and low-dose aspirin showed superiority in comparison to other drug classes due to their low toxicity profile and minimum adverse side-effects, high oral bioavailability and wide distribution, high adherence, and production of the most uniform response to reducing thrombotic events and platelet counts. The interferon drug class shows unique potential for the treatment of ET and should be placed above the second-line treatment standard of anagrelide due to its benefits in treatment of younger and pregnant patients. Interferons are the only class of drug for the treatment of ET that did not increase the risk of drug-related leukemogenic transformations. Despite non-adherence due to side-effects and lack of an oral administration, interferons are superior to anagrelide due to their longer dosing interval and immunological attack on JAK2 stem cells. Treatment of ET with anagrelide has shown similar efficacy to hydroxyurea and interferons in platelet reduction and rivals hydroxyurea in the prevention of thrombotic risk. Despite this benefit, the risk of bleeding associated with anagrelide is a significant disadvantage. Hydroxyurea and low-dose aspirin remain

the current standard of treatment for patients with ET, although new approaches may soon be available.

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## LIST OF ABBREVIATIONS

AUC.....	Area Under the Curve
CALR.....	Calreticulin
cAMP.....	Cyclic Adenosine Monophosphate
CML.....	Chronic Myeloid Leukemia
ET.....	Essential Thrombocythemia
IPSET.....	International Prognostic Score for ET
JAK.....	Janus Kinase
PMF.....	Primary Myelofibrosis
PV.....	Polycythemia Vera
MPL.....	Myeloproliferative Leukemia Virus Oncogene
MPN.....	Myeloproliferative Neoplasm
STAT.....	Signal Transducer and Activator of Transcription
TERT.....	Telomerase Reverse Transcriptase
TPO.....	Thrombopoietin
WHO.....	World Health Organization

## **INTRODUCTION**

### **Specific Aims**

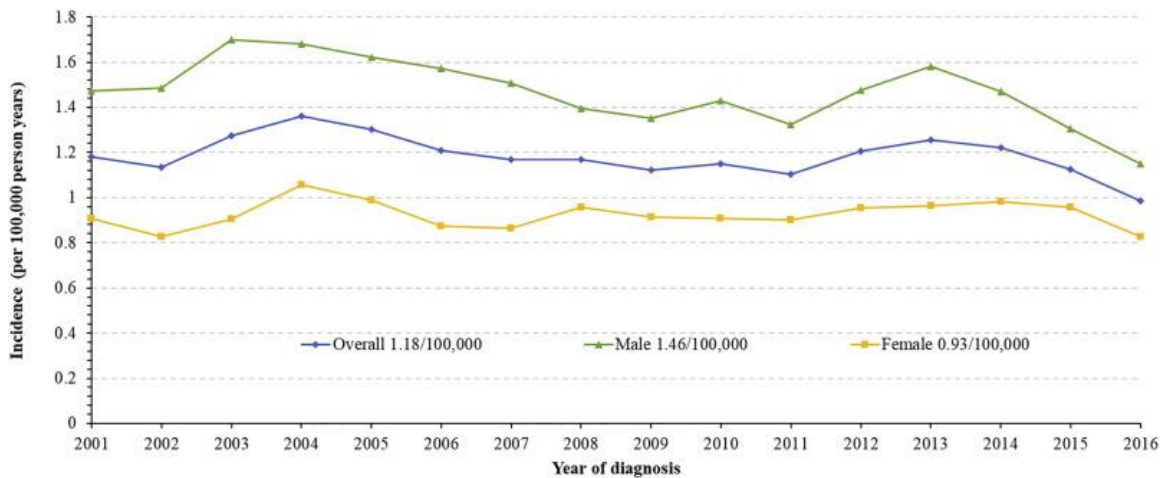
In the first part of this thesis a brief overview and history of essential thrombocythemia (ET) will be provided. The criteria for diagnostics and the recent changes brought forth by the World Health Organization will be examined and explained to further understand the complexity of this disorder. An in-depth review of the mutations and etiologies will be assessed to form a better understanding of the mechanistic actions of pharmacologic treatments. A detailed discussion into the prognosis and incidence rates of ET will also be reviewed. The next part of the thesis will compile a comprehensive list of the current and novel pharmacological treatments for ET and evaluate them both in monotherapy and in combination. A conclusion will be drawn about the best available therapy after careful consideration of pharmacokinetics, pharmacodynamics, patient evaluation, and side effects. Non-adherence rates for each type of therapy will also be considered, as rates in patients on cytoreductive treatments is reportedly as high as 28% and is especially high in younger patients where route and schedule of drug administration are key factors (Le Calloch et al. 2018).

### **Brief Overview and History of Essential Thrombocythemia**

Essential thrombocythemia is a rare myeloproliferative neoplasm (MPN) affecting roughly 1.18 out of every 100,000 people in the United States (Shallis et al. 2020). Since the discovery of myeloproliferative diseases in 1951 by William Dameshek, ET is one of the four classical hematopoietic tumors (Dameshek 1951). Polycythemia vera (PV) and primary myelofibrosis (PMF) being two other classical hematopoietic tumors that are more

closely related to ET in that they are classified as Philadelphia chromosome negative myeloproliferative neoplasms (Shallis et al. 2020). The last of the classic four myeloproliferative neoplasms is chronic myeloid leukemia (CML), which is classified as Philadelphia chromosome positive (Shallis et al. 2020).

There has been much debate over the years since its discovery in 1951 as to why the incidence of ET is rising. As the World Health Organization (WHO) changes and revises the diagnostic criteria for MPN's, the diagnoses have become more specific to each MPN. A study published in France in 2009 showed that there was an increase in the incidence of ET during 2005 to 2007 when compared to the 1980 to 2004 incidence rate (François Girodon et al. 2009). They theorized that the slight increase in incidence rates from 1.2 to 3 per 100,000 inhabitants on the World Standard Population, were due to the 2008 WHO revisions of MPN diagnostic criteria (François Girodon et al. 2009). However, a more recent study published in 2020 uses the 2016 WHO revisions and has demonstrated that the incidence rates from 2001 to 2016 for all MPNs, have remained stable, as seen in Figure 1, at around 1.18/100,000. (Shallis et al. 2020).



**Figure 1. Incidence of Essential Thrombocythemia in Male and Female Patients.** This image shows data collected from the Surveillance, Epidemiology, and End Results (SEER) study regarding the annual incidence of ET during 2001 to 2016. These data show the incidence of newly diagnosed ET in male, female, and all patients per 100,000 subjects on an annual basis. In recent years the incidence of ET in the general population appears to have remained stable with a slight dip in the last year 2016. Image adapted from Shallis et al. 2020.

The most recent revision of diagnostic criteria for ET and the other MPNs occurred in 2016 and has since produced changes in the treatment of MPNs. As new evidence is compiled on the diagnostic characteristics of the MPNs and new mutations are found, therapeutic discoveries are closing in on improving the quality of treatments. The Philadelphia chromosome negative MPNs are now known to be associated with three different molecular drivers: JAK2, CALR, and MPL (Grinfeld et al. 2018). Recent studies have shown that targeting these pathways could lead to more specific therapies and better treatment options for patients.

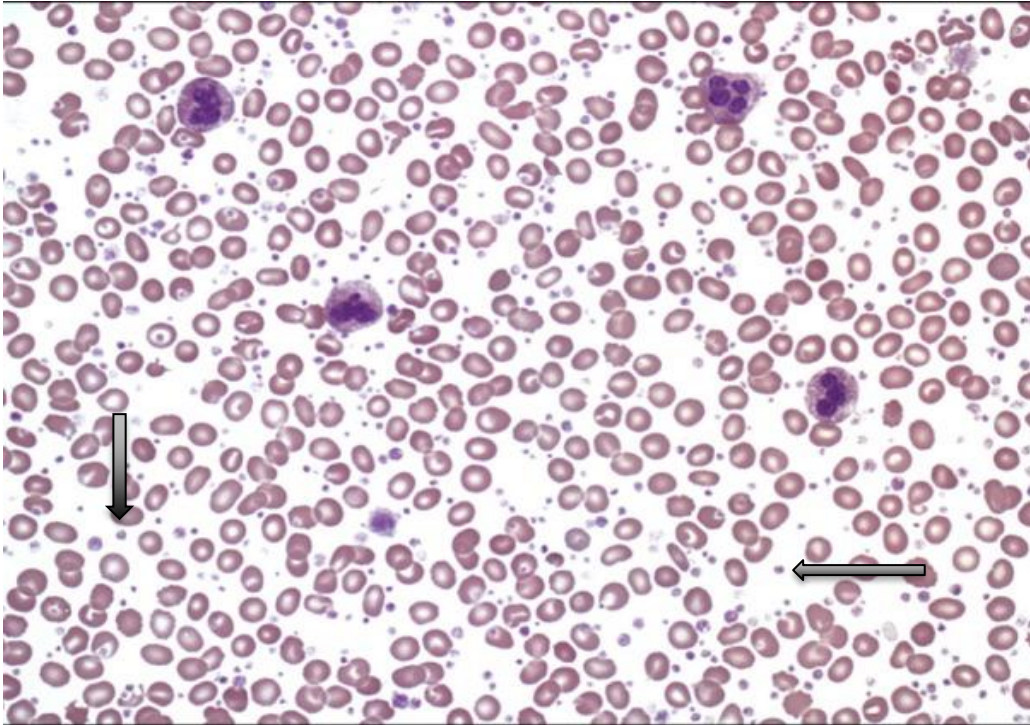
## **CHARACTERISTICS OF ESSENTIAL THROMBOCYTHEMIA**

### **Diagnostic Criteria for Essential Thrombocythemia**

The most recent 2016 WHO revisions classify ET as having four major criteria or three major criteria and one minor criteria. The major criteria for ET include platelet counts in excess of or equal to  $450 \times 10^9/L$ , proliferation of bone marrow megakaryocytes and loose clusters, Janus Kinase 2 (JAK2)/Calreticulin (CALR)/myeloproliferative leukemia virus oncogene (MPL) mutations, and not meeting the criteria of other myeloid neoplasm. The minor criteria required another clonal marker present (Tefferi and Barbui 2019). Other clonal markers that may aid in the diagnosis of ET is a polyclonal activation pattern in T cells but not granulocytes or platelets (Briere and El-Kassar 1998). Ruling out the possibility of reactive thrombocytosis from secondary causes such as trauma or infection that may cause platelet counts higher than  $450 \times 10^9/L$  is another alternative minor criterion. A bone marrow biopsy is the most accurate approach for diagnosing and distinguishing ET from other MPNs (Tefferi and Barbui 2019). This criteria is significantly different from that of the 2001 revision in that the platelet count was required to be in excess of  $600 \times 10^9/L$  and that of the 2008 revision in which the JAK2-V617F mutation was only required for PV diagnosis (Shallis et al. 2020).

Diagnosis of ET in patients presents as characteristic elevated platelets and potentiates risk of thrombosis and an increased risk of aggregation of platelets (Pedersen et al. 2018). Figure 2 illustrates the increased number of platelets characteristic of ET. It is worth noting that while blood smears are not a single determinant of ET, they are used for initial diagnosis of thrombocytosis with a confirmation by bone marrow biopsy. It is from

this point that the doctor may begin diagnosis of ET through the given criteria and elimination of other MPNs.



**Figure 2. Peripheral Blood Smear of Patient with ET.** The image shows a Wright's stain taken from a patient with ET. There are notably higher platelet counts at the time of diagnosis. A few examples of platelets have been indicated by arrows. Image taken from Byun et al. 2014.

