Case Report

Acta Haematologica

Acta Haematol 2019;141:261–267
DOI: 10.1159/000496821

Received: October 23, 2018
Accepted after revision: January 11, 2019
Published online: April 9, 2019

Colony-Forming Cell Assay Detecting the Co-Expression of *JAK2*^{V617F} and *BCR-ABL1* in the Same Clone: A Case Report

Elena Tirrò[a, b] Stefania Stella[a, b] Michele Massimino[a, b] Valentina Zammit[c] Maria Stella Pennisi[a, b] Silvia Rita Vitale[a, b] Chiara Romano[a, b] Sandra Di Gregorio[a, b] Adriana Puma[a, b] Francesco Di Raimondo[c, d] Fabio Stagno[c] Livia Manzella[a, b]

[a]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy; [b]Center of Experimental Oncology and Hematology, A.O.U. "Policlinico-Vittorio Emanuele", Catania, Italy; [c]Division of Hematology, A.O.U. "Policlinico-Vittorio Emanuele", Catania, Italy; [d]Department of Surgery and Medical Specialties, University of Catania, Catania, Italy

Keywords

Essential thrombocythaemia · Chronic myeloid leukaemia · *JAK2*^{V617F} · *BCR-ABL1*

Abstract

BCR-ABL1-negative myeloproliferative disorders and chronic myeloid leukaemia are haematologic malignancies characterised by single and mutually exclusive genetic alterations. Nevertheless, several patients co-expressing the *JAK2*^{V617F} mutation and the *BCR-ABL1* transcript have been described in the literature. We report the case of a 61-year-old male who presented with an essential thrombocythaemia phenotype and had a subsequent diagnosis of chronic phase chronic myeloid leukaemia. Colony-forming assays demonstrated the coexistence of 2 different haematopoietic clones: one was positive for the *JAK2*^{V617F} mutation and the other co-expressed both *JAK2*^{V617F} and the *BCR-ABL1* fusion gene. No colonies displayed the *BCR-ABL1* transcript alone. These findings indicate that the *JAK2*^{V617F} mutation was the founding genetic alteration of the disease, followed by the acquisition of the *BCR-ABL1* chimeric oncogene. Our data support the hypothesis that a heterozygous *JAK2*^{V617F} clone may have favoured the bi-clonal nature of this myeloproliferative disorder, generating clones harbouring a second transforming genetic event.

© 2019 S. Karger AG, Basel

Introduction

Myeloproliferative disorders (MPDs) are chronic haematological malignancies characterised by the clonal expansion of a transformed haematopoietic stem cell. Detection of a balanced translocation involving the long arms of chromosomes 9 and 22 that generates the abnormal Philadelphia (Ph) chromosome allows their classification into Ph-negative and Ph-positive diseases. The former include polycythaemia vera, essential thrombocythaemia (ET) and

E.T., S.S. and M.M. contributed equally to this work.

KARGER

E-Mail karger@karger.com
www.karger.com/aha

© 2019 S. Karger AG, Basel

Elena Tirrò
Department of Clinical and Experimental Medicine
University of Catania, Via Santa Sofia, 78
IT–95123 Catania (Italy)
E-Mail ele_tir@yahoo.it

Downloaded by:
Linköpings Universitetsbibliotek
130.236.82.7 - 4/11/2019 11:40:09 AM

