Prognostic Value Of Response To First-Line Hydroxyurea According To IPSET Stratification In Essential Thrombocythemia

Marta Santaliestra[1*], Marta Garrote[2*], María Soledad Noya[3], Manuel Pérez-Encinas[4], Alicia Senín[5], Raúl Pérez-López[6], Francisca Ferrer-Marín[7], Gonzalo Carreño-Tarragona[8], Gonzalo Caballero[9], Elena Magro[10], Patricia Vélez[11], Miguel Ángel Cortés Vázquez[11], Ana Moretó[12], Anna Angona[13], Irene Pastor-Galán[14], José María Guerra[15], Carmen García Hernández[16], María Isabel Mata[17], Ruth Stuckey[18], María Teresa Gómez-Casares[18], Laura Fox[19], Beatriz Cuevas[20], Valentín García-Gutiérrez[21], Ana Triguero[2], Eduardo Arellano-Rodrigo[2], Juan Carlos Hernández-Boluda[14], Alberto Alvarez-Larrán[2,22]

[1]Hospital Mutua de Terrassa, [2]Hospital Clínic Barcelona. [3]Complexo Hospitalario Universitario de A Coruña. [4]Hospital Clínico Universitario de Santiago de Compostela. [5]Hospital de Mar, Barcelona. [6]Hospital Virgen de la Arrixaca, Murcia. [7]Hospital Morales Messeguer, CIBERER, UCAM, IMIB, Murcia. [8]Hospital Universitario 12 de octubre, Madrid. [9]Hospital Universitario Miguel Servet, Zaragoza. [10]Hospital Príncipe de Asturias, Alcalá de Henares. [11]Hospital Universitario Marqués de Valdecilla, IDIVAL, Santander. [12]Hospital Universitario Cruces, Barakaldo. [13]Insitut Català d'Oncologia, Hospital Josep Trueta, Girona. [14]Hospital Clínico Universitario, Valencia. [15]Hospital Son Llatzer, Palma de Mallorca. [16]Hospital General Universitario de Alicante. [17]Hospital Costa del Sol, Marbella. [18]Hospital Universitario de Gran Canaria Doctor Negrín, Las Palmas de Gran Canaria. [19]Hospital Universitario Vall d'Hebron, Barcelona. [20]Hospital Universitario de Burgos. [21]Hospital Universitario Ramón y Cajal, IRYCIS, Universidad de Alcalá, Madrid. [22]Universitat de Barcelona (UB), Barcelona, Spain

*Marta Santaliestra and Marta Garrote have equally contributed

Corresponding author: Alberto Alvarez-Larrán, MD, Hematology Department, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain. Universitat de Barcelona (UB), Barcelona, Spain.

E-mail: aalvar@clinic.cat

Running title: Response to hydroxyurea according to IPSET

Key words: essential thrombocythemia, thrombosis, prognostication, myeloproliferative neoplasms, therapy

Abstract

Hydroxyurea (HU) constitutes the first-line treatment in most patients with essential thrombocythemia (ET), but criteria for changing therapy are not clearly established. The prognostic value of complete hematological response (CHR) and resistance/intolerance to HU was assessed in 1080 patients from the Spanish Registry of ET, classified according to revised IPSET-Thrombosis stratification (Very low- n= 61, Low- n=83, Intermediate- n= 261, and High-risk n=675). With a median therapy duration of 5 years, CHR was registered in 720 (67%) patients (1-year probability 51%) and resistance/intolerance in 219 (20%) patients (5-years probability 13%). After correction by other risk factors, High-risk patients achieving CHR showed a reduced risk of arterial thrombosis (HR: 0.35, 95%CI: 0.2-0.6, p=0.001) and a trend towards lower risk of venous thrombosis (HR: 0.45, 95%CI: 0.2-1.02, p=0.06) whereas no association was observed for intermediate- or low-risk patients. In comparison with non-responders, intermediate- and high-risk patients achieving CHR had longer survival and lower myelofibrosis incidence. Development of resistance/intolerance to HU, mainly cytopenia, was associated with higher probability of myelofibrosis but no effect on survival or thrombotic risk was demonstrated. In conclusion, CHR with HU is associated with better outcomes and might be an early indicator for selecting candidates to second-line clinical trials.

Introduction

Essential thrombocythemia (ET) is the most frequent chronic myeloproliferative neoplasm and the one with the best prognosis with survival being mainly determined by vascular complications and progression to secondary myelofibrosis (MF) or, less frequently, to acute myeloid leukemia (AML) (1-3). ET patients have traditionally been stratified into two thrombotic risk categories based on whether they are older than 60 years and have a history of thrombosis (4). More recently the revised IPSET-thrombosis scoring system, which accounts for the increased risk linked to the *JAK2* mutation, has been incorporated in most expert recommendations (5-8).

Hydroxyurea (HU) constitutes the first-line treatment of choice in the majority of patients with ET (7,8). Although most patients achieve adequate disease control under treatment with HU, some patients require therapy change due to inadequate response or intolerance. Definitions of response to treatment and criteria for resistance/intolerance to HU have been proposed by the European LeukemiaNet (ELN) and might help in the decision of changing therapy in ET (9,10). However, there is scarce information on the prognostic significance of these definitions in routine clinical practice since most studies have included a limited number of patients (11,12). Furthermore, the prognostic value of the response according to ET genotype or the revised-IPSET thrombosis risk score has not been studied.

In the present study, we have evaluated the prognostic value of achieving a complete hematological response (CHR) and developing resistance/intolerance to HU in a contemporary series of 1080 patients included in the Spanish Registry of Essential

Thrombocythemia. We also conducted subgroup analysis according to both classical and revised IPSET-thrombosis risk stratification.

Patients and Methods

The Spanish Registry of Essential Thrombocythemia is a nationwide, non-interventional prospective study started in 2015 by the Spanish Group of Ph-negative Myeloproliferative Neoplasms (GEMFIN). Patients diagnosed with ET after the year 2000 were eligible for inclusion. ET diagnosis was established locally at the hospital where the patient was being treated according to the prevailing World Health Organization (WHO) criteria at the time of diagnosis. Prefibrotic myelofibrosis were not allowed to be included. The registry allows the inclusion of previously diagnosed and newly diagnosed patients. When ET diagnosis was previous to the inclusion in the registry, clinical data were collected retrospectively and prospectively updated for therapy changes and complications. All methods were performed in accordance with the relevant guidelines and regulations including Spanish Medicines Agency approval (September 9th, 2015) and Hospital del Mar Ethic Committee approval (CEIC-Parc de Salut Mar resolution on November 10th, 2015, Protocol number GEE-AAS-2015-01). Approval from local IRBs was also obtained in each participating center following Spanish guidelines for prospective studies. Informed consent for participation in the Registry was obtained in all patients.