### **Megakaryocyte and Platelet Production Before Essential Thrombocythemia**

To understand the nature and treatment of ET it is best to understand megakaryocyte and platelet production under normal circumstances. Thrombopoietin is the growth-factor responsible for the megakaryocyte lineage (Deutsch and Tomer 2006). The binding of thrombopoietin to the c-Mpl receptor signals the maturation of megakaryocyte progenitors which give rise to platelets (Deutsch and Tomer 2006). Megakaryocytes are

produced in the bone marrow from hematopoietic stem cells (Deutsch and Tomer 2006). Regulation of megakaryocyte differentiation occurs through thrombopoietin at the c-Mpl receptor, in response to decreased platelets that bind free thrombopoietin, preventing its binding to the c-Mpl receptor (Deutsch and Tomer 2006). It is important to understand this fundamental mechanism of megakaryocyte and platelet production as it is key in the overproduction of platelets characteristic with ET.

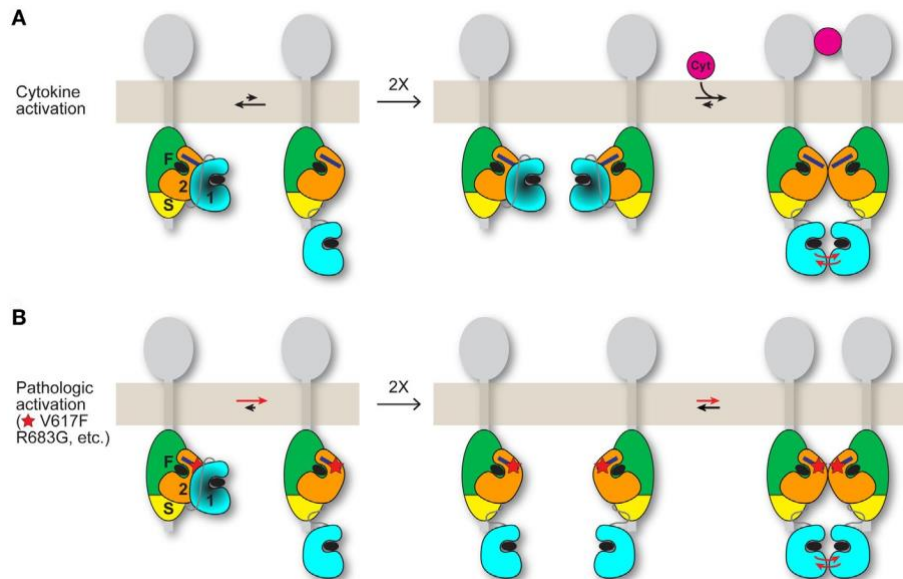
### **Mutations and the Molecular Mechanisms of Essential Thrombocythemia**

The first major break-through in the treatment of ET occurred with the differentiation between CML and the other three MPNs. CML is a Philadelphia chromosome positive disorder while ET is a Philadelphia chromosome negative disorder (Shallis et al. 2020). The translocation of the short arms of chromosome 22 to chromosome 9 results in a shorter than normal chromosome 22, giving rise to the new gene, BCR-ABL1 (Arber et al. 2016). This mutation gives rise to hematopoietic stem cell proliferation through the tyrosine-kinase pathway in CML. ET mutations give rise to similar hematopoietic stem cell proliferation but act through different mutations all of which activate the Janus kinase pathway (Arber et al. 2016).

The Janus Kinase family (JAK) consists of three JAKs (1-3) and Tyrosine kinase 2 (Hubbard 2018). Together they serve as activators through important cytokine receptors such as interleukins, interferons, growth hormones, erythropoietin and leptin (Hubbard 2018). When one of these signals binds to the extracellular receptor of a JAK, dimerization between the two JAK components occurs (Hubbard 2018). Trans-phosphorylation of the two dimer pieces activates the JAKs which can then phosphorylate signal transducer and

activator of transcription (STAT) (Hubbard 2018). STAT then can enter the nucleus and cause changes in transcription which may upregulate activity (Hubbard 2018).

One of ETs characteristic mutations needed for diagnosis is a JAK2 mutation at the JAK2 V617F location of hematopoietic cells (Arber et al. 2016). The pathological nature of this mutation is thought to have relevance in the JH2 domain (orange region) of JAK2, as seen in Figure 3 below (Hubbard 2018). JH1 (blue region) is the domain responsible for trans-phosphorylation and therefore stabilization of the JAK2 (Hubbard 2018). The JH2 domain acts as the autoinhibitory mechanism when there are cytokines (Hubbard 2018). In the presence of the JAK2 V617F mutation when there are no cytokines the JH2 domain is thought to act as mediator for dimerization of the JAK2 (Hubbard 2018). This in turn will activate JH1 and the JAK pathway (Hubbard 2018). The activation of the JAK2 pathway leads to an increase in the cellular proliferation lines of hematopoietic cells (Salhorta and Oo 2014). In the presence of the JAK2 V617F mutation the increased production of hematopoietic cells can be associated with elevated platelet counts, leukocytosis, and bone marrow fibrosis that is commonly seen in MPNs (Salhotra and Oo 2014).



**Figure 3. Mechanism of JAK2 and V617F Mutation.** Part A of the image shows the addition of cytokines (magenta) upon the extracellular JAK2 regions (grey) to induce the change in a normal functioning JAK2. The four domains of the JAK2 are FERM (green), SH2L (yellow), JH2 (orange), and JH1 (blue). Part B shows the capacity for the V617F (red star) mutation to stabilize the activated JAK2 through the JH1 domains in the absence of cytokines and initiate the phosphorylation sequence. The red arrow indicates the preference of the JH1 domain to remain active in the presence of the mutation. The mutation creates a ligand independent JAK. Image adapted from Hubbard et al. 2018.

There are other mutations though that can arise and result in diagnosis of ET in a JAK2 V617F negative patient. These mutations can occur in calreticulin (CALR) or myeloproliferative leukemia virus oncogene (MPL) as driver mutations (Araki and Komatsu 2020). The CALR mutation was discovered in 2013 and its role in ET has since been defined. The CALR mutation is a calreticulin (CALR) that encodes for a mutant chaperone located in the endoplasmic reticulum (Araki and Komatsu 2020). The mutation is caused by a frameshift in exon 9 from either nucleotide insertion or deletion and results in a gain of function which acts to inhibit the proline-rich section that typically would inhibit the thrombopoietin receptor MPL (Araki and Komatsu 2020). The domain at which

the frameshift occurs is the C-terminus where the wild-type CALR contains a series of negatively charged amino acids; the mutant form of CALR is replaced by positively charged amino acids (Araki and Komatsu 2020).

The CALR mutation involving chromosome 9 acts by granting cytokine-independent growth to cells through the thrombopoietin receptor which is activated by thrombopoietin (TPO) (Araki and Komatsu 2020). In the wild-type, MPL would be inhibited by a proline-rich segment on the gene product but, the CALR mutation removes this inhibition through the addition of another in-gene inhibitor that acts to block the proline-rich segment's activity (Araki and Komatsu 2020). The increased thrombopoietin receptor activation will cause activation of the JAK2 pathway which is independent of the JAK2 V617F mutation (Araki and Komatsu 2020).

Mutations in the thrombopoietin receptor gene (MPL) may also occur independently of the CALR mutations. The MPL mutation can occur at many different locations within the CALR gene, with W515L and W515K being the most common and W515A, W515R and S505N being the least common (Elsayed, Ranavaya, and Jamil 2019). These mutations have been shown to increase the receptor sensitivity to TPO (Elsayed, Ranavaya, and Jamil 2019). The activation of hypersensitive MPL receptor will now activate the JAK2 pathway when TPO binds. This in turn will produce the upregulation of megakaryocytes, platelet production, and other hemopoietic stems cells independently of the amount of the TPO cytokine (Lambert et al. 2012).

<b>Wild-type Gene</b>	<b>Gene Product</b>	<b>Gene Function</b>	<b>ET-associated Mutation</b>	<b>Effect of Mutation on structure</b>	<b>Effect of Gene Mutation on Hematopoietic Stem cell Proliferation</b>
JAK2 (No V617F)	Inactive JH1 domain in the absence of cytokines	Selective activation of hematopoietic stem cells in the presence of cytokine growth-factors via auto-phosphorylation of JH1	JAK2 V617F	Stabilization of JH1 domain in the absence of cytokines to induce auto-phosphorylation	Increased hematopoietic cell proliferation and lineages
CALR (negatively charged amino acids in C-terminus of gene product)	Encodes for a calreticulin protein and chaperone in ER	Calcium regulation via calreticulin and chaperone, cell growth and proliferation, cell migration, cell adhesion, and apoptosis	CALR (frameshift insertion or deletion resulting in positive amino acid in the C-terminus)	Addition of an auto-inhibiting segment that acts on the inhibitor of the MPL thrombopoietin receptor to allow direct interactions with CALR and MPL	Increased hematopoietic stem cell proliferation and lineage via MPL and CALR mutant interactions
MPL	Sensitive thrombopoietin receptor	Control of platelet count and blood cell production	W515L, W515K, W515A, W515R, S305N	Decreased sensitivity to thrombopoietin	Production of abnormal megakaryocytes

**Table 1. Overview of ET-associated mutations and Wild-type Genes.** The table illustrates the different genetic mutations that are associated with ET as well as the wild-type function and production. The mutations have similar results of ET symptomology with elevated platelet counts but the pathways and functions of the mutations vary.

### **Prognosis and Etiology of Essential Thrombocythemia**

In order to estimate survival rates of patients with ET doctors have created the International Prognostic Score for ET (IPSET). The IPSET uses a series of diagnostic factors to create a score for the severity of risk based on a point system. Age, leukocytosis, and history of thrombosis are the key features in the IPSET score but other independent

factors such as sex have also been identified to shorten survival of patients with ET (Tefferi et al. 2017).

ET may occur at any age but is predominantly diagnosed between the ages of 65-70 years old (Brière 2007). A more recent publication found that the average age of diagnosis is decreasing to around 58 years. The study used data collected from 1076 patients in the Mayo Clinic (Tefferi and Pardanani 2019). These discrepancies in data may be due to changes in the diagnostic standards of the WHO and recent updates in technology. Age of diagnosis does seem to show a significant difference in prognosis though. Those diagnosed at age 60 years and older showed markedly decreased life spans and are placed into high risk categories for thrombosis and leukemic transformations (Tefferi and Pardanani 2019). A diagnosis at age of 50 years and younger led to a normal life span and appeared to have no significant threats within the first decade following diagnosis, however, longer follow ups did reveal shorter lifespans and more complications than their healthy counterparts (Tefferi et al. 2017). The Mayo Clinic study on MPNs revealed that patients with ET may have an average overall survival of 18 years (Szuber et al. 2019). As data is compiled and more risk assessments completed, it can be concluded that the life span and survival of ET patients are significantly shortened when compared to those without ET.