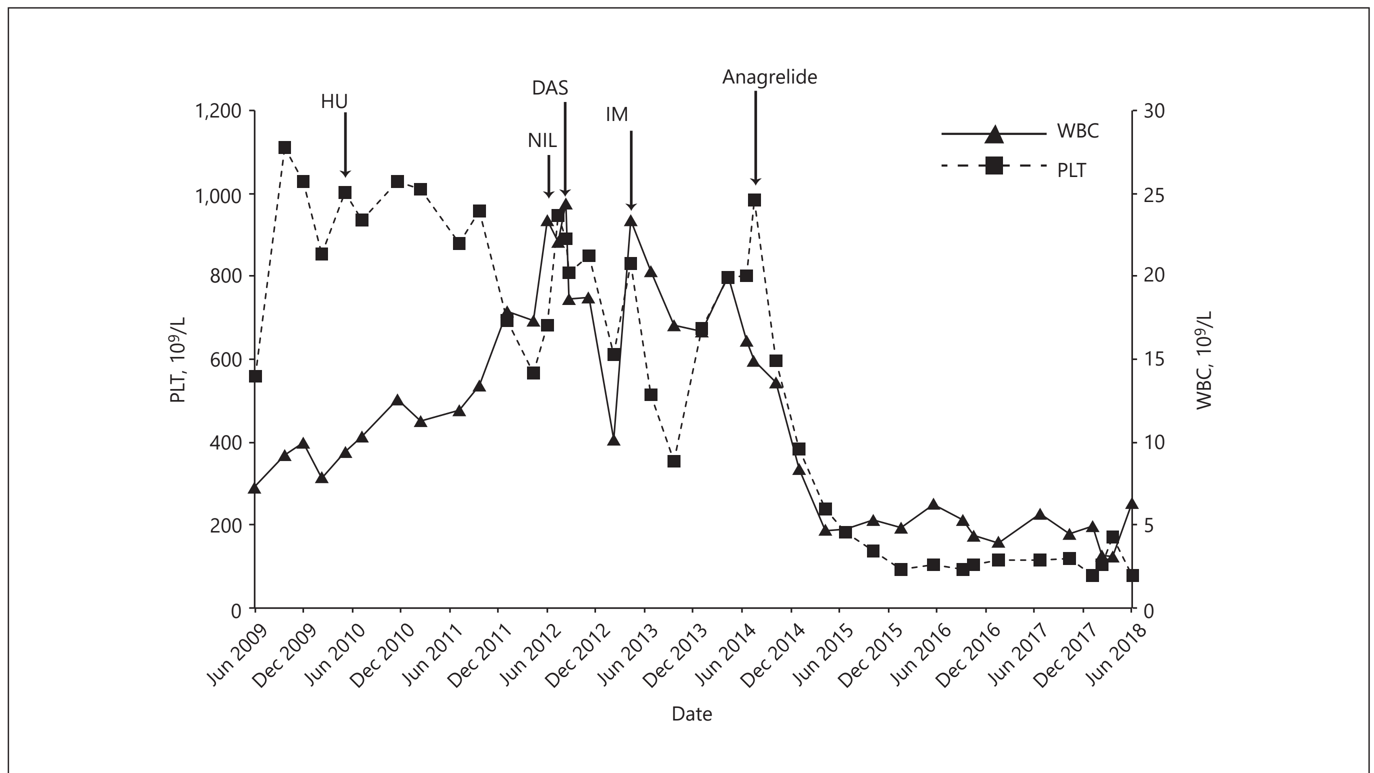

Fig. 1. The patient's PTL and WBC count parameters over time are shown in relationship to TKIs (NIL, DAS and IM), HU or anagrelide treatments. PLT, platelet; WBC, white blood cell; TKI, tyrosine kinase inhibitor; NIL, nilotinib; DAS, dasatinib; IM, imatinib; HU, hydroxyurea.

primary myelofibrosis (PMF), while the latter are represented by chronic myeloid leukaemia (CML).

Ph-negative MPDs are characterised by the presence of alterations in the sequence of the Janus kinase 2 (*JAK2*), calreticulin (*CALR*) or myeloproliferative leukaemia protein (*MPL*) genes. Specifically, a point mutation in exon 14 of *JAK2* (g.1849 G>T) causing the substitution of a valine with a phenylalanine (*V617F*) is the most common genetic event detected in Ph-negative MPDs. The *JAK2*^{V617F} mutation is found in over 90% of polycythaemia vera patients and in 35–50% of individuals diagnosed with ET or PMF [1].