By November 2023, a total of 3364 patients were included in the registry with 2356 (70%) of them being treated with HU. Patients treated with first line HU in whom full information regarding response to treatment was available were selected for the present study (n=1080). The majority of patients were already treated with HU prior to inclusion

in the registry with 537, 216 and 149 patients starting HU the previous year, between the second and third year, and three or more years before inclusion, respectively. Prospective follow-up was available in 744 (69%) patients with a median duration of 3.4 years. Data were entered by the attending physician or local investigators into an electronic case report form (e-CRF) accessible at the scientific area of GEMFIN website.

Response to HU was categorized according to ELN criteria (9). CHR was defined as normalization of the platelet count ($<400x10^9$/l) in the absence of disease-related symptoms, with a normal spleen size and a leukocyte count $<10x10^9$/l. Any response that did not satisfy CHR criteria was classified as a non-response. Loss of response was recognized when a responder no longer met the criteria for response in two consecutive measurements separated by at least 1 month.

Modified ELN definitions of resistance/intolerance to HU required the fulfilment of at least one of the following criteria: platelet count $>600x10^9$ /l after 3 months of at least 2 g/d or maximum tolerated doses of HU; platelet count $>400x10^9$ /l combined with leukocyte count $<2x10^9$ /l or hemoglobin <100 g/l at any dose of HU; presence of leg ulcers or other unacceptable mucocutaneous manifestations at any dose of HU (10).

The main outcomes of the study were survival, thrombotic events under HU, bleeding, and disease progression. Arterial thrombosis included coronary artery disease, stroke/transient ischemic attack, peripheral artery disease and other arterial territories. Venous thromboembolic events included superficial thrombophlebitis, deep vein thrombosis, pulmonary thromboembolism, splanchnic vein thrombosis, and other venous territories. Both major and minor bleeding were included in analysis.

Period at risk of thrombosis was defined as the time elapsed from HU start to thrombotic event, end of HU therapy, death, or last visit, whichever occurred first. For survival and disease progression, time at risk was calculated from HU start to last contact. Probabilities of thrombosis, disease progression and survival were calculated according to the achievement of CHR and the development of resistance/intolerance to HU. Subgroup analyses according to revised IPSET-thrombosis categories and the two-tier classical score assigned at start of first line therapy with HU were also explored. Time to event curves were calculated by the Kaplan-Meier method with the log-rank test for comparisons. Multivariate analyses of factors predicting the main outcomes were done by Cox regression. All the statistical analyses were performed with SPSS, version 25.

Results

Clinical and hematological characteristics of the patients

Main clinical and hematological characteristics at diagnosis of 1080 patients treated with first line HU in whom full information regarding response to treatment was available are shown in table 1.

Median time elapsed from diagnosis to HU start was 33 days, with 867 (80%) patients beginning on HU in the first year after diagnosis. At time of cytoreduction, 144 (13%) and 936 (87%) patients corresponded to low-risk and high-risk categories, respectively, according to classical risk stratification. When the revised IPSET-Thrombosis was used, the corresponding figures were 61 (6%), 83 (8%), 261 (24%), and 675 (62%) cases in the very-low, low, intermediate, and high-risk categories, respectively. Main reason for starting HU included: age older than 60 years (n=713), thrombosis (n=138), bleeding (n=8), extreme thrombocytosis (n=129), microvascular disturbances (n=41), other (n=22), and not determined (n=29). Concomitant antiplatelet therapy and anticoagulation were used in 991 and 199 patients, respectively.

Clinical-hematological response and resistance/intolerance to HU

Median duration of therapy with HU was 5 years (IQR: 2.4-8.2). CHR was achieved by 720 (67%) patients, while the remaining patients were classified as non-responders. Median time to CHR was 352 days, with the probability of achieving a CHR at 1 and 2 years being 51% and 61%, respectively. Response duration was assessed in 583 cases, with 134 of patients loosing CHR during follow-up. The probability of maintaining CHR at 1, 3, and 5 years was 94%, 84% and 78% respectively.

Resistance/intolerance to HU was registered in 219 (20%) patients including 30 (3%), 80 (7%), 63 (6%), and 54 (5%) cases with persistent thrombocytosis despite maximum tolerated dose of HU, cytopenia at the lowest dose to maintain response, leg ulcers, or unacceptable mucocutaneous toxicity, respectively. *CALR/MPL* genotypes were more frequently observed among patients developing persistent thrombocytosis or cytopenia (supplemental table 1). Patients with persistent thrombocytosis and unacceptable mucocutaneous toxicity were significantly younger whereas cytopenia and leg ulcers were associated with older age (supplemental table 1). The probability of resistance/intolerance to HU at 1, 3, and 5 years was 4%, 9%, and 13%, respectively.

HU was stopped in 317 (29%) patients. The most frequent reasons for discontinuation included toxicity (131 cases), followed by inadequate response (45 cases), and disease progression (31 cases).

Survival

With a median follow-up of 6.6 years, 245 patients died resulting in a median projected survival of 16 years. The causes of death included infection (n=52), cardiovascular events (n=31), disease progression (n=19), second primary neoplasia (n=33), other (n=92), not reported (n=18).

Median survival was significantly longer in patients achieving CHR than in non-responders (16 years and 14 years in responders and non-responders, respectively, p= 0.04). CHR was associated with a lower risk of death (HR: 0.65, 95%CI: 0.5-0.8 p<0.001) after correction by classical risk stratification (HR: 5.05, 95%CI: 2.7-9.3 p=0.001) or IPSET-thrombosis stratification (HR: 1.7, 95%CI: 1.4-2.0, p<0.001). Subgroup analysis showed a significantly lower survival only in intermediate- and high-risk revised IPSET-thrombosis

groups not achieving CHR (figures 1 and 2). There was no significant association between development of resistance/intolerance to HU and survival.

Thrombosis and bleeding

A total of 69 arterial thrombotic events were registered while on first-line HU, with the probability of arterial thrombosis at 5 and 10 years of 4.9% and 10.1% respectively. CHR was associated with a lower probability of thrombosis (10-years probability of arterial thrombosis: 19.2% and 7.7% for patients not achieving and those achieving CHR respectively p=0.001). The protective effect of CHR on arterial thrombosis was restricted to high-risk patients according to either classical risk stratification or revised IPSET-thrombosis (Figure 3 and Table 2). Multivariate analysis of patients included in the high-risk IPSET category showed a lower risk of arterial thrombosis for those patients achieving CHR (HR: 0.35, 95%CI: 0.2-0.6, p=0.001) after correction by age (HR: 1.06, 95%CI: 1.02-1.1, p=0.001), history of arterial thrombosis (HR: 3.3, 95%CI: 1.8-6.05, p<0.001), and presence of cardiovascular risk factors (p not significant). No risk factors for arterial thrombosis could be identified on multivariate analysis of intermediate-risk patients including CHR, age and cardiovascular risk. There was no association among the development of resistance/intolerance to HU and the probability of arterial thrombosis (data not shown).