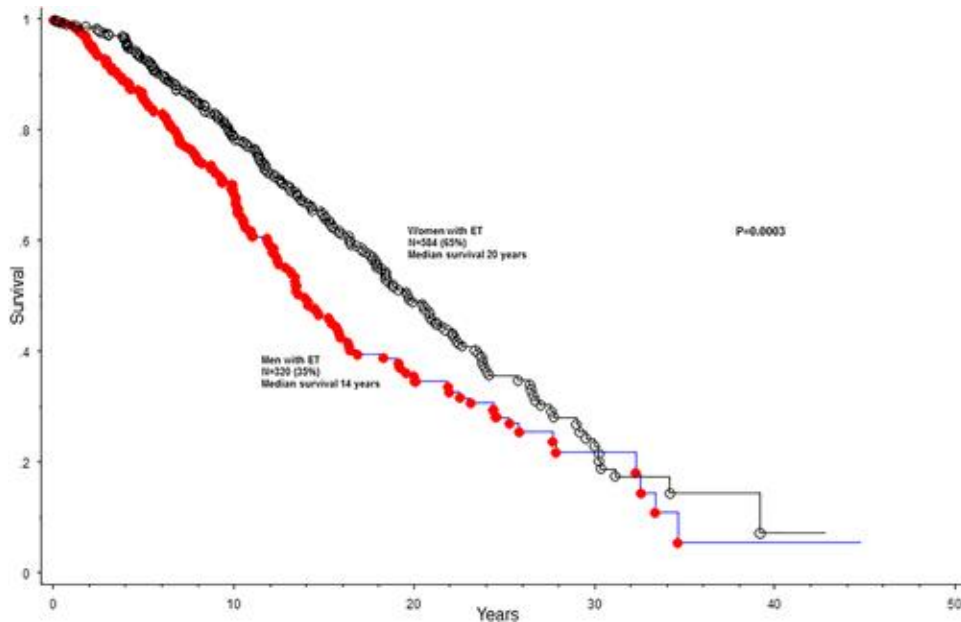
One major risk that ET patients face is the threat of thrombotic complications. It therefore makes sense that a previous history of thrombosis would place patients in higher risk categories and shorten the survival chances of these patients (Tefferi 2012). One study performed by Stefano et al. (2008) showed that after one thrombotic event the likelihood

of a reoccurrence was 5.9% in patients <60 years old and 8.9% in patients >60 years old (Stefano et al. 2008). Thrombotic events for this study were defined as ischemic strokes, transient ischemic attacks, acute myocardial infarction, unstable angina pectoris, peripheral arterial thrombosis, retinal artery/vein occlusion, deep venous thrombosis, and pulmonary embolism (Stefano et al. 2008). A patient with an age greater than 60 years old and history of thrombotic events has a 1.7 times increased risk of reoccurrence (Stefano et al. 2008). The study used a retrospective design, following patients between the years of 1985 to 2005 at major hematology centers (Stefano et al. 2008). A thrombotic event was required to be reported if it occurred within the 2 years before the diagnosis of the patient or after the referral to the hematology center (Stefano et al. 2008). Their data concluded that major risk factors for a reoccurrence of thrombotic events and therefore a decreased prognosis was an age over 60 years with prior history of thrombosis (Stefano et al. 2008).

Hyperleukocytosis, a white blood cell count of  $15 \times 10^9/l$ , was reported to be associated with a worse prognosis if found at time of diagnosis with ET (F. Girodon et al. 2010). Gangat et al. (2007) found similar results as well as confirmed the strong correlation between low hemoglobin and hyperleukocytosis in predicting a low prognostic value for ET patients. Based on a multivariate analysis, leukocytosis and low hemoglobin, used together with the age variable and a platelet count of  $\geq 1000 \times 10^9/l$ , showed a lower prognostic score than when compared to patients lacking these (Gangat et al. 2007).

Gender also plays a key role in the prognostic score of ET patients. Women, particularly those diagnosed at older ages, were found to have a longer survival rate and better prognostic score and less complications resulting in premature death, than their male

counterparts (Tefferi et al. 2017). The findings were further indicative of a difference between sexes based on a multivariate analysis of the IPSET risk categories comparing high-risk, intermediate-risk, and low-risk men and women with ET (Tefferi et al. 2017). In the high-risk IPSET category, men were shown to have the highest survival disadvantage when compared to their women counterparts (Tefferi et al. 2017). Women were more likely to receive a diagnosis though than men with 67% of the patients diagnosed in a Mayo Clinic study being women (Tefferi and Pardanani 2019). It is estimated that ET's ratio is two females to every one male (Brière 2007). While gender does seem to be a factor in the prognostic score of ET, there is no evidence that it has any significance in the leukemic transformation of ET (Gangat et al. 2007).



**Figure 4. Survival Years by Gender of Patients with ET.** This graph indicates the years of survival after diagnosis of males (red) and females (black) with ET. The median population age of the surveyed 904 patients was 58 years old (18-96 years old) and 65% of the population was female. Women appear to be diagnosed later than their male counterparts with an average female diagnosis occurring between 58 to 60 years of age and a male diagnosis occurring around 56 to 58 years of age. The median survival after diagnosis of ET is 14 year in men and 20 years in women. Image taken from Tefferi et al. 2017.

### **Mutation's Differing Effects on the Prognosis and Etiology of Essential Thrombocythemia**

Each mutation profile can yield a different subtype of ET in patients and present with different stratifications of the disorder. Kim et al. (2015) sought to analyze the different mutation profiles and disease subgroups. In the 199 patients that the study reviewed 79 were diagnosed with ET (Kim et al. 2015). The mutation profiles of these patients showed that 50 (63.3%) had the JAK2 V617F mutation, 9 (11.4%) had the type 1

CALR frameshift, 5 (6.3%) had the type 2 CALR frameshift, only 2 (2.5%) had the MPL mutation, and 13 (16.5%) were triple negative (Kim et al. 2015).

Patients with the CALR mutations had profiles that tended to be more male predominant with lower leukocytosis and hemoglobin levels, but higher platelet counts than patients with JAK2 mutations (Kim et al. 2015). These values were compared to their JAK2 mutated counterparts. The CALR mutation profile placed ET patients in a lower risk category on the prognosis scale (Kim et al. 2015). The results from Kim et al. (2015) supports a similar study conducted by Rumi et al. (2014). The study showed that patients with the JAK2 mutation were in fact older, had higher leukocytosis and hemoglobin levels, and lower platelet counts when compared to their CALR counterparts (Rumi et al 2014). This study reviewed 745 ET patients of whom, 466 (62%) had the JAK2 V617F mutation, 176 (24%) had the CALR exon 9 mutation, 28 (4%) had the MPL exon 10 mutation, and 75 (10%) were triple negative (Rumi et al. 2014). It is worth noting that this study determined a significant proportion of ET patients with the JAK2 V617F mutation progressed to PV while none of the CALR patients did. This finding shows that the CALR mutation results in a different disease profile from that of the JAK2 mutation (Rumi et al. 2014). Further research on treatment efficacy in patients stratified by their mutation profiles is needed to determine if the optimum treatment should differ based on mutational subtypes.

<b>Study</b>	<b>JAK2 V617F</b>	<b>CALR (type 1 and 2)</b>	<b>MPL Exon 10</b>	<b>Triple Negative</b>	<b>Total # Patients</b>
Kim et al.	50 (63.3%)	14 (17.7%)	2 (2.5%)	13 (16.5%)	79
Rumi et al.	466 (62%)	176 (24%)	28 (4%)	75 (10%)	745

**Table 2. Comparison of Mutation Profiles from Two Separate Studies.** The table above illustrates the similar percentages of mutation types found in patients with ET from two different studies despite the smaller sample size of the first study. The geographic locations of the studies varied differed as well with Rumi et al. conducting their study in Italy and Kim et al. conducting their study in South Korea.

### **An Overview of the Treatments for Essential Thrombocythemia**

When viewed in clinical practice, the physician may have a few options for the treatment of ET. As seen earlier in the risk stratification levels determined by the IPSET criteria, a patient may be low risk, intermediate risk, or high risk (Tefferi and Pardanani 2019). Each stratification comes with its own diagnosis and prognosis; therefore, it should make sense that treatment will vary with each level.

If the patient presents at the lowest level of risk stratification with little to no risk of a cardiovascular event, the clinician may decide on only monitoring the current platelet and hemoglobin counts (Tefferi and Pardanani 2019). Should the patient move to a higher risk level, the clinician may select a treatment of low-dose aspirin and hydroxyurea to reduce risk of thrombosis, as this combination produces efficacy with lower side effects than other cytoreductive therapies (Tefferi and Pardanani 2019).

Should a patient acquire intolerance to hydroxyurea as a first-line drug, there are other treatment options available for ET (Tefferi and Pardanani 2019). Anagrelide is a second-line drug approved by the FDA for treatment of ET in the event of intolerance to hydroxyurea (Tefferi and Barbui 2019). Based on several comparative studies of anagrelide

vs hydroxyurea, it has been determined that hydroxyurea is the better choice as a first-line drug for ET (Tefferi and Pardanani 2019).

In more recent years as research on ET has increased, many new drugs have begun to surface as potential candidates for treatment. Pegylated Interferon-alfa 2a is one such drug that causes a substantial reduction in hematocrit levels, but the risk of side effects is still being assessed (Tefferi and Barbui 2019). Alkylating agents like busulfan have often been considered a last resort option for patients with advanced ET and hydroxyurea intolerance (Tefferi and Barbui 2019). Other potential candidates include JAK inhibitors like ruxolitinib and momelotinib as well as telomerase inhibitors like imetelstat (Tefferi and Barbui 2019). Histone-deacetylase inhibitors are also novel drug candidates that have been shown to reduce ET allele burden and other symptoms (Gunawan et al. 2018). The allelic burden is a concept referring to the proportion of mutant genes to wild-type in the JAK2 hematopoietic stem cells. Higher counts of mutated cells lead to a higher risk of symptoms (Passamonti and Rumi 2019). Reducing the proportion of mutated hematopoietic stem cells lowers the population of abnormal cells that lead to excessive platelet counts and increased risk of thrombosis (Passamonti and Rumi 2009).

It is important to note that due to its low prevalence, ET falls under the category of an “orphan disease” and is therefore covered by the Orphan Drug Act. According to the FDA an orphan disease is one that effects fewer than 200,000 patients and thus qualifies for tax cuts and 50% payment of research on drug treatments through federal grants and programs (Commissioner 2019). This federal program incentivizes research for rare diseases that may not be able to attract large nongovernmental funding. Almost all of the

drugs for ET fall under the Orphan Drug Act, spurring more research for treatment discoveries.

## **EVALUATION OF CURRENT AND POTENTIAL DRUG TREATMENTS**

### **Low-Dose Aspirin and Cytoreductive Hydroxyurea**

The standard first-line treatment for essential thrombocythemia is administration of low-dose aspirin (81 mg) and hydroxyurea because of their low side effect profiles, good efficacy, oral administration route, and low intolerance rates (Tefferi and Pardanani 2019). Patients at the low risk level of ET are usually monitored by health care providers or started on a daily low-dose treatment of aspirin alone (Tefferi and Pardanani 2019). In more severe cases of ET the doctor may decide to place the patient on a daily regimen of hydroxyurea and low-dose aspirin (Tefferi and Pardanani 2019).