The *CALR* gene displays indels on exon 9 that have been reported in 20–30% of ET and PMF patients. Finally, 2–5% of ET and PMF patients harbour activating mutations in the *MPL* gene located on exon 10 and involving the tryptophan residue in position 515 [2].

CML is characterised by the Ph chromosome that juxtaposes, in frame, the first exons of the breakpoint cluster region (*BCR*) gene with the 3′ coding sequence of the *ABL* proto-oncogene [3]. The ensuing chimeric *BCR-ABL1* transcript encodes for an oncoprotein displaying constitutive tyrosine kinase activity [4–7]. Inhibition of BCR-ABL1 kinase activity has, therefore, become the treatment of choice for newly diagnosed CML [8, 9].

Ph-negative and Ph-positive MPDs are considered mutually exclusive. Nevertheless, there have been several reports of patients co-expressing the *JAK2*^{V617F} mutation and the *BCR-ABL1* transcript, with discordant results as to the order in which these alterations were acquired and their expression in the same or in distinct clones [10, 11]. Here, we present the case of a male subject with an initial ET diagnosis displaying the *JAK2*^{V617F} mutation who subsequently developed CML with the appearance of the *BCR-ABL1* fusion gene.

Case Report

In June 2009, a 61-year-old man was referred to the Division of Haematology of the A.O.U. "Policlinico-Vittorio Emanuele" in Catania because a complete cell blood count showed an elevated platelet (PLT) number (560 × 10^9/L), with haemoglobin levels (14.8 g/dL), haematocrit (43.3%) and white blood cell (WBC) counts (7.38 × 10^9/L) within the normal range (Fig. 1). A bone

Downloaded by:
Linköpings Universitetsbibliotek
130.236.82.7 - 4/11/2019 11:40:09 AM

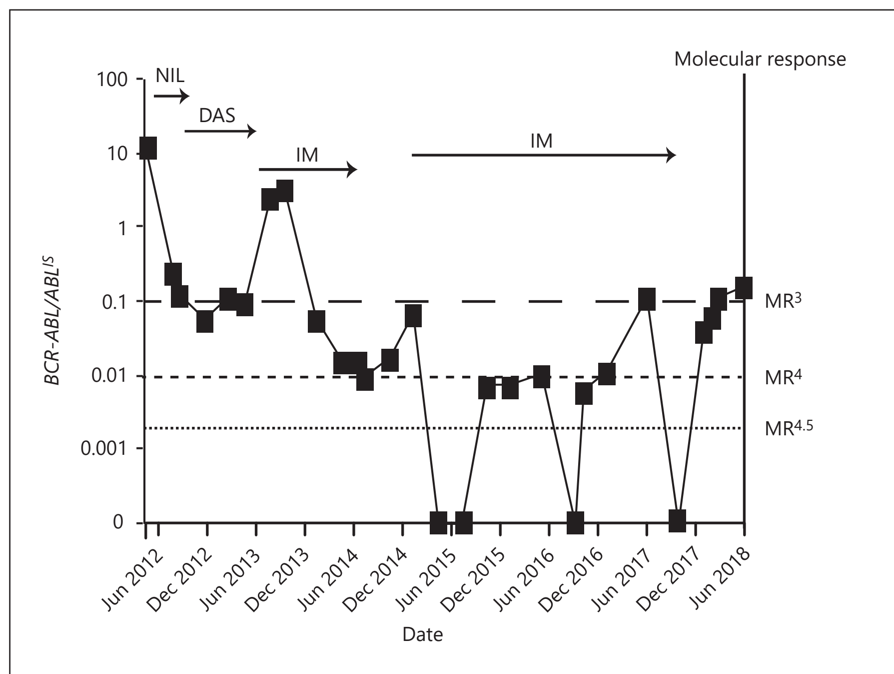

Fig. 2. *BCR-ABL1/ABL^{IS}* ratio over the depicted time period in relation to the specified TKI treatments. Dotted lines represent achievement of a major (MR^3) or a deep MR (MR^4 and $MR^{4.5}$). TKI, tyrosine kinase inhibitor; MR, molecular response.