Venous thrombotic events were registered in 37 patients resulting in a venous thrombosis probability of 3.3% and 4.5% at 5 and 10 years, respectively. Four out of 37 (u11%) venous thrombosis corresponded to superficial thrombophlebitis. CHR was associated with a lower probability of venous thrombosis only in patients included in the high risk IPSET category (Figure 4 and table 2). Multivariate analysis of patients included

in the high-risk IPSET category showed a trend to a lower risk of venous thrombosis for those patients achieving a CHR (HR: 0.45, 95%CI: 0.2-1.02, p=0.06) after correction by age (HR: 1.06, 95%CI: 1.01-1.1, p=0.01), history of venous thrombosis (HR: 5.1, 95%CI: 2.02-12.6, p=0.001), and *JAK2* mutational status (p not significant). There was no association among the development of resistance/intolerance to HU and the probability of venous thrombosis (data not shown).

A total of 52 patients presented at least one bleeding event under HU. There were not a significant association between the probability of bleeding and the achievement of CHR or the development of resistance/intolerance to HU.

Disease progression

Fifty-eight patients progressed to MF, resulting in a cumulative incidence of MF of 8.9% after 10 years from HU start. CHR was associated with a significant lower probability of disease progression to MF (10-years probability 5.5% and 16% in responders and non-responders, respectively, p<0.001). The absence of CHR was associated with a higher probability of MF in intermediate- and high-risk categories according to the revised IPSET-thrombosis (table 3).

MF probabilities according to development of resistance/intolerance to HU in the different IPSET-revised thrombosis risk groups are shown in table 4. When the different criteria included in the definition of resistance/intolerance to HU were analyzed separately, only cytopenia and leg ulcers were associated with a significantly higher probability of MF (Supplemental table 2). Cytopenia at the lowest dose to achieve response was associated with a higher probability of MF in both intermediate- (10-years

probability of MF: 20% and 10% for those developing cytopenia or not, p=0.003) and high-risk patients (38% and 5% respectively, p<0.001).

Thirty-three patients progressed to AML (n=26) or MDS (n=7) resulting in a cumulative probability of 1.2% and 3.8% at 5 and 10 years from HU start respectively. CHR was not associated with the probability of AML/MDS in the overall group of patients. Subgroup analysis showed a higher probability of AML/MDS in intermediate risk patients not achieving CHR (table 3). Development of cytopenia under HU was associated with a significant higher probability of AML/MDS in the overall group of patients (Supplemental Table 2). After subgroup analysis according to risk stratification, MDS/AML probability remained significant only in high-risk patients (10-years probability 17% and 2% for those developing cytopenia or not, respectively p=0.004).

Discussion

In the present study we have analyzed the prognostic impact of achieving CHR and resistance/intolerance to HU criteria defined by the ELN in a contemporary series of patients included in the Spanish registry of ET. To the best of our knowledge, it constitutes the largest series to date on this topic and the first in which the response to first-line HU is evaluated according to the different risk groups defined by the revised IPSET-thrombosis score. Failure to achieve CHR was associated with worse survival, increased risk of arterial thrombosis, and a higher probability of transformation to MF.

The most innovative finding of the present work is the prognostic value of CHR in specific ET groups as defined by the revised IPSET-thrombosis stratification. In high-risk patients (age > 60 years and *JAK2*-mutated or any age with history of thrombosis), achieving CHR was associated with a lower risk of arterial thrombosis, supporting that CHR should be a treatment endpoint in the routine management of these patients. In the intermediate risk group, enriched by *CALR*-mutated patients older than 60 years, achieving CHR was not associated with a lower probability of thrombosis which supports the ELN recommendation of not pursuing normalization of the platelet count when such a strategy results in toxicity (13). Moreover, CHR was associated with better survival and a lower probability of MF in high- and intermediate-risk patients, illustrating the usefulness of the CHR definition to identify a subgroup of patients who are candidates for second-line treatment. For this subgroup of patients, new therapies aimed at modifying the natural history of the disease are needed, especially to delay MF progression (14).

Two previous studies using the ELN definitions of response failed to demonstrate any prognostic value for CHR (11,12). However, this discrepancy could be justified by differences in the number of patients included, treatment intensity, and statistical design. One study included 166 patients from two Spanish reference centers with 70% of them achieving CHR after 1 year of treatment, while in a second study on 416 patients treated in 3 Italian centers, CHR was reported in only 25% of patients (11,12). Such a marked difference was explained by the different platelet count target employed in these two studies (< 400x109/l and <600x109/l, respectively). Moreover, the inclusion of an additional group with partial response, evaluation of the response at a fixed time, or risk assessment according to duration of response or sustained response might also have contributed to the differences observed in comparison with our study. It should be noted that we included 1080 patients from hospitals across Spain, which constitutes a representative sample of clinical practice in our country. In this regard, the overall CHR rate of 67% (51% at 12 months of therapy) aligns with treatment intensity aimed at normalizing the platelet count in most centers. To the best of our knowledge, this is the first study to identify, in real life, the prognostic value of CHR in ET patients. Our findings are in line with recently published results in PV patients from the MAJIC PV trial in which CHR improved event free survival whichever treatment modality is used in second line (15).

Finally, we have evaluated the prognostic significance of the development of resistance/intolerance to HU according to the various criteria included in the ELN definition. Notably, we have confirmed previous findings from our group showing that cytopenia, especially anemia in combination with thrombocytosis, is associated with disease progression to both MF and AML (12). Specifically, the prognostic value of

cytopenia was restricted to intermediate- and high-risk patients for MF progression and to high-risk patients for AML-MDS. These findings confirm a hierarchy of importance of the different criteria included in the ELN definition with cytopenia showing the highest prognostic value, especially in intermediate- and high-risk IPSET groups. If these observations also operate in lower risk categories should be explored in future studies involving higher number of patients.

ELN response criteria were established for its implementation in the evaluation of response in clinical trials while the resistance/intolerance criteria were designed to assist in deciding whether to change treatment. Consequently, most clinical trials evaluating second line drugs have used the resistance/intolerance criteria in their inclusion criteria (16-18). However, our findings underscore that achieving CHR holds significant value in guiding treatment decisions for patients illustrating that both CHR and resistance/intolerance criteria could be utilized for clinical trial eligibility. Moreover, it seems that the CHR definition may outperform the resistance/intolerance criteria in selecting candidates for second line trials. Supportive evidence favoring this strategy includes an earlier identification of candidates, as illustrated by 49% and 4% of non-responders and resistant/intolerant patients at 1 year, respectively, as well as the broader prognostic value for CHR in main clinical outcomes such as arterial thrombosis, disease progression and survival.