Aspirin inhibits platelet activation while hydroxyurea targets DNA synthesis directly. Once taken, the mechanism of action of hydroxyurea involves a conversion to a free radical nitroxide which is taken up by proliferating megakaryocytes (Yarbro 1992). The free radical binds at the active site of ribonucleotide reductase in the proliferating megakaryocytes preventing the formation of deoxyribonucleotides from ribonucleotide precursors and therefore selectively inhibiting DNA synthesis (Yarbro 1992). Cell death during the S phase is produced and there is a reduction in the number of circulating megakaryocytes, termed cytoreduction (Yarbro 1992). Hydroxyurea also works to inhibit DNA repair (Yarbro 1992). The mechanism for aspirin involves the acetylation of cyclooxygenase in the platelets (Schrör 1997). The acetylation of the serine in the cyclooxygenase prevents arachidonic acid from binding at the binding site and forming thromboxane (Schrör 1997). Without thromboxane the platelets cannot be activated and

aggregation cannot occur (Schrör 1997). This irreversible, long-lasting inhibition of platelet cyclooxygenase helps prevent the formation of thromboses.

In recent studies of low-dose aspirin and ET there has been much debate on the exact regimen to follow for required platelet suppression. The treatment of ET following a thrombotic event may change the drug of choice for platelet suppression (Landolfi and Gennaro 2008). Clopidogrel may be a drug of choice following an acute coronary syndrome while dipyridamole may help in the event of ischemic strokes or transient ischemic attacks (Landolfi and Gennaro 2008). The safety and efficacy of these drugs has not been assessed in enough detail with ET patients taking cytoreductive drugs and more data are needed (Landolfi and Gennaro 2008). Once daily low-dose aspirin defined as 81 mg, while a useful treatment, seems to lack the ability to combat the high platelet turnover of ET (Larsen et al. 2019). Studies on patients with ET have shown that a regimen of low-dose aspirin given twice daily may prove more beneficial. In one such study by Larsen et al. (2019), they were able to show that twice daily low-dose aspirin increased platelet inhibition, proving to have a more consistent effect than once-daily treatments in patients with ET. Their data compared ET patients using first the once daily treatment for 7 days and then, after a 14-day aspirin washout, a twice daily 7-day regimen (Larsen et al. 2019). The findings showed lower thromboxane B2 and platelet aggregation in the group treated twice daily (Larsen et al. 2019). Aggregation was assessed using whole blood impedance aggregometry with arachidonic acid as the agonist and thromboxane B2 was measured using an enzyme linked immunosorbent assay (Larsen et al. 2019). Another similar study by Rocca et al. (2020), used three dosing regimens in patients with ET; once daily, twice

daily, and three times daily low-dose aspirin. Their study showed that the twice daily and thrice daily regimen significantly reduced thromboxane B2 when compared with the once daily regimen after two weeks of 100 mg daily dosing (Rocca et al. 2020). The study further showed that while the thrice daily did reduce thromboxane B2 more than the twice daily, the abdominal discomfort score for the thrice daily regimen was higher (Rocca et al. 2020). Their data indicate that once daily dosing regimen for patients with ET is not as effective in reducing platelet activation as the twice daily and any further reductions in dosing regimen did not significantly reduce platelet activation (Rocca et al. 2020). While both studies failed to report any evidence on major side effects outside of abdominal pain, Bhatt et al. (2008) showed some of the adverse side-effects associated with low-dose aspirin. Patients who take low-dose aspirin should be aware of an increased risk of upper gastrointestinal bleeding and abdominal discomfort. Dosing over 81 mg for long-term use may not be recommended (Bhatt et al. 2008).

In a study conducted by Rodriguez et al. (1998) on the pharmacokinetics of orally administered hydroxyurea compared to intravenous hydroxyurea in 29 patients with solid malignancies, the  $T_{max}$  was found to be 1.22 hours with a delay in appearance of 0.22 hours for the oral drug (Rodriguez et al. 1998). This indicates a relatively rapid absorption rate with the peak plasma concentration reaching 794  $\mu\text{mol/L}$  after a 2000-mg oral dose (Rodriguez et al. 1998). The bioavailability for oral hydroxyurea was found to be complete at around 100% (Rodriguez et al. 1998). Hydroxyurea was excreted via renal and nonrenal methods and has a clearance of 7.7 L/hour (Rodriguez et al. 1998). The distribution was found throughout all tissues but was concentrated in erythrocytes and leukocytes

(Rodriguez et al. 1998). Drug in the plasma was 75-80% bound to plasma proteins (Rodriguez et al. 1998). The dosing regimen for this study used 80 mg every three days for three weeks totaling to 2000 mg orally (Rodriguez et al. 1998).

Hydroxyurea is typically given in dosages ranging from 15-20 mg/kg/day with the appropriate dosing regimen dependent on platelet values (Finazzi and Barbui 2001). Studies show that the median length of time for the onset of treatment efficacy varies depending on platelet counts but high risk patients were reported to have satisfactory platelet counts after a median of 30 days (Cortelazzo et al. 1995).

Toxicity and severe adverse side effects due to hydroxyurea are reportedly rare but more recent studies have shown this may not always be the case. Some studies have analyzed the claims of hydroxyurea increasing the transformation rate to acute leukemia. The findings of Finazzi and Barbui (2001) indicate the risk of acute leukemic transition in older patients with high-risk profiles is outweighed by the potential benefits of hydroxyurea. There is still much debate over the use of hydroxyurea in younger patients with low-risk profiles though (Finazzi and Barbui 2001). Nielsen and Hasselbach's (2003) research on leukemic transformation used data collected from 83 patients, 58 of these patients were treated with hydroxyurea and 18 of them had received hydroxyurea and busulphan. Follow-ups were conducted for an average of 7.8 years and any incidence of transformation to acute myeloid leukemia or myelodysplasia was noted (Nielsen and Hasselbalch 2003). Their research suggests that hydroxyurea has leukemogenic tendencies and increases risk of acute myeloid leukemia transformation by 14% (Nielsen and Hasselbalch 2003). This risk is severely increased to 30% if busulphan, another form of

chemotherapy, is used before treatment with hydroxyurea (Nielsen and Hasselbalch 2003). They concluded that hydroxyurea is not recommended in younger patients due to acute leukemic transformation after extended usage (Nielsen and Hasselbalch 2003).

Hydroxyurea has also been associated with cutaneous ulcers and lesions after continued long-term use (Quattrone et al. 2013). These side effects may become extremely relevant in younger patients diagnosed with ET who start hydroxyurea at young ages (Quattrone et al. 2013). Hydroxyurea most often affects those tissues with high cellular turnover and can lead to startling adverse effects despite doctor's claims to its safety (Quattrone et al. 2013). A study published by Quattrone et al. (2013) shows a multitude of cutaneous ulcerations in ET patients caused by underlying vasculopathy to lower extremities. The vasculopathy results in increased ulcerations and poor survival rates (Quattrone et al. 2013). There are several case studies that have reported cutaneous lesions on patients diagnosed with MPNs and started on hydroxyurea. Most of these patients were diagnosed relatively young (late 40s to early 50s) and had started long term hydroxyurea treatment before the lesions had appeared (Saravu et al. 2006). The span of treatment time with hydroxyurea ranged from 2-7 years and had an accumulated dosage of 1-3kg before the lesions were found (Saravu et al. 2006). When taken off hydroxyurea, all patients demonstrated healing of the ulcers (Saravu et al. 2006). In a review conducted by the Mayo Clinic, data showed 14 patients developing ulcers after an average of 6-years use of hydroxyurea (Best et al. 1998). Multiple ulcers were shown to have appeared in over half the patients and when hydroxyurea was ceased the ulcers healed (Best et al. 1998). While not all patients on hydroxyurea develop cutaneous lesions and ulcers, it is clear that after

long-term usage patients are at an increased risk of this adverse dermatologic effect (Quattrone et al. 2013). The damage caused to DNA repair may play a key role in the development of cutaneous ulcers in patient taking hydroxyurea (Quattrone et al. 2013).

Some lesser adverse effects caused by hydroxyurea may occur as well. Although they are subtle and many are not life threatening, they do appear more commonly than cutaneous lesions and acute leukemic transformation. Rodriguez et al. (1998) reported patients most often experiencing nausea/vomiting, as well as diarrhea, and less frequently neutropenia and thrombocytopenia. The findings suggests that these effects, ranging from mild to moderate, appeared in a little over half the patients monitored (Rodriguez et al. 1998).

Hydroxyurea and low-dose aspirin are commonly used together in treatment of ET (Tefferi and Barbui 2019). Recent studies have looked into the effect of cytoreduction alone vs cytoreduction in combination with low-dose aspirin to analyze the potential risks and benefits of the two treatment options. Alvarez-Larrán et al. (2013) conducted one such study, viewing 247 high-risk ET who had no prior history of a thrombosis occurrence. The occurrence of a thrombosis event was used as the dependent variable to monitor the effect of the cytoreduction alone vs the low-dose aspirin and cytoreduction (Alvarez-Larrán et al. 2013). Their findings indicated that the likelihood of thrombotic events was reduced slightly, but not significantly, by the addition of low-dose aspirin to hydroxyurea (Alvarez-Larrán et al. 2013). Patients on cytoreduction alone had an average of 24.4 thrombotic events per 1000 person-years while those in the combination therapy had 14.4 events every 1000 person-years (Alvarez-Larrán et al. 2013). They then conducted the same study in

another cohort aged 60 years and older (Alvarez-Larrán et al. 2013). The results were surprisingly different and showed a significant decrease in thrombotic events for low-dose aspirin and cytoreduction treatment together (Alvarez-Larrán et al. 2013). The event rate was 8.6 for the combination vs 29.2 for hydroxyurea alone per every 1000 person-years (Alvarez-Larrán et al. 2013). It was concluded from these data that cytoreductive treatment with low-dose aspirin is beneficial in patients with ET over 60 in preventing thrombotic events.

The same study also reviewed the side-effect risk differences of cytoreduction alone vs when paired with low-dose aspirin. Alvarez-Larrán et al. (2013) discovered that the threat of major bleeding on low-dose aspirin was a severe problem despite the efficacy in preventing thrombotic events. The incidence of bleeding was 14.4 on low-dose aspirin and cytoreduction and 1.4 without low-dose aspirin (Alvarez-Larrán et al. 2013). Similar values were reported for the 60 and over cohort. The study showed 12 patients had an incidence of major bleeding and two of them died as a result (Alvarez-Larrán et al. 2013). The patients were retrospectively studied with follow ups of 763 person-years for cytoreduction and low-dose aspirin and 685 person-years for cytoreduction alone (Alvarez-Larrán et al. 2013).

### **Cytoreductive Treatment Using Anagrelide**

Anagrelide is the second drug of choice in the event of intolerance to hydroxyurea. The mechanism of action by which anagrelide reduces platelet counts in ET patients involves megakaryocytes (Tefferi et al. 1997). It appears that anagrelide inhibits

megakaryocyte differentiation and produces prolonged phosphorylation of the eIF2 $\alpha$  by potentially inhibiting phosphoprotein phosphatase (Ahluwalia et al. 2015). The prolonged phosphorylation of eIF2 $\alpha$  causes upregulation of downstream signals which induce the cellular stress response (Ahluwalia et al. 2015). Induction of the cellular stress response in megakaryocytes leads to cellular apoptosis and thus cytoreduction (Ahluwalia et al. 2015). This is different from that of hydroxyurea which acts to inhibit early megakaryocyte proliferation. Anagrelide may also act through inhibition of the GATA-1 and FOG-1 upstream regulators associated with megakaryocyte development thus inhibiting the production of platelets (Ahluwalia et al. 2015). Increased levels of thrombopoietin, a cytokine associated with the regulation of platelet growth, has confirmed this theory. Thrombopoietin acts as a ligand, binding to receptors on platelets and undergoing endocytosis (Palmlblad et al. 2008). Fewer platelets in circulation results in more thrombopoietin in circulation (Palmlblad et al. 2008). Spencer and Brogden (1994) also showed that another mechanism may be key in protecting high-risk ET patients from thrombosis. They further examined the effects of anagrelide on platelet aggregation and shape. The inhibition of cyclic adenosine monophosphate (cAMP) phosphodiesterase by anagrelide decreases the enzymatic breakdown of cAMP raising its level in platelets (Spencer and Brogden 1994). Increased cAMP prevented the aggregation of platelets and thus decreased risk of thrombosis (Spencer and Brogden 1994). These studies were only carried out in animals due to the high dosage required to induce these changes (Spencer and Brogden 1994).

