



Original Article

Is Allogeneic Transplantation an Option in Patients Affected by Concurrent Myelofibrosis and Chronic Myeloid Leukemia (CML)?

Federica Sorà^{1,2}, Patrizia Chiusolo^{1,2}, Francesco Autore¹, Sabrina Giammarco¹, Luca Laurenti^{1,2}, Idanna Innocenti¹, Elisabetta Metafuni¹, Eugenio Galli¹, Andrea Bacigalupo^{1,2} and Simona Sica^{1,2}.

¹ Dipartimento di Diagnostica per Immagini, Radioterapia Oncologica ed Ematologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy.

² Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica del Sacro Cuore, Roma, Italy.

Competing interests: The authors declare no conflict of Interest.

Abstract. Classification of myeloproliferative neoplasms is based on hematologic, histopathologic, and molecular characteristics, including the BCR-ABL1 and JAK2 V617F or MPL and CALR. Although the different gene mutations ought to be mutually exclusive, several cases with co-occurring BCR-ABL1 and JAK2 V617F or CALR have been identified with a frequency of 0.2-2.5% in the European population. The tyrosine kinase abnormalities appeared to affect independent subclones because imatinib mesylate (IM) treatment induced Ph⁺-CML remission, whereas the JAK2V617F clone either persisted or clinically expanded after a major response of Ph⁺-clone.

Allogeneic stem cell transplantation is at present the only potentially curative therapy for these patients after therapy with ruxolitinib and TKI inhibitor. We describe the case of 3 young people treated in our institution for the coexistence of BCR/ABL chronic myeloid leukemia and another Philadelphia chromosome-negative (Ph⁻) Chronic myeloproliferative disease. They received ruxolitinib, imatinib/nilotinib, and allogeneic transplantation with safe and efficient results.

Keywords: Chronic myeloid leukemia; Myelofibrosis; JAK; Allogeneic transplantation.

Citation: Sorà F., Chiusolo P., Autore F., Giammarco S., Laurenti L., Innocenti I., Metafuni E., Galli E., Bacigalupo A., Sica S. Is allogeneic transplantation an option in patients affected by concurrent myelofibrosis and Chronic Myeloid Leukemia (CML)? *Mediterr J Hematol Infect Dis* 2021, 13(1): e2021062, DOI: <http://dx.doi.org/10.4084/MJHID.2021.062>

Published: November 1, 2021

Received: July 11, 2021

Accepted: October 15, 2021

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by-nc/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Correspondence to: Federica Sorà, MD. Dipartimento di Diagnostica per Immagini, Radioterapia Oncologica ed Ematologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy. Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica del Sacro Cuore, Roma, Italy. Largo Gemelli 1, 00168 Roma. Tel: 0039-6-30154180. E-mail: federica.sora@unicatt.it

Introduction. Chronic myeloproliferative diseases (CMPDs) are clonal disorders of hematopoietic stem cells classified according to their clinical and phenotypical features and genetic aberrations.¹ CMPDs are usually divided according to the presence of Philadelphia chromosome/BCR-ABL fusion (Ph⁺) as chronic myeloid leukemia (CML) and the Philadelphia chromosome-negative (Ph⁻) CMPD (polycythemia vera (PV), essential thrombocythemia (ET), primary

myelofibrosis (PMF). In the Ph negative category, a crucial role is linked to detecting the three major subcategories of gene mutations: JAK2/CALR/MPL mutation. Recently, some authors simultaneously reported on few well-documented cases with concurrent JAK2V617F and BCR-ABL translocation.²⁻⁷ The tyrosine kinase abnormalities appeared to affect independent subclones because imatinib mesylate (IM) treatment induced Ph⁺-CML remission, whereas the

JAK2V617F clone either persisted or clinically expanded after a major response of Ph+ CML to IM.⁸⁻⁹

Allogeneic hematopoietic cell transplantation (HCT) is currently the only treatment modality that offers potentially curative therapy for patients with myeloproliferative neoplasms (MPN) being largely abandoned in Ph+ MPD in the era of targeted therapy but still valid in primary myelofibrosis (PMF) and secondary myelofibrosis developing after polycythemia vera (post-PV MF) or essential thrombocythemia (post-ET MF) based on attributable risk score and patient's profile.