The REVEAL study clearly demonstrated that persistent leukocytosis is a risk factor for thrombosis in polycythemia vera while different studies point to a possible role for leukocytes in ET (19-20). PT1 subanalysis showed that platelet count outside the normal range was associated with the risk of major hemorrhage whereas persistent leukocytosis

correlated with thrombosis and major hemorrhage (21). We were not able to separately analyze the value of leukocytosis and persistent thrombocytosis under HU since the blood count values were not available. Another limitation of our work includes the lack of data in a proportion of patients precluding the evaluation of the prognostic value of response duration. We were unable to demonstrate any association between the resistance/intolerance criteria and survival, which may be attributed to heterogeneity of resistance/intolerance criteria, a high proportion of patients experiencing extra-hematological toxicity and limited follow-up.

In conclusion, CHR with first-line HU is a relevant prognostic factor that correlates with significant better survival and a lower rate of disease progression to MF in patients categorized as high- and intermediate-risk according to revised IPSET-thrombosis stratification. Moreover, high risk patients achieving CHR face a lower risk of arterial thrombosis. The results of the present study support that failure to achieve CHR could be considered as an eligibility criterion in second-line clinical trials.

Acknowledgements: We are indebted to all members of GEMFIN participating in the Spanish Registry of Essential thrombocythemia. We thank MFAR staff for their technical assistance.

Author Contributions: AAL designed the study, collected the data, performed the statistical analysis, analyzed and interpreted the results and wrote the paper. MS, MG, EAR and JCHB: collected the data, analyzed and interpreted the results, and wrote the paper. MSN, MPE, AS, RPL, FFM, GCT, GC, EM, PV, MACV, AM, AA, IPG, JMG, CGH, MIM, RS, MTGC, LF, BC, VGG, and AT collected the data, interpreted the results and approved the final version.

Competing interests: The Spanish registry of Essential thrombocythemia is financed with GEMFIN's own funds without direct collaboration from any pharmaceutical company. This work has been funded by Instituto de Salud Carlos III (ISCIII) through the projects PI21/00231, PI21/00347, and PI21/00538 and co-funded by the European Union.

Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Tefferi A, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L, et al. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood. 2014; 124: 2507–2513.
2. Cervantes F, Alvarez-Larran A, Talarn C, Gomez M, Montserrat E. Myelofibrosis with myeloid metaplasia following essential thrombocythaemia: actuarial probability, presenting characteristics and evolution in a series of 195 patients. Br J Haematol. 2002; 118: 786–790.
3. Godfrey AL, Green A, Harrison CN. Essential thrombocythemia: challenges in clinical practice and future prospects. Blood. 2023; 141: 1943–1953.
4. Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M, et al. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. J Clin Oncol. 2011; 29: 761–770.
5. Barbui T, Finazzi G, Carobbio A, Thiele J, Passamonti F, Rumi E, et al. Development and validation of an international prognostic score of thrombosis in World Health Organization-essential thrombocythemia (IPSET-thrombosis). Blood. 2012; 120: 5128–5133.
6. Barbui T, Vannucchi AM, Buxhofer-Ausch V, De Stefano V, Betti S, Rambaldi A, et al. Practice-relevant revision of IPSET-thrombosis based on 1019 patients with WHO-defined essential thrombocythemia. Blood Cancer J. 2015; 5:e369.
7. Barbui T, Tefferi A, Vannucchi AM, Passamonti F, Silver RT, Hoffman R, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms:

revised management recommendations from European LeukemiaNet. Leukemia. 2018; 32:1057-1069.

8. Mesa RA, Jamieson C, Bhatia R, Deininger MW, Fletcher CD, Gerds AT, et al. NCCN guidelines insights: myeloproliferative neoplasms, version 2.2018. J Natl Compr Canc Netw. 2017; 15:1193–1207.
9. Barosi G, Birgegard G, Finazzi G, Griesshammer M, Harrison C, Hasselbalch HC, et al. Response criteria for essential thrombocythemia and polycythemia vera: result of a European LeukemiaNet consensus conference. Blood. 2009; 113: 4829–4833.
10. Barosi G, Besses C, Birgegard G, Briere J, Cervantes F, Finazzi G, et al. A unified definition of clinical resistance/intolerance to hydroxyurea in essential thrombocythemia: results of a consensus process by an international working group. Leukemia. 2007; 21: 277–280.
11. Carobbio A, Finazzi G, Antonioli E, Vannucchi AM, Barosi G, Ruggeri M, et al. Hydroxyurea in essential thrombocythemia: rate and clinical relevance of responses by European LeukemiaNet criteria. Blood. 2010; 116:1051-1055.
12. Hernández-Boluda JC, Alvarez-Larrán A, Gómez M, Angona A, Amat P, Bellosillo B, et al. Clinical evaluation of the European LeukaemiaNet criteria for clinicohaematological response and resistance/intolerance to hydroxycarbamide in essential thrombocythaemia. Br J Haematol. 2011; 152:81-88.
13. Alvarez-Larrán A, Sant'Antonio E, Harrison C, Kiladjian JJ, Griesshammer M, Mesa R, et al. Unmet clinical needs in the management of CALR-mutated essential thrombocythaemia: a consensus-based proposal from the European LeukemiaNet. Lancet Haematol. 2021; 8:e658-e665.

14. Kishtagari A, Gerds AT. Unmet Need in Essential Thrombocythemia and Polycythemia Vera. Hematol Oncol Clin North Am. 2021; 35:295-303.
15. Harrison CN, Nangalia J, Boucher R, Jackson A, Yap C, O'Sullivan J, et al. Ruxolitinib Versus Best Available Therapy for Polycythemia Vera Intolerant or Resistant to Hydroxycarbamide in a Randomized Trial. J Clin Oncol. 2023; 41:3534-3544.
16. Verstovsek S, Komatsu N, Gill H, Jin J, Lee SE, Hou HA, et al. SURPASS-ET: phase III study of ropeginterferon alfa-2b versus anagrelide as second-line therapy in essential thrombocythemia. Future Oncol. 2022; 18: 2999–3009.
17. Harrison CN, Mead AJ, Panchal A, Fox S, Yap C, Gbandi E, et al. Ruxolitinib vs best available therapy for ET intolerant or resistant to hydroxycarbamide. Blood. 2017; 130: 1889–1897.
18. Yacoub A, Mascarenhas J, Kosiorek H, Prchal JT, Berenzon D, Baer MR, et al. Pegylated interferon alfa-2a for polycythemia vera or essential thrombocythemia resistant or intolerant to hydroxyurea. Blood. 2019; 134: 1498–1509.
19. Gerds AT, Mesa R, Burke JM, Grunwald MR, Stein BL, Squier P, et al. Association between elevated white blood cell counts and thrombotic events in polycythemia vera: analysis from REVEAL. Blood. 2024; 143:1646-1655.
20. Carobbio A, Ferrari A, Masciulli A, Ghirardi A, Barosi G, Barbui T. Leukocytosis and thrombosis in essential thrombocythemia and polycythemia vera: a systematic review and meta-analysis. Blood Adv. 2019; 3:1729-1737.
21. Campbell PJ, MacLean C, Beer PA, Buck G, Wheatley K, Kiladjian JJ, et al. Correlation of blood counts with vascular complications in essential thrombocythemia: analysis of the prospective PT1 cohort. Blood. 2012; 120: 1409–1411.