Anagrelide undergoes rapid absorption when given orally and its effects and adverse reactions are thought to be directly linked to dosage. The starting doses of anagrelide vary by country with the United States giving the highest dose of 2.0 mg/day and Japan and Europe recommending 1.0 mg/day while even lower doses of 0.5 mg/day may be given (Hashimoto et al. 2020). Besses et al. (2012) conducted a study to analyze the pharmacokinetics of anagrelide in younger (18-50) vs older (65+) patients. To account for dosing differences in patients, the researchers normalized plasma concentrations of anagrelide and 3-hydroxy-anagrelide to a dose of 1 mg twice daily (Besses et al. 2012). Their results indicated that anagrelide had a higher concentration maximum ( $C_{max}$ ) of 3.63 ng/ml in the older patients than the 2.66 ng/ml shown in younger patients as well as a higher area under the curve ( $AUC_T$ ) which was 10.3 ng x h/ml vs 6.4 ng x h/ml respectively (Besses et al. 2012). There was no significant difference in anagrelide's half-life between elderly and young patients (Besses et al. 2012). Differences in anagrelide concentrations may be due to a lesser first pass effect in elderly patients (Besses et al. 2012). The distribution for anagrelide was found to be widespread through-out all tissues with a volume distribution ( $V_d$ ) of about 139-277 L in healthy adults and normalized to 12 L/kg (Besses et al. 2012). It is key to note that the first pass metabolism of anagrelide by hepatic CYP1A2 produces a more potent active metabolite, 3-hydroxy-anagrelide (Besses et al. 2012). The bioavailability of oral anagrelide was found to be around 70% and the time to peak plasma level was between 1 to 2 hours for both anagrelide and 3-hydroxy-anagrelide (Besses et al. 2012). The half-life of anagrelide is 1.7 hours while the half-life of its more potent metabolite is 3.9 hours (Wagstaff and Keating 2006). Due to the nature of 3-

hydroxy-anagrelide it is important to also look at its effects. Besses et al. (2012) found that the peak plasma concentration of 3-hydroxy-anagrelide was higher in younger patients with a  $C_{max}$  of 7.26 ng/ml vs 4.19 ng/ml in elderly patients. The  $AUC_T$  was also higher in younger patients, 27.6 ng x h/ml vs 17.4 ng x h/ml seen in the elderly patients (Besses et al. 2012). Surprisingly though, the half-life of 3-hydroxy-anagrelide was found to be significantly longer in elderly patients (Besses et al. 2012). Clearance of 3-hydroxy-anagrelide is also achieved by CYP1A2 (Besses et al. 2012). Their findings indicated, however, that based on platelet counts there was no reason to adjust the dosages of anagrelide due to age (Besses et al. 2012). Reports for the average time of satisfactory platelet counts were concluded to be around 3 to 4 weeks following the initiation of daily dosing (Mazur et al. 2004).

The onset time and risk of adverse effects of anagrelide are thought to be directly dependent on the dose. In a study by Ito et al. (2019) the efficacy and safety of anagrelide was reviewed. They reported slightly lower adverse effects than previous studies and theorized the cause to be a slightly lower dosage of 1.44 mg/day unlike other studies which used higher dosages (Ito et al. 2019). Anagrelide is often used as the second line defense for ET due to its adverse side effects (Tefferi and Barbui 2019). Older patients may experience more severe side effects of several types (Wagstaff and Keating 2006). Side effects that are commonly seen in patients may range from headaches, nausea, diarrhea, and abdominal pain to more severe cardiac effects like palpitations and sometimes heart failure (Wagstaff and Keating 2006). The severity of these adverse side effects may diminish with continued usage of anagrelide (Wagstaff and Keating 2006).

Anagrelide is one of the more common second-choice alternatives prescribed by doctors despite studies proving its noninferiority in the treatment of ET (Tefferi and Barbui 2019). Its usage as a potential first-line therapy has been studied in the ANAHYDRET and EXELS trials comparing hydroxyurea to anagrelide, both alone and in combination with aspirin. These two studies were among the largest and most comprehensive, giving rise to valuable comparative information on the treatment of ET. The ANAHYDRET study was a phase 3 trial examining specifically the claim that anagrelide was equally efficacious and as tolerable as hydroxyurea. Their findings confirmed earlier proof that anagrelide is noninferior to hydroxyurea (Gisslinger et al. 2013). Over the course of the study, Gisslinger et al. (2013) noted that there was no significant difference in the treatment effects of anagrelide and hydroxyurea. The study observed 259 patients, 122 taking anagrelide and 137 taking hydroxyurea, for a total of 730 patient years (Gisslinger et al. 2013). Gisslinger et al. (2013) reviewed platelet counts, hemoglobin levels, leukocyte counts, and other related ET events at 6 months, 12 months, and 36 months. As compared to hydroxyurea treatment, anagrelide resulted in a similar platelet reduction, a slightly greater decrease in hemoglobin levels, and no change in leukocyte levels which decreased in the hydroxyurea cohort (Gisslinger et al. 2013). The analysis of ET related events revealed that major incidences of thrombosis were similar in two groups, 3.32% of anagrelide patients and 3.42% of hydroxyurea patients (Gisslinger et al. 2013). There was also no significant difference between minor events with 10.6% in anagrelide patients and 8.1% in hydroxyurea patients (Gisslinger et al. 2013). Gisslinger et al. (2013) did note that there was a slight difference in bleeding events, both major and minor, with anagrelide patients

having more bleeding events (Gisslinger et al. 2013). The direct mechanism of this side-effect is unknown, but it is believed that following an overdose of anagrelide, platelet function is severely inhibited (Spencer and Brogden 1994). Large doses of anagrelide have shown to increase cAMP levels and impair platelet aggregation and shape in animal studies resulting in bleeding (Spencer and Brogden 1994).

The EXELS study is, to date, the largest study conducted on the treatment of ET using anagrelide or hydroxyurea, both in combination with aspirin. A total of 3,598 patients were recruited with high-risk ET and monitored in a phase IV study (Jean-Jacques Kiladjian et al. 2013). The key focus of the study was to review the safety of long-term usage of anagrelide in high-risk ET patients (Jean-Jacques Kiladjian et al. 2013). Results of the study showed similar results to the ANAHYDRET study including higher incidence of hemorrhage (Jean-Jacques Kiladjian et al. 2013). The EXELS study noted the higher hemorrhage rates with anagrelide in combination with low-dose aspirin (Jean-Jacques Kiladjian et al. 2013). In an evaluation of EXELS, Birgegard et al. (2014) concluded that younger patients were able to tolerate the side effects associated with anagrelide better than their older counterparts and continued treatment longer (Birgegard et al. 2014).

All of the studies presented comparing anagrelide and hydroxyurea are in an agreement that hydroxyurea seems to be the best first-line choice of the two treatment options. In the meta-analysis by Samuelson et al. (2006), the researchers point out the key issue that the risk of bleeding is significantly increased when anagrelide is used in combination with low-dose aspirin. These data were supported by Birgegard et al. (2014) as well. The risk of transformation to myelofibrosis may also be increased in treatment

with anagrelide and low dose aspirin as shown in the Primary Thrombocythemia 1 study (Harrison et al. 2005). The mechanism behind this observation is still unknown and deserves further research (Harrison et al. 2005). Conflicting data published by Kellner et al. (2020) showed that over a 10-year study span of patients with ET anagrelide had lower thrombotic risk, higher overall survival, and higher progression-free cases than hydroxyurea and low-dose aspirin. Their results indicated that the lower thrombotic risk may be due to the lower incidence of minor thrombotic events in the anagrelide cohort (Kellner et al. 2020). The increased incidence of major arterial thrombotic events in the anagrelide cohort was theorized to be due to the lack of aspirin usage (Kellner et al. 2020). Kellner et al. (2020) also report twice as many hydroxyurea patients transforming to myelofibrosis but the Primary Thrombocythemia 1 study showed more patients in the anagrelide and low-aspirin group developing myelofibrosis (Harrison et al. 2005). The discrepancies in data may be due to the combination therapy of anagrelide and low-dose aspirin and patient characteristics (Kellner et al. 2020).

### **Potential and Current Usage of Interferons in the Treatment of Essential Thrombocythemia**

Interferon- $\alpha$  is one of the three main treatments for MPNs despite not being approved by the FDA for usage in ET. Clinical trials are currently underway to demonstrate the potential usage of interferons for ET. While the exact mechanism of interferons is still under some debate, research in recent years have revealed its key role in the treatment of MPNs. The mechanism of action through which interferons act is the suppression of

hematopoietic progenitor cell differentiation (Birgegård 2016). Interferon- $\alpha$  is the type 1 interferon that is most often used in treatment (J.-J. Kiladjian, Chomienne, and Fenaux 2008). Interferon- $\alpha$  has many target actions that may explain efficacy for MPNs. Those actions include suppression of megakaryopoiesis and the hematopoietic progenitors for erythrocytes and granulocytes (Stein and Tiu 2013). This action induces cyto-reduction of platelets and has been shown to reduce the JAK2V617F allelic burden (ratio of mutant genes to wild-type genes) that is sometimes associated with ET (Stein and Tiu 2013). As previously discussed in the section on mutations and molecular mechanisms of ET, interferons act as key signals for the activation of the JAK/STAT pathways (Hubbard 2018). The JAK2V617F mutation produces hematopoietic stem cells that are hypersensitive to interferon- $\alpha$  (Bywater et al. 2019). This hypersensitivity creates enhanced STAT1 signaling during interferon- $\alpha$  treatment (Bywater et al. 2019). Bywater et al. (2019) theorized the mechanistic reduction of the JAK2V617F allelic burden to be induced by interferon- $\alpha$  causing DNA damage to the JAK2V617F hematopoietic stem cells via specific activation of reactive oxygen species. In addition to the reduction of the JAK2V617F allelic burden, Kiladjian et al. (2008) showed the megakaryocytic lineage also had a unique specificity for interferon- $\alpha$ . Treatment with interferon- $\alpha$  inhibited thrombopoietin-induced signaling and thus decreased megakaryopoiesis (J.-J. Kiladjian, Chomienne, and Fenaux 2008). The inhibition and decrease of the megakaryocytes are associated with morphological and biochemical changes (J.-J. Kiladjian, Chomienne, and Fenaux 2008). This sensitivity to interferon- $\alpha$  also resulted in suppression of the MPN clones via an immune response (J.-J. Kiladjian, Chomienne, and Fenaux 2008). The action

of interferon- $\alpha$  is unique compared to anagrelide and hydroxyurea in that it may not only alter morphologies of megakaryocyte clones, but it may also lead to an immune response against the MPN clones.