Molecular studies showed the prognostic role of "driver mutations" as *JAK2*, *MPL1*, and *CALR* and additional somatic DNA mutations, including *ASXL1*, *EZH2*, *IDH1/2*, and *SRSF2*. Thus, the combination and number of mutations are more relevant than a given single mutation. Mutations also appear to impact the outcome of hematopoietic cell transplantation (HCT). The availability of the JAK 1/2 inhibitor ruxolitinib, the first agent approved for molecularly targeted therapy in patients with PMF or with PV intolerant to other therapies, has modified treatment strategies for these patients. Randomized trials have shown that ruxolitinib, in addition to alleviating symptoms and reducing splenomegaly in a large proportion of patients, also prolongs survival.¹⁰⁻¹¹

Since the rarity of concomitant presence of BCR-ABL positive CML and myelofibrosis, only a few data are available of the combination therapy and stem cell transplantation (SCT) on the role on the outcome.

This report is the first to describe patients diagnosed with two concomitant chronic myeloproliferative diseases treated with tyrosine-kinase inhibitors and JAK2 inhibitors or anagrelide for whom allogeneic SCT was either performed or planned.

Patients and Methods.

Case report 1. In 1997, a 24-year-old woman presented with elevated platelet counts without clinical features. Bone marrow morphological analysis was performed, and a diagnosis of ET was made. Cytogenetic analysis showed normal karyotype, and the molecular study was negative for BCR-ABL1 rearrangement. The patient was firstly treated with interferon-alpha with benefits, and a successful pregnancy was carried out. In 2003, after an increase of hematocrit value over 50%, she received five phlebotomies, and anagrelide was introduced. JAK2 V617F mutation was detected for the first time in 2008. Bone marrow biopsy, performed in 2012, documented post-ET myelofibrosis (IMF-2), and the JAK2 V617F allelic ratio was 73%. While on anagrelide, in January 2014, she showed persistent leucocytosis, and PCR analysis showed a BCR-ABL1 transcript (b3a2). Cytogenetic analysis showed a normal karyotype; FISH analysis confirmed t(9:22) translocation. As a consequence, imatinib therapy was administered at 400

mg daily, associated with anagrelide. Major molecular response (MMR) was rapidly achieved at six months and deep molecular response (DMR) (MR4.5) at 12 months, with no effect on the myelofibrosis. The allelic ratio during imatinib and anagrelide treatment was checked every three months and remained stable over 50% for all the period. NGS analysis for subclonal mutations was performed, and we found SF3B1 c.1849G>T with a low VAF (9%).

In June 2018, anagrelide was discontinued because of progressive anemia while in DMR for BCR-ABL transcript with increasing bone marrow fibrosis. Shortly after anagrelide discontinuation, thrombocytosis required hydroxyurea and in December 2018 bone marrow biopsy showed blastic evolution (17% of blasts) of post-ET myelofibrosis and acquisition of cytogenetic abnormality in 17 out of 20 metaphases (46,XX,-12,+der(12)(12qter->q10::8q10->qter),-4,+22,del(20)(q11)). In July 2019, she received an allogeneic peripheral blood SCT from an HLA-matched unrelated donor (MUD) after a TBF conditioning regimen.¹² The status at ASCT was DMR of CML, JAK2 V617F mutation with allele burden of 58%. Full donor chimerism was documented at day+30 after transplant. The patient is alive in complete remission of both diseases with stable full donor chimerism at 24 months after SCT without signs of GVHD.

Case report 2. In July 2014, a 52-year-old man presented with splenomegaly and elevated white blood cell count (170000/microl). He also showed splenomegaly (20 cm below costal margin), fatigue, peripheral edema, and weight body loss (10 kg in six months). The molecular study disclosed BCR-ABL rearrangement (b3a2), and a diagnosis of CML chronic phase was made according to ELN criteria.¹³ Bone marrow biopsy confirmed the diagnosis of a myeloproliferative disorder with marked fibrosis and sclerosis. Cytogenetic analysis showed a normal karyotype, and the molecular study was negative for the JAK2 V617F mutation. Sokal, Hasford, EUTOS risk score were high. The patient started Nilotinib 600mg with a rapid peripheral blood cell count normalization and reduction of the spleen size. After six months, despite MMR for BCR-ABL transcript, systemic symptoms and splenomegaly were unchanged, and bone marrow fibrosis persisted. In February 2015, Calreticulin (*CALR*) gene type1 was detected. The analysis was performed with PCR followed by capillary electrophoresis, and the ratio between the mutated gene and wild type (30%) remained stable from diagnosis until transplant. Moreover, NGS analysis for subclonal mutations was performed, and the following genes were found to be mutated: *ASXL1* c.302C>T (VAF 37%) and *ZRSR2* c.5558-1G>T (VAF 10%). He added ruxolitinib at the dose of 20mg/d. After three months of ruxolitinib, there was a mild decrease in splenomegaly, but no