409

Legends for the figures

Figure 1: Survival according to complete hematological response in intermediate-risk patients with essential thrombocythemia treated with first line hydroxyurea. Median survival was 17 years and 11 years in responders and non-responders respectively, p value <0.001. Black solid line corresponds to responders. Red solid line corresponds to non-responders. Risk stratification by revised IPSET-thrombosis score.

Figure 2: Survival according to complete hematological response in high-risk patients with essential thrombocythemia treated with first line hydroxyurea. Median survival was 15.5 years and 12.5 years in responders and non-responders, respectively, p value =0.02. Black solid line corresponds to responders. Red solid line corresponds to non-responders. Risk stratification by revised IPSET-thrombosis score.

Figure 3: Cumulative incidence of arterial thrombosis according to complete hematological response in high-risk patients treated with first line hydroxyurea (p<0.001). Black solid line corresponds to responders. Red solid line corresponds to non-responders. Risk stratification by revised IPSET-thrombosis score.

Figure 4: Cumulative incidence of venous thrombosis according to complete hematological response in high-risk patients treated with first line hydroxyurea (p=0.035). Black solid line corresponds to responders. Red solid line corresponds to non-responders. Risk stratification by revised IPSET-thrombosis score.

Table 1: Main clinical characteristics at diagnosis in 1080 patients included in the Spanish Registry of Essential Thrombocythemia treated with first line hydroxyurea	
Age, median (interquartile range)	68 (60-76)
Female sex, n (%)	660 (61)
Bleeding before diagnosis, n (%)	41 (4)
Thrombosis before diagnosis, n (%)	194 (18)
Cardiovascular risk factors	
Diabetes, n (%)	180 (17)
Therapy for hypertension, n (%)	601 (56)
Smoking, n (%)	111 (10)
Symptoms at diagnosis	
Microvascular disturbances, n (%)	182 (17)
Arterial thrombosis, n (%)	56 (5)
Venous thrombosis, n (%)	23 (2)
Bleeding, n (%)	32 (3)
Hemoglobin g/l, median (interquartile range)	142 (132-151)
Leukocyte count x10^9/l, median (interquartile range)	8.9 (7.3-10.9)
Platelet count, median (interquartile range)	728 (608-912)
Genotype*	
*JAK2*V617F, n (%)	690 (64)
CALR, n (%)	259 (24)
MPL, n (%)	38 (3.5)
Triple negative, n (%)	87 (8)
Classical risk stratification	
Low, n (%)	210 (19)
High, n (%)	870 (81)
Revised IPSET-Thrombosis stratification	
Very low, n (%)	88 (8)
Low, n (%)	122 (11)
Intermediate, n (%)	239 (22)
High, n (%)	631 (58)

*Six additional cases were double positive: *JAK2*V617F+/*CALR+* n=3, *JAK2*V617F+/*MPL+* n=2, *CALR+*/*MPL+* n=1

Table 2: Cumulative incidence of thrombosis according to hematological response in 1080 patients with essential thrombocythemia treated with first line hydroxyurea

Risk stratification		ARTERIAL THROMBOSIS			VENOUS THROMBOSIS		
Classical	CHR	Events/patients	10-years probability	P value	Events/patients	10-years probability	P value
Low	Yes	3/72	6.6%	0.9	4/72	0%	0.6
	No	2/72	9.6%		1/72	3.3%	
High	Yes	36/648	**6.9%**	**<0.001**	19/648	3.8%	0.07
	No	28/288	**21.8%**		13/288	7.3%	
Revised IPSET-T	CHR	Events/patients	10-years probability, %	P value	Events/patients	10-years probability, %	P value
Very low	Yes	1/28	7%	0.4	0/28	-	-
	No	0/33	-		0/33	-	
Low	Yes	2/44	4.3%	0.7	4/44	7.2%	0.4
	No	2/39	14%		1/39	0%	
Intermediate	Yes	6/156	6%	0.07	4/156	4.4%	0.8
	No	8/105	11%		3/105	3.8%	
High	Yes	30/492	**7.2%**	**<0.001**	15/492	**3.7%**	**0.035**
	No	20/183	**27.9%**		10/183	**9.3%**	

433

Table 3: Cumulative incidence of disease progression to myelofibrosis (MF) or myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) according to risk stratification and hematological response in 1080 patients with essential thrombocythemia treated with first line hydroxyurea