The efficacy of interferon- $\alpha$  is similar to both those of anagrelide and hydroxyurea in terms of platelet reduction, but the onset of action may take longer (Birgegård 2016). Interferon- $\alpha$  has a high bioavailability after parenteral administration (Radwanski et al. 1987). The distribution for interferon- $\alpha$  is relatively similar to hydroxyurea but smaller than that of anagrelide at a mean estimate of 12 to 40 L (without a given bodyweight) and normalized for body weight at 1.4L/Kg in patients with hepatitis C (Radwanski et al. 1987). Lengfelder et al. (1996) reported that a daily dose of 3 mill IU produced a successful cytoreductive response in 90% of patients. In long-term maintenance studies of interferon- $\alpha$  it was found that of 35 patients who began 6-month induction period with 21 mill IU 90% of them had weekly platelet values under  $600 \times 10^9/L$  (Lengfelder, Griesshammer, and Hehlmann 1996). After the induction period they were placed on a maintenance program of 3 mill IU (Sacchi et al. 1991). Of the 35 patients 24% were required to take it daily, 61% only took it 3 times a week, and 15% took it once a week (Sacchi et al. 1991). Given that interferon- $\alpha$  has no oral formulation and must be administered via injection, adherence may be a disadvantage with this treatment form. The bioavailability of each injection area will also differ dependent on where it is injected. Intramuscular (IM) is 83%, subcutaneous (SubQ) is 90%, and intravenously is just under 100% (Radwanski et al. 1987). Radwanski et al. (1987) also demonstrated that 30 minutes after intravenous injection, interferon- $\alpha$  was distributed very rapidly with a distribution half-life of 2 hours.

The intramuscular and subcutaneous injections showed absorption half-lives of 2 to 3 hours (Radwanski et al. 1987). After the starting dose of  $5 \times 10^6$  IU/m<sup>2</sup>, interferon- $\alpha$  has an intramuscular C<sub>max</sub> of 42.1 IU/ml at six hours and a subcutaneous C<sub>max</sub> is 45.5 IU/ml at eight hours (Radwanski et al. 1987). Radwanski et al. (1987) also noted the elimination half-lives to be 2.2 hours intramuscularly and 2.9 hours subcutaneously (Radwanski et al. 1987). The clearance of interferon- $\alpha$ , while little is known about the mechanism, is roughly 2 times that of the glomerular filtration rate and the drug has been found in the urine of test animals (Radwanski et al. 1987).

Adverse side effects from interferon- $\alpha$  has contributed to 20-25% of the patient non-adherence rates in ET (Birgegård 2016). Kiladjian et al. (2008) also reported similar data showing a non-adherence rate of 25% in patients with ET taking interferon- $\alpha$ . Of this 25%, 12.5% of them experience side effects within the first year to cause the cessation (J.-J. Kiladjian, Chomienne, and Fenaux 2008). They have shown that the most common adverse side effects are flu-like symptoms ranging from headaches and body aches to fevers and fatigue (J.-J. Kiladjian, Chomienne, and Fenaux 2008). The symptoms are dose dependent and can start 1 to 3 hours after injection with a decrease in intensity within several weeks (J.-J. Kiladjian, Chomienne, and Fenaux 2008). Hematological toxicity is rarely reported in patients with ET. Patients most frequent reasons for withdrawal from interferon- $\alpha$  treatment are the muscle aches and fatigue (J.-J. Kiladjian, Chomienne, and Fenaux 2008). Other less frequent side effects that patients report are anxiety, depression, and mood changes, which may affect patients' social environments (J.-J. Kiladjian, Chomienne, and Fenaux 2008). There have also been reports of skin irritations,

autoimmune abnormalities, and in some cases cardiac and neurological toxicities (J.-J. Kiladjian, Chomienne, and Fenaux 2008).

Through advancements in pharmaceuticals, the pegylation of interferon- $\alpha$  2a has made vast improvements in the administration and side effects experienced by patients (J.-J. Kiladjian, Chomienne, and Fenaux 2008). Due to the slower clearance of pegylated interferon- $\alpha$  2a, the drug effect lasts longer and therefore the regimen only requires a dosage once per week (Glue et al. 2000) Glue et al. (2000) showed the half-life to be 4.6 hours and the clearance one-tenth that of non-pegylated interferon. They concluded that once weekly injections were enough to sustain the treatment regimen and needed serum levels (Glue et al. 2000). Side effects and adverse events also showed a notable difference between nonpegylated interferon- $\alpha$  and the pegylated version (Glue et al. 2000) Yacoub et al. (2019) performed the largest study to date to investigate pegylated interferon- 2a in high risk ET patients who had intolerance to hydroxyurea. Their review on the safety of pegylated interferon- $\alpha$  2a revealed that the discontinuation rates in several studies reviewing the treatment ranged from 13.9% to 24.3% (Yacoub et al. 2019). The data indicated that the lower the dosage the fewer discontinuations (Yacoub et al. 2019). As far as adverse events anemia was the most common hematological type and abdominal pains were the most common non-hematological form (Yacoub et al. 2019). Of the 65 patients, 40 discontinued treatment due to study closure or treatment completion, 7 were discontinued due to adverse effects, and 7 more were discontinued due to disease progression, indicating the majority of patients were able to complete the study without severe side effects (Yacoub et al. 2019). While the elimination of adverse side effects has

still yet to be seen in the treatment of ET with interferon- $\alpha$ , there is clear evidence that the pegylation of interferon- $\alpha$  may show promise in the reduction of the most severe adverse effects. Some flu-like symptoms still persisted in the pegylated version of interferon- $\alpha$ , but patients reported more tolerability than previously recorded in safety studies (Gowin et al. 2017). Samuelsson et al. (2006) warns, that while some studies indicate mildly adverse effects, their experience in the field and previous studies of anagrelide showed that patients have a tendency to report higher adverse effects. Their study revealed a 50% dropout rate for pegylated interferon- $\alpha$  2a (Samuelsson et al. 2006).

In more recent years a monopegylated version called Ropeginterferon- $\alpha$  2b has been introduced in clinical trials as well. Pegylated interferon- $\alpha$  2a contains a polyethylene glycol chain on the interferon- $\alpha$  (Zalipsky 2020). The molecule itself weighs roughly 40 kDa (Zalipsky 2020). The ropeginterferon- $\alpha$  differs from the pegylated version in that a single polyethylene glycol chain is attached to a proline which is added to the interferon- $\alpha$  (Zalipsky 2020). The molecular weight is 60 kDa (Zalipsky 2020).

The route of administration is still an injection but now patients are given a prefilled pen which can be taken home for ease of administration and increase in adherence rates (How and Hobbs 2020). The half-life of ropeginterferon is 6-10 days subcutaneously and allows for a bi-weekly schedule of dosing (Gisslinger et al. 2016) . Adverse effects were mild compared to those of interferon and pegylated interferon and the discontinuation rate was only 8% (Gisslinger et al. 2016). The adverse effects that did occur were mild flu-like symptoms, fatigue, thrombocytopenia, and leukopenia as well as abnormalities in liver function test (Gisslinger et al. 2016). Ropeginterferon-  $\alpha$  2b is currently under review for

a biological license application by the FDA. This would make it the first interferon approved and developed for treatment of MPNs (How and Hobbs 2020).

Studies recently involving the comparison of hydroxyurea and interferon- $\alpha$  have shed light on the possibility of interferon- $\alpha$  as a treatment standard for ET and even first-line therapy in younger and pregnant patients (Barbui et al. 2018). Studies reviewing hydroxyurea and interferon usage have shown that unlike hydroxyurea treatment, treatment with interferons have been reported to reduce allelic burdens (ratio of mutant genes to wild-type genes) of CALR and JAK2V617F mutations in hematopoietic stem cells. In some cases the JAK2V617F allelic burden was reported as undetectable in these cells and remained even so with the cessation of treatment (Foucar and Stein 2017). Mascarenhas et al (2016) in an interim analysis noted that there is no significant difference in the hematologic response between hydroxyurea and interferon treatment. More analysis would be needed on adverse effects and quality of life to determine a difference in first line therapy (J. O. Mascarenhas et al. 2016). In a more recent study, Yacoub et al. (2019) evaluated the efficacy of pegylated interferons in the event of intolerance to hydroxyurea and found them to be beneficial but questioned the toxicity profile. They concluded that while interferons are a good resource for high-risk ET patients that are intolerant to hydroxyurea, it is necessary for patient screening and gradually increasing dosage for the management of adverse events (Yacoub et al. 2019). Other studies have shown an even greater efficacy for interferons when used in combination with ruxolitinib, a janus kinase inhibitor (Hasselbalch and Holmström 2019). It is key to note that unlike hydroxyurea, interferon has not been reported to cause leukemic transformation and malignancies

associated with its use (Zhang and Duan 2014). In their study, Zhang and Duan (2014) showed that in comparison to hydroxyurea, interferon- $\alpha$  showed increased hematologic remissions, lower chance of thrombosis, as well as possible induction of remission on a molecular level in JAK2V617F + patients (Zhang and Duan 2014). Lane and Mullally (2013) reported similar results in JAK2V617F + patients as well, showing molecular remission and possible eradication of the JAK2 long-term hematopoietic stem cells (Lane and Mullally 2013). Another study by Verger et al. (2015) showed significant hematological responses in patients with CALR mutations as well as JAK2 (Verger et al. 2015). These recent studies on the usage of interferons indicate a key resource for the treatment of ET. The ability of interferons to reduce mutational allelic burdens occurs a on molecular level and is an extremely useful tool for the long-term control of ET. More research is needed on peginterferon and ropeginterferon in order to fully understand the risks and benefits of this drug class.

### **Investigational Treatment of Essential Thrombocythemia with Janus Kinase Inhibitors**

Janus kinase inhibitors are currently under investigational review for the treatment of MPNs and ET (Geyer and Mesa 2014). Ruxolitinib is the most recent and currently only janus kinase inhibitor still under-going evaluation and clinical trials for the treatment of ET and PV (Geyer and Mesa 2014). Current uses of janus kinase inhibitors are for the treatment of myelofibrosis, a disease often associated with the progression of ET into more

severe symptoms. Ruxolitinib has been approved for this indication in the United States (Geyer and Mesa 2014).

The mechanism of action of janus kinase inhibitors for MPNs is thought to be through selectively inhibiting JAK signal transduction (Cazzola and Kralovics 2014). The JAK2 subclass of janus kinases is the most important in MPNs because this is the location of the JAK2 V617F mutation (Cazzola and Kralovics 2014). It appears that no matter which mutation is present, the JAK-STAT pathway is overly active in the megakaryocytes of these MPNs (Cazzola and Kralovics 2014). Activation of growth and differentiation of megakaryocytes occurs via the coupling of cytokine ligand-binding to extracellular JAK and the intracellular tyrosine phosphorylation (Hubbard 2018). The auto-phosphorylation of the two JAK dimers leads to activation of the STAT pathway and hematopoietic cell phosphatase (Hubbard 2018). Once the STAT pathway is activated, changes in transcription factors occur to upregulate the proliferation of hematopoietic stem cells (Hubbard 2018). Hematopoietic cell phosphatase is an enzyme that serves as a negative feed-back regulator to decrease the growth of hematopoietic stem cells (Ihle et al. 1994). The JAK inhibitors are designed to act specifically via induced fit at an ATP-binding site that is unique to the JAK2 family (Lucet et al 2006). Binding of the inhibitor prevents the activation loop from opening to expose the activation site and thus the inhibitor fits into the active site and stops the activation of JAK2 (Lucet et al. 2006). Ruxolitinib is an important JAK inhibitor because it inhibits the JAK2/JAK1 complex selectively at JAK2 (Verstovsek et al. 2010).