significant change in bone marrow fibrosis was detected. However, a dose reduction of ruxolitinib and nilotinib was required to manage hematologic toxicity (grade 3 anemia according to CTCAE). In March 2019, constitutional symptoms increased, and severe hepatomegaly developed, and the patient became transfusion dependent. The patient was eligible for allogeneic SCT and received peripheral blood SCT from a MUD with TBF as a conditioning regimen. At day +30, a mixed chimaerism was found (88%), while at day +60, full donor chimaerism was obtained. The posttransplantation course was remarkable for acute renal and respiratory failure, followed by several complications, including CMV, cardiac failure, and GVHD. The patient died from multi-organ failure seven months after SCT and no evidence of diseases.

Case report 3. In June 2015, a 58-year-old woman was referred from another hospital with chronic phase CML diagnosed two years before, low Sokal risk and BCR-ABL b3a2. Despite deep molecular response (MR4) during treatment with nilotinib, thrombocytosis persisted. At the time of our evaluation, white blood cell count and hemoglobin level were normal, but platelet count was persistently high ($600 \times 10^9/l$) despite normal iron balance. For these reasons, she was re-evaluated, and JAK2 V617F mutation was detected, and the allelic burden remained stable over 50%. Cytogenetic analysis showed normal karyotype. Spleen size was enlarged, and bone marrow biopsy showed marked fibrosis. Due to the patient's refusal, ruxolitinib was not started. A MUD search was initiated. Progressive splenomegaly and fatigue further developed in 2018. NGS analysis was performed, and the following subclonal mutations were found: ASXL1 c.2110G>A (VAF 48%), TET2 c.5162T>C, c.5623T>C and c86C>G with VAF of 47%, 38% and 47% respectively. Finally, in February 2019, ruxolitinib was started at the dose of 20mg without hematologic toxicity, control of fatigue, but no spleen size reduction. This patient was classified as DIPSS grade 2 MFI; she was still in DMR for CML and, in March 2021, received an allogeneic SCT from a MUD. Full donor chimerism was documented at day+30 after transplant. The patient is alive in complete remission with stable full donor chimerism at four months after SCT without signs of GVHD.

Discussion. From 2007 onward, almost 70 cases of coexistent MPD have been reported in the medical literature. Their incidence varies from 0.2% to 2.5% with a median age of 68 years, predominantly males, and some authors suggested that the incidence may vary according to different phases of CML.¹⁴ The first report, published by Kramer et al.³ in 2007, describes a 50 years old man who, three years after CML diagnosis successfully treated with imatinib and in cytogenetic and

molecular response, developed an increase in LDH plasma level and a decrease in platelet count. The patient also presented an enlarged spleen and bone marrow fibrosis and a positive JAK2-V617F mutation. In this report, the author showed that bone marrow fibrosis was not a consequence of progressive CML. Also, imatinib, a selective inhibitor of ABL, KIT, and PDGF receptor, reduces the content of bone marrow fibers CML. However, the response to imatinib could accelerate the outgrowth of IMF, modifying the balance of the concurrent MPD. Furthermore, JAK2 mutation was already present at CML diagnosis in a retrospective analysis of frozen samples.

In 2007, Inami et al. and Bornauer et al.⁴⁻⁵ published the cases of few patients simultaneously presenting JAK2-V617F mutation and BCR-ABL rearrangement. Also in this case, molecular abnormalities seem to affect two different clones, and the molecular response to imatinib induced the clinical manifestation of myelofibrosis, shifting the balance in favor of the JAK2 V617F clone. From the HANNOVER registry in 2008, Hussein et al.² reported four additional cases. Finally, Pieri et al.¹⁵ described double mutated myeloproliferative disease in a cohort of CML patients with an incidence of 8 out of 314 patients in a chronic phase (2.55%).