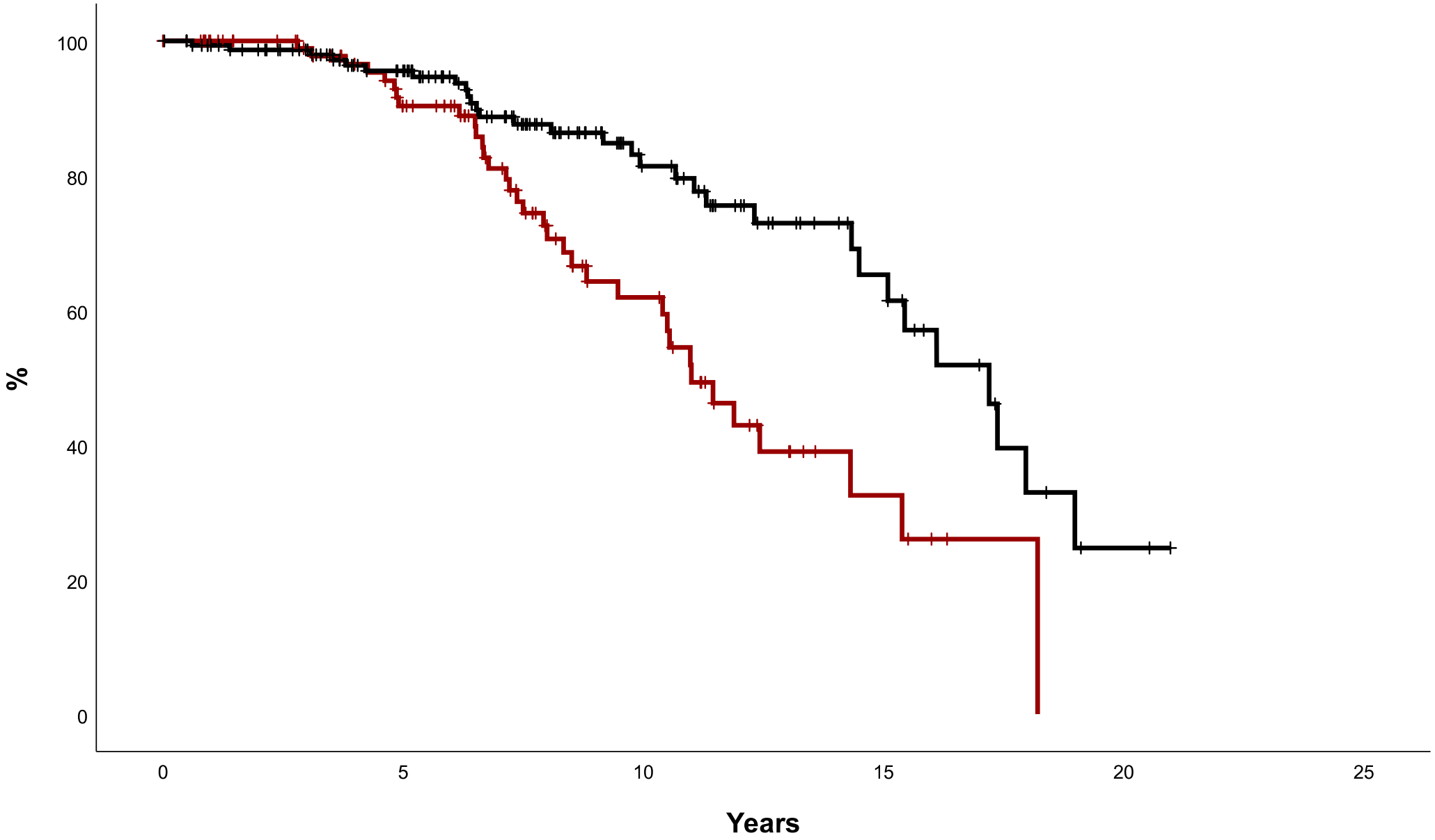
Risk stratification		MYELOFIBROSIS			MDS/AML		
Classical	CHR	Events/patients	10-years probability	P value	Events/patients	10-years probability	P value
Low	Yes	3/72	7%	0.4	3/72	8%	0.05
	No	6/72	6%		0/72	-	
High	Yes	21/648	5%	**<0.001**	17/648	3%	0.07
	No	28/288	18%		13/288	5%	
Revised IPSET-T	CHR	Events/patients	10-years probability, %	P value	Events/patients	10-years probability, %	P value
Very low	Yes	0/28	-	0.1	1/28	0%	0.3
	No	4/33	12%		0/33	-	
Low	Yes	3/44	11%	0.9	2/44	13%	0.1
	No	2/39	6%		0/39	-	
Intermediate	Yes	5/156	5%	**0.01**	2/156	2%	**0.001**
	No	13/105	23%		8/105	7%	
High	Yes	16/492	6%	**0.03**	15/492	3%	0.8
	No	15/183	15%		5/183	3%	