Ruxolitinib is given orally making it easy for patients to maintain the daily dosing schedule. In two recent Phase III trials on ruxolitinib the maximum dosage given was 20 mg twice daily (Mesa, Yasothan, and Kirkpatrick 2012). The FDA dosing label recommends the dosing be determined by platelet counts, 20 mg twice daily for platelet counts higher than  $200 \times 10^9/L$  and 15mg twice daily for lower counts. As the platelet counts decline every  $25 \times 10^9/L$  so should the dosage decline by 5mg each time until the desirable platelet count is achieved at which point the dosing is continued at the same amount ("US Food and Drug Administration" 2011) .

In recent clinical trials of ruxolitinib the pharmacokinetics of the drug were determined in healthy volunteers. The oral bioavailability was determined to be high (Shi et al. 2011). The time to peak plasma concentration after oral ingestion is 1 to 2 hours and the drug in plasma is 97% bound to albumin (Shi et al. 2011). The volume of distribution is found to be between 1.0 and 1.94 L/Kg at steady state in healthy individuals (Shi et al. 2011). Ruxolitinib is metabolized via CYP3A4 into two active metabolites (J. Mascarenhas and Hoffman 2012). The half-life of ruxolitinib alone is 3 hours while total half-life including the metabolites is 5.8 hours. Clearance of the drug differs between men (17.7 L/h) and women (22.1 L/h) and is lower in PV patients (12.7 L/h) (J. Mascarenhas and Hoffman 2012). According to the FDA drug label on ruxolitinib, in the two Phase III clinical trials that were performed, adverse effects such as thrombocytopenia, anemia, and neutropenia were seen as early as 6 weeks into the treatment ("US Food and Drug Administration" 2011). The length of time for desired platelet counts to be achieved occurred within 8 weeks (David Green 2017).

Recent data has shown that JAK2 inhibitors may have limited efficacy in MPN patients including those with ET. Vannucchi and Harrison (2017) explain in their study on the emerging treatments for MPNs that results with JAK2 inhibitors have been bleak. In early Phase I and II clinical trials either the efficacy of the JAK2 inhibitors was low or the toxicities limited the length of time a patient could adhere (Vannucchi and Harrison 2017). JAK2 inhibitors like fedratinib and pacritinib were placed on hold after clinical trials in primary myelofibrosis (PMF) raised safety concerns (Vannucchi and Harrison 2017). Adverse side-effects like Wernicke's encephalopathy, increased bleeding, and cardiac events were seen in treatment using these drugs (Vannucchi and Harrison 2017). Ruxolitinib was selected for clinical trials with ET due to an increased survival advantage of PMF patients and the reduction in thrombotic events in PV patients (Vannucchi and Harrison 2017). The limited side-effect profile in the treatment of PMF and PV with ruxolitinib raised no concern for the safety of patients (Vannucchi and Harrison 2017). The COMFORT-I and COMFORT-II studies, however, both showed that due to adverse effects and lack of efficacy over half of the patients discontinued ruxolitinib in 3 years and 25% more discontinued after 5 years (Schain et al. 2019). After the discontinuation of ruxolitinib in this study, treatment options were limited to hydroxyurea and glucocorticoids (Schain et al. 2019). Another key problem with JAK2 inhibitors is that most are not specific to JAK2 and may also block JAK1 and JAK3. This could lead to more severe side effects like anemia (Dahlström et al. 2020). Momelotinib is another JAK inhibitor that has shown limited potential in the treatment of MPNs. Like ruxolitinib, momelotinib is a selective JAK1 and JAK2 inhibitor, however, momelotinib has no difference in affinity for JAK1 or

JAK2 (Verstovsek et al. 2017). In a phase II study on the treatment of ET and PV using momelotinib, patients were given 100 mg and 200 mg orally once daily (Verstovsek et al. 2017). The study showed that only 2 patients with PV met the criteria for an efficacious treatment and none of the ET patients met the criteria (Verstovsek et al. 2017). This study demonstrated the limited efficacy of momelotinib in the treatment of ET and PV (Verstovsek et al. 2017).

Current studies are reviewing the treatment of high-risk ET patients with ruxolitinib. These patients are typically intolerant to hydroxyurea and often are on a last treatment effort. In a phase II study of ruxolitinib, 24 of the 39 patients monitored continued treatment after the study cutoff (Verstovsek et al. 2014). The study showed ruxolitinib decreased platelet counts and 13.2% showed significant reduction in platelet counts below  $400 \times 10^9/L$  (Verstovsek et al. 2014). Ruxolitinib was also efficacious in the reduction of white blood cells and splenomegaly associated with ET (Verstovsek et al. 2014). In the MAJIC-ET phase II study the results indicated that ruxolitinib was less efficacious than hydroxyurea and that it should remain a second-choice treatment option (Gunawan et al. 2018). One valuable asset of ruxolitinib that was noted was the decrease in symptom prevalence, frequency, and severity, termed the symptom burden, by 32% as compared to the 0% of hydroxyurea in the MAJIC-ET study (Gunawan et al. 2018). Another study showed that in a one-year treatment with ruxolitinib, the 16 high-risk ET and PV patients had no reports of thrombotic events and the reduction of platelets allowed PV patients to cease phlebotomy treatments (Ekinici and Merter 2019). However, in elderly patients there was an increased risk of serious infection and development of pneumonia did occur in 2

patients (Ekinici and Merter 2019). Treatment of ET with JAK inhibitors is still an open topic of research due the potential shown by ruxolitinib. While many JAK inhibitors, like fedratinib and pacritinib, have been removed from clinical trials due to adverse effects and low efficacies, ruxolitinib have proven of some value in the treatment of ET. It is clear that more research is needed on JAK inhibitors and their potential role in the treatment of ET.

<b>Study</b>	<b>Reference</b>	<b>Drug</b>	<b>Outcome</b>
COMFORT-I (PV and ET)	Schain et al. 2019	Ruxolitinib	Not efficacious due to adverse side effects
COMFORT-II (PV and ET)	Schain et al. 2019	Ruxolitinib	Not efficacious due to adverse side effects
Verstovesk et al. 2010	Verstovesk et al. 2010	Ruxolitinib	Not efficacious due to adverse side effects
Verstovesk et al. 2009	Verstovesk et al. 2009	Pacritinib	Discontinued Clinical trials due to severe adverse effects
Verstovesk et al. 2017 (PV and ET)	Verstovesk et al. 2017	Momelotinib	Not efficacious in ET patients

**Table 3. Overview of JAK Inhibitor Clinical Trials.** The table above illustrates the different clinical trials of recent JAK inhibitors in the treatment of MPNs. The data is adapted from Sonbol et al. 2013.

### **Imetelstat and the Potential of Telomerase Inhibition in the Treatment of Essential Thrombocythemia**

Imetelstat is a telomerase inhibitor that is of potential use in the treatment of ET. Telomerase is a key enzyme in MPNs causing the malignant properties of cancers (Dahlström et al. 2020). In normal human differentiated cells telomerase is a silent RNA-dependent polymerase that can cause cancers when active through telomere lengthening (Dahlström et al. 2020). The catalytic component of telomerase is called telomerase reverse transcriptase (TERT) and it is key in the development and progression of cancers (Dahlström et al. 2020). TERT has been shown to be active in megakaryocytes of patients

who have been diagnosed with ET (Baerlocher et al. 2019). This leaves a clear mechanism of action for imetelstat in the treatment of ET. Baerlocher et al. (2019) determined that imetelstat acts to suppress the growth of megakaryocytes in ET patients and its effect is independent of mutation form or previous treatments. They theorized the mechanism of action to be uniquely linked to the increasing levels of TERT seen in ET patients and the effects of imetelstat consistently decreased TERT through-out the study of ET patient cells (Baerlocher et al. 2019).

Baerlocher et al. (2019) assessed the efficacy and safety of imetelstat in the treatment of ET. In their study they administered imetelstat intravenously to 18 patients with 7 receiving a starting dose of 7.5 mg/kg and 11 receiving 9.4 mg/kg (Baerlocher et al. 2019). The data showed the hematologic response rate to be 100% (platelet count lower than  $600 \times 10^9/L$ ) and a complete response rate (platelet count lower than  $400 \times 10^9/L$ ) rate was achieved in 16 patients within 6 weeks of initial dosing (Baerlocher et al. 2019). The higher dosage correlated with quicker response times (Baerlocher et al. 2019). Long-term data indicated that intermittent dosing was required to maintain the hematologic response (Baerlocher et al. 2019). While imetelstat did reduce the allelic burden of all mutations, it appeared to be more proficient in the reduction of JAK2 V617F mutated patients (Baerlocher et al. 2019). The main concern for adverse effects were fatigue, nausea, abnormal liver function, and neutropenia (Baerlocher et al. 2015). Research by Dahlström et al. (2020) suggests the possible combining of JAK inhibitors and telomerase inhibitors for the treatment of ET. Their data were validated by an increase in CD34+ cells from the JAK inhibitors that were blocked by the addition of telomerase inhibitors (Dahlström et al.

2020). It is believed that JAK inhibitors act on gene KLF4 increasing its expression to induce cyto-reduction but, the increased expression may lead to an induction of cells to de-differentiate to CD34+ leukemic stem cells. In the first 9 weeks of treatment with JAK inhibitors CD34+ cells rose from 2% to 90% (Dahlström et al. 2020). The addition of telomerase inhibitors is theorized to target the telomerase of these cells and decrease the JAK inhibitor induced CD34+ effect (Dahlström et al. 2020). The exact mechanism by which this occurs is still under review. More data are needed to verify this, but researchers hypothesize that telomerase inhibitors may decrease the adverse side-effects associated with JAK inhibitors and increase their efficacy (Dahlström et al. 2020).

## **DISCUSSION**

Based on the FDA drug labels and data collected from clinical studies, a comparative table of the key drugs for the treatment of ET has been composed. It is important to note that some of the data obtained for the table came from clinical trials on MPNs. PV and ET are very similar in treatment and outcome and therefore clinical trials are easily comparable and in certain instances interchangeable. The data for ruxolitinib mostly pertained to patients of post-ET and post-PV who were in the myelofibrosis stage. These data are still relevant to this thesis because most patients in the late phase of ET and PV have exhausted all other treatment options and therefore ruxolitinib is a treatment of last resort for these patients. Current studies on ruxolitinib in the treatment of ET are still ongoing. Momelotinib was not included in the comparison chart due to its lack of efficacy in the treatment of ET during phase II studies.

Of the three orally administered drug, hydroxyurea, anagrelide, and ruxolitinib, hydroxyurea has the highest bioavailability. Unlike these low molecular weight drugs that can be given orally, the interferon class has a high molecular weight limiting its administration to injections via doctor visits. Oral drugs have an increased adherence to dosing regimens due to self-administration. The delivery device for ropeginterferon tries to solve this problem, as it is a refillable pen that can be self-administered bi-weekly at home instead of a clinic visit.

Hydroxyurea and anagrelide's hematologic effects are both seen within a month of administration, whereas the effects of the interferon class occur more slowly, averaging around 2 to 3 months for high-risk patients (David Green 2017). Anagrelide and ruxolitinib

achieve peak plasma concentrations the most rapidly of the oral drugs and anagrelide also has the shortest elimination half-life. However, the breakdown of anagrelide and ruxolitinib into active metabolites prolongs their pharmacodynamic effects. The clearance of both anagrelide and ruxolitinib is mediated in the liver by CYPs, which increases risk of adverse effects and toxicity due to drug interactions. While the complete mechanism of clearance is unknown for hydroxyurea, it is theorized to use a hepatic route that may become saturable, increasing the risk of toxicity as well.