The optimal first-line treatment for these patients was not clearly described, but the response of BCR-ABL burden to different TKI (imatinib, dasatinib, nilotinib) was optimal in all patients reported. The increase of JAK allele burden following the successful treatment of CML was characterized by the appearance of constitutional symptoms and spleen enlargement resulting from proliferative competition between the two clones. More recently, Iurlo et al. and Zhou et al.⁶⁻⁸ described additional cases of concomitant MPD in whom the use of combination therapy with ruxolitinib, associated with imatinib or dasatinib, in three patients was safe and effective, but with no data with a prolonged follow up are available.

Finally, Martin Cabrera et al.⁹ analyzed all patients with suspected MPD for BCR ABL, JAK2 V617F, and MPL by RT PCR analysis, they found 23 out 10875 patients positive at diagnosis for BCR ABL and JAK2 V617F (0.2%), but no patients positive for MPL and BCR ABL. In these patients, the molecular analysis suggested that JAK2 V617F and BCR-ABL mutations developed from different clones except for two patients in which common ancestors were supposed. Additional genetic abnormalities were also reported suggesting genomic instability.¹⁴⁻¹⁵

Thus there are patients with concurrent MPD, although rare, two different clones are involved, the clinical presentation may vary being CML or JAK2 V617F or MPD alternatively detected first or during treatment, suggesting a shift in favor of the expansion of

one clone over the other particularly when CML was the prior disease treated with TKI. Latency seems to be different; in fact, a decade in average was necessary for CML to occur after JAK2 + MPD but only 5.4 years when JAK2 + MPD followed CML in a recent analysis from Bader et al.¹⁷

In this scenario, we found three additional patients with concurrent MPD with JAK2 mutation in 2 cases and 1 case expressing CALR. CML was easy to manage either with imatinib or nilotinib, but JAK2 V617F or Calr MPD was uneasily controlled by conventional therapy, including hydroxiurea, transfusions, and JAK2 inhibitors such as ruxolitinib in all patients. All of them developed overt IMF. Considering their age and despite deep control of CML, all of them were considered eligible for allogeneic HSCT as the only curative option in intermediate- or high risk myelofibrosis. In the first patient, the indication for HSCT was supported by the blastic phase. Meanwhile, in the other ones, the presence of detrimental NGS mutation reinforced the HSCT need.

References:

1. Tefferi A, Thiele J, Orazi A, Kvasnicka HM, Barbui T, Hanson CA et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. *Blood* 2007; 110: 1092-1097. <https://doi.org/10.1182/blood-2007-04-083501> PMID:17488875
2. Hussein K, Bock O, Seegers A, Flasshove M, Henneke F, Büsche G et al. myelofibrosis evolving during Imatinib treatment of a chronic myeloproliferative disease with co-existing BCR-ABL translocation and JAK2V617F mutation. *Blood* 2007; 109: 4106-4107. <https://doi.org/10.1182/blood-2006-12-061135> PMID:17449802
3. Kramer A, Reiter A, Kruth J, Erben P, Hochhaus A, Muller M et al. JAK2-V617F mutation in a patient with Philadelphia-chromosome-positive chronic myeloid leukaemia. *Lancet Oncol* 2007; 8: 658-660. [https://doi.org/10.1016/S1470-2045\(07\)70206-1](https://doi.org/10.1016/S1470-2045(07)70206-1)
4. Bornhauser M, Mohr B, Oelschlaegel U, Bornhauser P, Jacki S, Ehninger G et al. Concurrent JAK2(V617F) mutation and BCR-ABL translocation within committed myeloid progenitors in myelofibrosis. *Leukemia* 2007; 21: 1824-1826. <https://doi.org/10.1038/sj.leu.2404730> PMID:17476275
5. Inami M, Inokuchi K, Okabe M, Kosaka F, Mitamura Y, Yamaguchi H et al. Polycythemia associated with the JAK2V617F mutation emerged during treatment of chronic myelogenous leukemia. *Leukemia* 2007; 21: 1103-1104. <https://doi.org/10.1038/sj.leu.2404591> PMID:17301812
6. Iurlo A, Gianelli U, Rapezzi D, Cattaneo D, Fermo E, Binda F et al. Imatinib and ruxolitinib association: first experience in two patients. *Haematologica*. 2014;6:e76-7. <https://doi.org/10.3324/haematol.2013.102525> PMID:24633869 PMID:PMC4040897
7. Soderquist CR, Ewalt MD, Czuchlewski DR, Geyer JT, Rogers HJ, Hsi ED et al. Myeloproliferative neoplasms with concurrent BCR-ABL1 translocation and JAK2 V617F mutation: a multi-institutional study from the bone marrow pathology group. *Mod Pathol*. 2018;5:690-704. <https://doi.org/10.1038/modpathol.2017.182> PMID:29327708 PMID:PMC6008160
8. Zhou A, Knoche EM, Engle EK, Fisher DA, Oh ST. Concomitant JAK2 V617F-positive polycythemia vera and BCR-ABL-positive chronic myelogenous leukemia treated with ruxolitinib and dasatinib. *Blood Cancer J*. 2015; 10:e351. <https://doi.org/10.1038/bcj.2015.77> PMID:26430722 PMID:PMC4635189
9. Martin-Cabrera P, Haferlach C, Kern W, Schnittger S, Haferlach T. BCR-ABL1-positive and JAK2 V617F-positive clones in 23 patients with both aberrations reveal biologic and clinical importance. *Br J Haematol*. 2017 Jan;1:135-139 <https://doi.org/10.1111/bjh.13932> PMID:26847954
10. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med*. 2012 ;366:799-807. <https://doi.org/10.1056/NEJMoa1110557> PMID:22375971 PMID:PMC4822164
11. Harrison C, Kiladjian J, Haifa K, Gisslinger H, Waltzman R, Stalbovska V et al. JAK Inhibition with Ruxolitinib versus Best Available Therapy for Myelofibrosis *N Engl J Med* 2012; 366:787-798 <https://doi.org/10.1056/NEJMoa1110556> PMID:22375970
12. Sora F, Grazia CD, Chiusolo P, Raiola AM, Bregante S, Mordini N, et al. Allogeneic Hemopoietic Stem Cell Transplants in Patients with Acute Myeloid Leukemia (AML) Prepared with Busulfan and Fludarabine (BUFLU) or Thiotepa, Busulfan, and Fludarabine (TBF): A Retrospective Study. *Biol Blood Marrow Transplant*. 2020 Apr;26(4):698-703. <https://doi.org/10.1016/j.bbmt.2019.12.725> PMID:31875522
13. Baccarani M, Saglio G, Goldman J, Hochhaus A, Simonsson B, Appelbaum F, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood*. 2006;108:1809-20. <https://doi.org/10.1182/blood-2006-02-005686> PMID:16709930
14. Makishima H, Jankowska AM, McDevitt MA, O'Keefe C, Dujardin S, Cazzolli H, et al. CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia. *Blood*. 2011;21:e198-206 <https://doi.org/10.1182/blood-2010-06-292433> PMID:21346257 PMID:PMC3110035
15. Pieri L, Spolverini A, Scappini B, Occhini U, Birtolo S et al. Concomitant occurrence of BCR-ABL and JAK2V617F mutation. *Blood*. 2011 22;12:3445-6. <https://doi.org/10.1182/blood-2011-07-365007> PMID:21940831
16. Dogliotti I, Fava C, Serra A, Gottardi E, Daraio F, Carnuccio F, et al. CALR-positive myeloproliferative disorder in a patient with Ph-positive chronic myeloid leukemia in durable treatment-free remission: a case report. *Stem Cell Investig*. 2017 Jun 23;4:57 <https://doi.org/10.21037/sci.2017.06.02> PMID:28725653 PMID:PMC5503914

17. Bader G, Dreiling B. Concurrent JAK2-Positive Myeloproliferative Disorder and Chronic Myelogenous Leukemia: A Novel Entity? A Case Report With Review of the Literature. *J Investig Med High Impact Case Rep.* 2019 Jan-Dec;7:2324709619832322
<https://doi.org/10.1177/2324709619832322>
PMid:30803277 PMCID:PMC6393948
18. Jain T, Mesa RA, Palmer JM. Allogeneic Stem Cell Transplantation in Myelofibrosis. *Biol Blood Marrow Transplant.* 2017 ;9:1429-1436.
<https://doi.org/10.1016/j.bbmt.2017.05.007>
PMid:28499938 PMCID:PMC8148877
19. Tiribelli M, Palandri F, Sant'Antonio E, Breccia M, Bonifacio M. The role of allogeneic stem-cell transplant in myelofibrosis in the era of JAK inhibitors: a case-based review. *Bone Marrow Transplant.* 2020 ;4:708-716.
<https://doi.org/10.1038/s41409-019-0683-1>
PMid:31534197 PMCID:PMC7113188