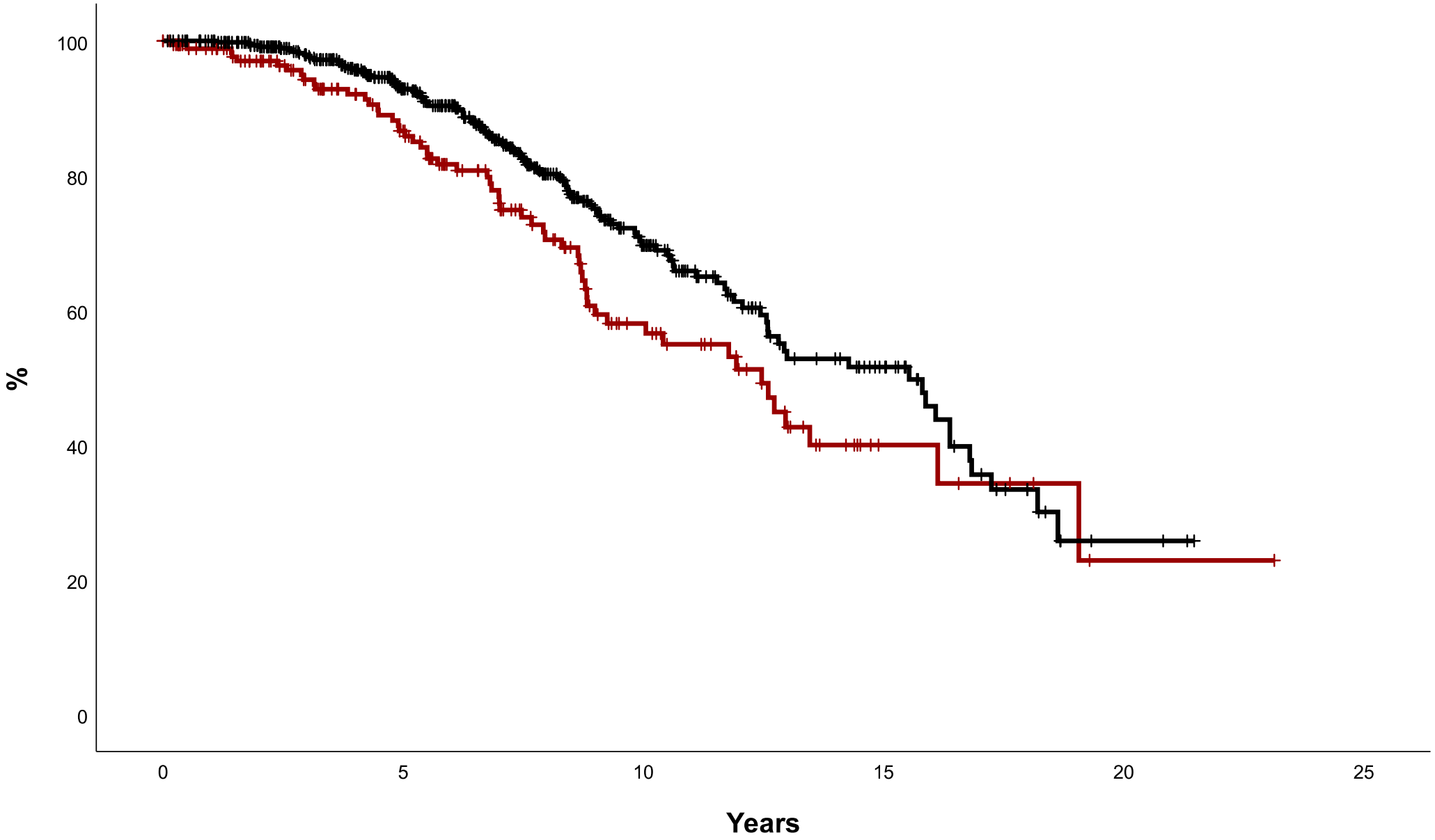
434

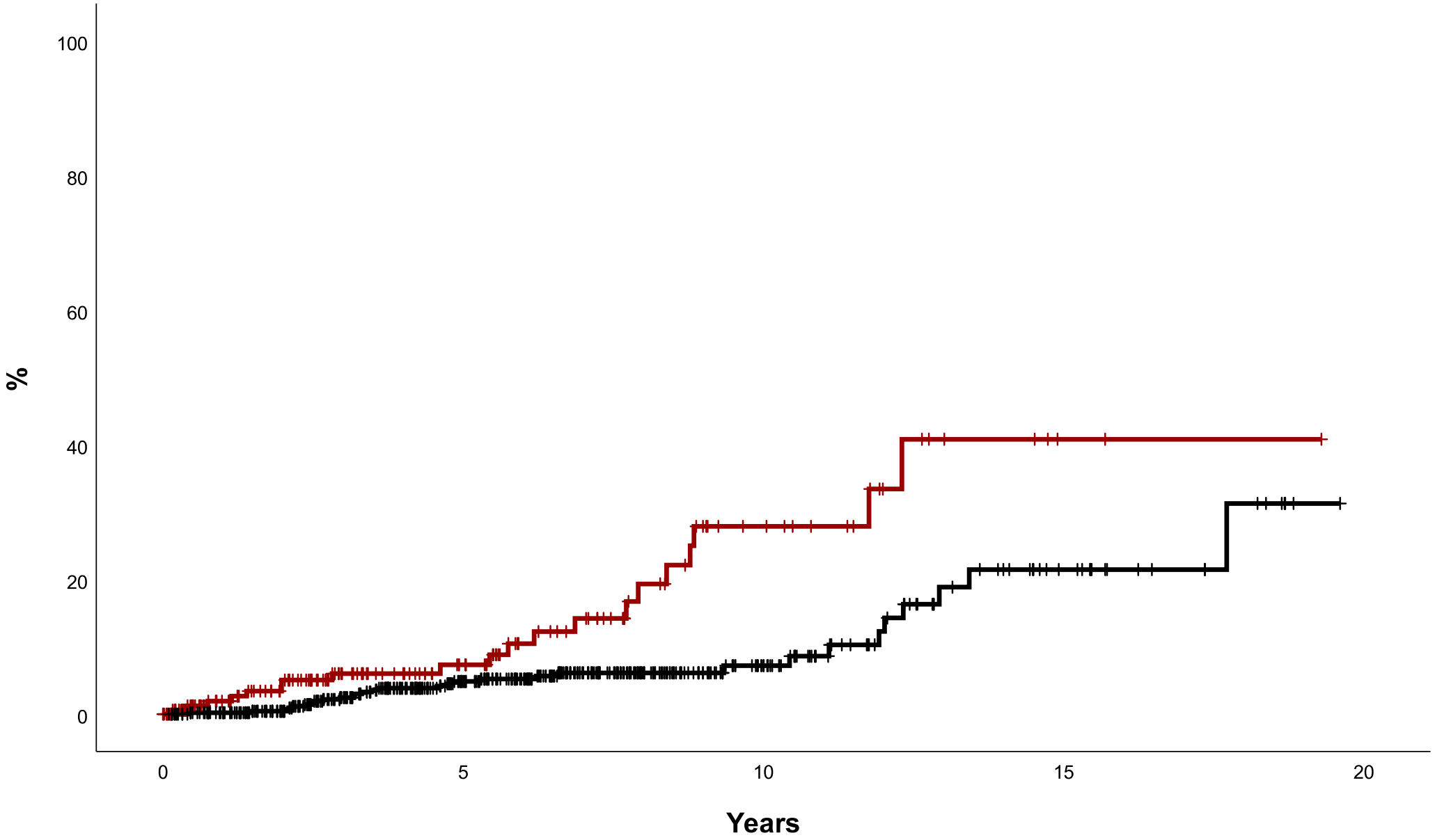
Table 4: Cumulative incidence of disease progression to myelofibrosis (MF) or myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) according to risk stratification and development of resistance/intolerance to first line hydroxyurea in 1080 patients with essential thrombocythemia

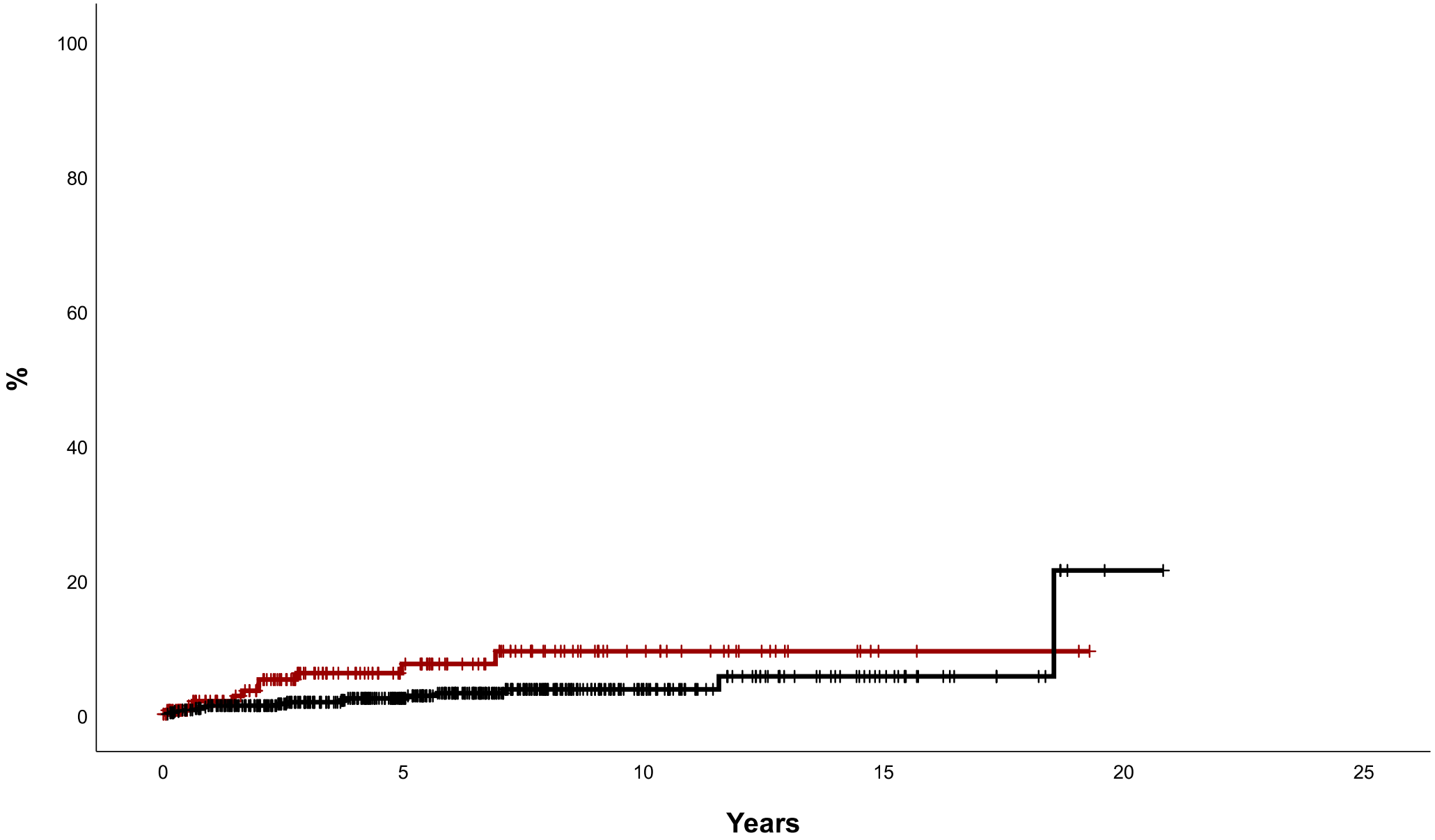
Risk stratification		MYELOFIBROSIS			MDS/AML		
Classical	R/I	Events/patients	10-years probability	P value	Events/patients	10-years probability	P value
Low	Yes	5/32	20%	**0.02**	1/32	6%	0.8
	No	4/112	3%		2/112	2%	
High	Yes	28/187	17%	**<0.001**	12/187	7%	0.2
	No	21/749	6%		18/749	2%	
Revised IPSET-T	R/I	Events/patients	10-years probability, %	P value	Events/patients	10-years probability, %	P value
Very low	Yes	2/15	14%	0.2	0/15	-	0.1
	No	2/46	3%		1/46	0%	
Low	Yes	3/17	20%	0.1	1/17	10%	0.6
	No	2/66	3%		1/66	4%	
Intermediate	Yes	10/72	14%	0.08	5/72	6%	0.6
	No	8/189	11%		5/189	3%	
High	Yes	18/115	19%	**<0.001**	7/115	7%	**0.2**
	No	13/560	5%		13/560	2%	