Pegylated interferon and ropeginterferon have the slowest clearances, leading to sustained serum levels for longer periods of time, so that they require less frequent maintenance dosing compared to other drugs. Adherence will decrease with increased frequency of dosing. It is key to note that most patients with ET are elderly and an increase in dosing frequency will be harder to remember, involve more trips to pharmacies, and lead to an over-all decrease in the adherence to the drug. Pegylated interferon may have a once weekly dosing schedule, but it does require frequent doctor visits for injections. The table below gives a more detailed look into the various ET treatment options and their pharmacokinetics.

<b>Drug</b>	<i>Hydroxyurea</i>	<i>Anagrelide</i>	<i>Interferon-<math>\alpha</math></i>	<i>Pegylated Interferon-<math>\alpha</math> 2a</i>	<i>Ropeginterferon-<math>\alpha</math> 2b</i>	<i>Ruxolitinib</i>
<b>Response time (when platelet count <math>\leq 450 \times 10^9/L</math>)</b>	Response time ~1 month	Response time ~7-14 days with full effect in 3 to 4 weeks	Response time ~ 2 months	Response time ~ 3 month	Unknown	Response time ~ 8 weeks
<b>Distribution/Vd</b>	0.48 to 1.62 L/kg in healthy adults	12 L/kg in healthy adults	12 to 40 L (body weights not given) and 1.4 L/kg in patients with hepatitis C	9 L (average body weight of 82.9 kg) and 0.99 L/kg in patients with hepatitis C	6.6 to 17 L (body weights not given)	1 to 1.94 L/kg in healthy adults
<b>Metabolism</b>	60% via saturable hepatic metabolism	Hepatic via CYP1A2 into 2 metabolites, an active and inactive form	Unknown	Unknown	Unknown	Metabolized by CYP3A4 into 2 active metabolites
<b>Bioavailability</b>	Oral ~100%	Oral ~70%	IM~83% SubQ~90%	SubQ~61%	SubQ~80% in monkeys	Oral~95%
<b>Half-life</b>	Half-life; 1.9-3.9 hours in adults	Half-life; 1.3-1.4 hours in adults	Half-life; IV~ 2 hours IM/SubQ~ 2 to 3 hours	Half-life; 4.6 hours	Half-life; 6 to 10 days SubQ	Half-life: 3 hours for drug and 5.8 hours for active metabolites
<b>Time to Peak Cp</b>	1-4 hours in adults	1-2 hours in adults	3-12 hours IM/SubQ	8-12 hours until Cmax and sustained for 48 to 72 hours	3-6 days	1-2 hours
<b>Clearance</b>	7.7 L/hr	156 L/hr	9.4 L/hr 100 times that of pegylated interferons	0.126 L/hr	0.023 to 0.061 L/hr	12.7 L/hr

**Table 4. Comparison of Drugs for Treatment of ET.** The table above provides the key pharmacokinetic and pharmacodynamic differences between treatment options for ET. Data for Hydroxyurea: Rodriguez et al. 1998 and “Drugs@FDA: FDA-Approved Drugs” n.d. Data for anagrelide: Besses et al. 2012 and Mazur et al. 2004 Data for Interferon- $\alpha$ : Radwanski et al. 1987 and García-García et al. 2016. Data for pegylated interferon- $\alpha$  2a: Glue et al. 2000 and Brennan, Xu, and Grippo 2012. Data for ropeginterferon- $\alpha$  2b: Gisslinger et al. 2016 and How and Hobbs 2020. Data for ruxolitinib: Shi et al. 2011 and J. Mascarenhas and Hoffman 2012.

Patient characteristics are an important consideration in the choice of treatment for ET. Hydroxyurea and low-dose aspirin are better suited treatments for the standard ET patient. They are diagnosed at an elderly age, typically 60+ years of age, and some form of risk factor, such as a history of thrombosis. This classifies them in a high-risk ET category. However, not all patients fit this mold, for younger patients or those that are pregnant hydroxyurea is not the best option. Its long-term effects, while not clearly defined in elderly, may be potentiated in younger patients leading to leukemic transformation and cutaneous lesions. The best option for these patients appears to be anagrelide or interferons.

JAK inhibitors, like ruxolitinib, are the only treatment form that show inferiority to hydroxyurea when used in monotherapy in comparison studies. This inferiority is thought to be remedied by the addition of telomerase inhibitors like imetelstat (Dahlström et al. 2020). When comparing the treatment options of ET for adverse effects, it is best to compare them to hydroxyurea as a base for the best available therapy to date. In a meta-analysis by Samuleson et al. (2015) anagrelide was proven to be inferior to hydroxyurea in an overall risk assessment of thrombosis, rates of major bleeding, and death outcome. The table below gives a visual representation of data collected from 3 of the most important anagrelide vs hydroxyurea studies up to date. The results indicate that hydroxyurea is superior to anagrelide.

Study	Thrombotic Event	Major Bleeding	Disease Related Death	Study Conclusion
(Gisslinger et al. 2013) Hydroxyurea n=131 Anagrelide n=122	Hydroxyurea: 28% of patients Anagrelide: 30% of patients	Hydroxyurea: 1.5% of patients Anagrelide: 4.0% of patients	Hydroxyurea: 3.8% of patients Anagrelide: 2.5% of patients	Study showed anagrelide to be non-inferior to hydroxyurea
(Harrison et al. 2005) Hydroxyurea n=404 Anagrelide n=405	Hydroxyurea: 7.8% of patients Anagrelide: 9.9% patients	Hydroxyurea: 2.0% of patients Anagrelide: 5.4% of patients	Hydroxyurea: 6.7% of patients Anagrelide: 7.7% of patients	Study showed hydroxyurea is superior to anagrelide

**Table 5. Comparison of Hydroxyurea and Anagrelide Studies.** The data above shows the incidence of thrombosis, major bleeding, and death outcome during two studies of anagrelide vs hydroxyurea. The death outcome in both studies was stratified to disease related vs unrelated death. Gisslinger et al. (2013) demonstrated that anagrelide was non-inferior to hydroxyurea in efficacy but adverse events were shown to be worse with anagrelide. Based on the data collected anagrelide is the inferior drug to hydroxyurea in terms of adverse effects.

While studies directly comparing hydroxyurea to interferons in efficacy have yet to be fully presented, there are some data indicating that hydroxyurea is superior to interferons. Adherence rates are indicative of the quality of life a patient is experiencing while taking a drug. High non-adherence rates may indicate that the drug is causing severe adverse events causing the patients to cease treatment. Multiple studies have reported the drop-out rate to be as high as 55% in a cohort of patients with PV and ET taking cytoreductive drugs (Le Calloch et al. 2018). Despite the high hematological response rates and reduced allelic burden, the reports of fatigue and other flu-like symptoms appear to dissuade most patients from continuing on with this form of treatment.

In the treatment of ET with JAK inhibitors, studies have concluded that hydroxyurea is the superior treatment. Studies show that the efficacy of JAK inhibitors is lacking in comparison to hydroxyurea (Dahlström et al. 2020). The addition of telomerase inhibitors, like imetelstat may increase the efficacy by reducing the CD34+ build up that

associated with JAK inhibition. On its own imetelstat has yet to be compared to hydroxyurea in full comparison studies. Imetelstat is given intravenously making it less likely to be adhered to by patients. The hematologic rates were 100% in ET patients on combination therapy with JAK inhibitors but adverse side effects were present with higher doses (Baerlocher et al. 2015). It is key to note that more data is needed to formulate any further conclusions about imetelstat and JAK inhibitors in comparison to hydroxyurea. While some studies have been published, there are not enough results to form a conclusive point of superiority on the combination therapy.

## **FUTURE STUDIES AND RESEARCH**

There is clear need for additional research on the treatment of ET. Hydroxyurea is currently the best option for treatment, but as prevalence of ET rises and younger patients are diagnosed there needs to be more treatment options with few side effects. Roppeginterferon shows promising results for the long-term treatment of ET, but not enough studies have been completed to show a comparison of safety and efficacy to hydroxyurea. Interferons are the most promising forms of treatment due to their sustained activity and immunological response for long-term treatment in conjunction with their hematologic response. However, the need for parenteral injection is a limitation and given the very high molecular weight of the drug class an alternative route of administration is unlikely.

More data are also needed on the effects of differing mutational profiles on the appropriate choice of treatments. There has been very little data to date that indicate any difference in treatment based on the mutation type in ET patients. Data indicate that differences in mutations may result in different subtypes of ET, as shown with slightly higher platelet counts and prevalence in one gender over the other in those with CALR mutations. It has also been noted that the JAK2 V617F mutation has higher transformation rates to PV and post-ET than the other mutations. These differences need to be further investigated to determine how symptom burden, drug of choice, and dosing affect the patient and their response to the given therapy.

## **CONCLUSIONS**

With all factors considered the best available therapy for the treatment of ET is hydroxyurea and low-dose aspirin. This treatment method is useful for the majority of ET patients, considering the typical diagnosis of ET is in elderly patients. The toxicity profile is relatively low, and the chance of severe adverse effects is less than with other available therapies. Any cutaneous lesions that may present themselves usually occur after extended long-term use and are typically found in patients who start hydroxyurea treatment at younger ages. Non-adherence to hydroxyurea due to adverse events is less common than in any other treatment form. Hydroxyurea is also key a treatment for ET due to its relatively rapid hematologic response and cytoreductive effect. The combinational therapy of hydroxyurea and low-dose aspirin produces the most uniform response of reducing thrombotic events and increasing the quality of life for the patient.

In the event that a patient is of younger age or pregnant or intolerant to hydroxyurea, treatment with interferons appears to be the next best option. While the route of administration and flu-like side effects may deter some patients and doctors from this treatment, the benefits out-weigh the risks. Despite its toxicity profile, interferons are the only treatment option that are known to have no leukemogenic effects and whose safety profile has been evaluated and approved for pregnant women. They also hold key value in an immunological attack on the JAK2 V617F hemopoietic stem cells for long-term treatment. Advances in the treatment of ET with interferons is making progress with increased time between maintenance doses for ropeginterferon with a delivery device for

patient self-administration. Treatment by interferons gives the patient the next best quality of life relative to the standard treatment of hydroxyurea and low-dose aspirin.

Anagrelide should serve as a last choice option in the event of hydroxyurea intolerance or lack of response to interferons. It shows similar efficacy to that of hydroxyurea with hematologic responses. Anagrelide almost rivals hydroxyurea in the prevention of thrombotic events, but the adverse effects are slightly worse. The combinational therapy of anagrelide and low-dose aspirin causes high bleeding rates and should be monitored frequently. Anagrelide and low-dose aspirin should not be used together as an initial form of second-line treatment but rather the patient should be started on anagrelide and monitored from there for signs of improvement. Should no signs of improvement be seen, the patient may be administered low-dose aspirin.

In conclusion it is in the best interest of the patient for the doctor to take a full-scale work-up of the patient before prescribing any treatment for ET. All factors including age of diagnosis and length of treatment should be considered as well as allelic mutation burdens. Hydroxyurea and low-dose aspirin remain the current gold-standard of treatment for patients with ET, although new approaches may soon be available.

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## CURRICULUM VITAE