R/I: resistance/intolerance to hydroxyurea. When different criteria included in the definition of R/I to hydroxyurea were analyzed separately only cytopenia remain significant for progression to MF in the following risk groups: classic high-risk ($p<0.001$), intermediate-risk revised IPSET($p=0.003$) and high-risk revised IPSET ($p<0.001$). Regarding AML cytopenia was associated with a higher probability only in high-risk groups according to stratification by both classic ($p=0.01$) and revised IPSET ($p=0.004$).


100
80
60
40
20
0
%
0
5
10
15
20
25
Years


100
80
60
40
20
0
%
0
5
10
15
20
25
Years


100
80
60
40
20
0
%
0
5
10
15
20
Years


100
80
60
40
20
0
%
0
5
10
15
20
25
Years

Supplemental table 1: Main clinical characteristics at diagnosis according to the development or not of resistance/intolerance to first-line hydrodroxyurea in 1080 patients included in the Spanish Registry of Essential Thrombocythemia

	R/I to HU (Any criterium)			Persistent Thrombocytosis			Cytopenia			Leg ulcers			Unacceptable mucocutaneous toxicity		
	No N=861	Yes N=219	p	No N=1050	Yes N=30	p	No N=1000	Yes N=80	P	No N=1017	Yes N=63	p	No N=1026	Yes N=54	p
Age, years	66	67	0.8	67	60	0.03	66	71	<0.001	68	71	<0.001	67	61	0.01
Sex															
Male	78%	22%	0.15	98%	2%	0.5	92%	8%	0.5	96%	4%	0.1	96%	4%	0.2
Female	82%	18%		97%	3%		93%	7%		93%	7%		94%	6%	
Hemoglobin, g/l	142	139	0.007	141	137	0.2	142	133	<0.01	141	140	0.8	141	144	0.2
Leukocytes, $x10^9$/l	9.4	9.6	0.4	9.4	9.8	0.5	94	9.8	0.2	9.4	9.4	0.9	9.4	9.0	0.2
Platelets, $x10^9$/l	779	907	<0.001	800	969	0.04	794	933	<0.001	798	921	0.02	803	828	0.5
Genotype*															
*JAK2*V617F	84%	16%		99%	1%		95%	5%		94%	6%		96%	4%	
CALR	72%	28%	<0.001	93%	7%	<0.001	89%	11%	0.001	92%	8%	0.2	94%	6%	0.4
MPL	60%	40%		90%	10%		82%	18%		95%	5%		92%	8%	
Triple negative	79%	21%		98%	2%		90%	10%		99%	1%		93%	7%	

*Six double positive cases (*JAK2*V617F+/*CALR+* n=3, *JAK2*V617F+/*MPL+* n=2, *CALR*+/*MPL+* n=1) were excluded from analysis. Resistance/intolerance to HU was defined as: platelet count >600x109 /l after 3 months of at least 2 g/d or maximum tolerated doses of HU; platelet count >400x109 /l combined with

leukocyte count <2x109 /l or hemoglobin <100 g/l at any dose of HU; presence of leg ulcers or other unacceptable mucocutaneous manifestations at any dose of HU

Supplemental Table 2: Cumulative incidence of disease progression to myelofibrosis (MF) or myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) according to hematological response (CHR) and resistance/intolerance to hydroxyurea (HU) in 1080 patients with essential thrombocythemia							
		MYELOFIBROSIS			MDS/AML		
		Events/patients	10-years probability	P value	Events/patients	10-years probability	P value
CHR	Yes No	24/720 34/360	5.5% 16%	**<0.001**	20/720 13/360	3.8% 3.7%	0.4
Resistance/intolerance to HU (any criteria)	No Yes	25/861 33/219	5.7% 17.4%	**<0.001**	20/861 13/219	2.5% 6.7%	0.2
Persistent thrombocytosis	No Yes	54/1048 4/30	8.7% 14.6%	0.2	32/1048 1/30	3.5% 8.3%	0.8
Cytopenia	No Yes	36/998 22/80	7% 29.7%	**<0.001**	25/998 8/80	3% 11%	**0.01**
Leg ulcers	No Yes	47/1016 11/62	8.2% 16.1%	**0.03**	30/1016 3/62	3.6% 6%	0.8
Unacceptable mucocutaneous toxicity	No Yes	56/1025 2/53	9.2% 5.3%	0.4	33/1025 0/53	4% -	0.2