marrow biopsy revealed hyper-cellularity with granulocytic and megakaryocytic hyperplasia and a normal karyotype. A diagnosis of ET was made, and treatment with low-dose aspirin was commenced to reduce the risk of thrombotic events. Subsequently, hydroxyurea was started at a dose of 1,000 mg/daily. Expression of the *JAK2* mutation was analysed by real-time RT-PCR (Q-PCR) with probes specific for the mutant and wild type sequences, and a low percentage of the mutant allele (25%) was detected.

Three years later, the WBC count began to increase and, in April 2012, the leukocyte count was 17.3×10^9/L. Blood smears and a bone marrow biopsy were compatible with a diagnosis of chronic phase (CP) CML, with bone marrow basophilia and 5% blasts. A cytogenetic analysis showed t(9;22)(q34;q11) in 20 out of 20 metaphase cells. Qualitative RT-PCR using standardized primers detected the presence of the e13a2 *BCR-ABL1* transcript, while a quantitative Q-PCR, performed as previously described [12], revealed a *BCR-ABL1/ABL^{IS}* of 16.09% (Fig. 2). The patient was thus diagnosed as having CP-CML with low Sokal, EURO and EUTOS risks in addition to the previously detected ET. At the time, a 50% increase in the mutant allele burden was noticed at a control of the *JAK2*^{V617F} levels.

Hence, in June 2012, the patient was started on the tyrosine kinase inhibitor (TKI) nilotinib (NIL) 300 mg twice daily. NIL therapy was ceased 3 months later as a result of anaemia (haemoglobin 9.3 g/dL) associated with leukocytosis (WBC 24.38×10^9/L) and thrombocytosis (PLT 889×10^9/L). Therefore, the patient was started on conventional dasatinib (DAS) (100 mg/day) and achieved a major molecular response (MMR) after 3 months of treatment. However, 3 months later, an increase in *BCR-ABL1* levels was detected by quantitative Q-PCR, and he lost his MMR but not the cytogenetic response. A mutational study failed to detect alterations in the *BCR-ABL1* sequence clearly associated with TKI resistance but identified 3 low-level mutations (T267S, Y353S and G463D). DAS therapy was maintained, the patient regained MMR, but, due to the development of severe pulmonary hypertension, the patient was switched to imatinib (IM) conventional therapy and regained a MMR. Treatment with IM progressively induced a further decrease in *BCR-ABL1* transcripts, and the patient achieved an MR^4, although, after 10 months of treatment, he developed a grade-3 haematological toxicity that caused a temporary IM discontinuation. The routine cell blood counts performed during IM therapy confirmed persistence of the thrombocytosis (PLT about 800×10^9/L). Thus, low-dose interferon and subsequently hydroxyurea were added to IM treatment. However, in October 2014, the patient stopped responding to this cytoreductive combination. Hence, anagrelide therapy was begun, and the PLT count normalized. Unfortunately, in April 2018 (after >8 years from the ET diagnosis and 6 years from the CML diagnosis), the patient developed a squamous non-small cell lung cancer (NSCLC) and was forced to cease IM because of the potential cross-reaction with the association of cisplatin and vinorelbine that he received as first-line treatment. After IM cessation, *BCR-ABL1/ABL^{IS}* levels began to increase, although, at the last control, the patient displayed *BCR-ABL1/ABL^{IS}* transcripts still compatible with a maintained complete cytogenetic response.

Results

To establish if the *JAK2*^{V617F} mutation and the translocation generating the BCR-ABL1 oncogene occurred in the same or in different clones, we analysed peripheral blood samples at ET and CML diagnosis and after 36 months of IM therapy. 1×10^5 primary mononuclear cells were seed-

Downloaded by:
Linköpings Universitetsbibliotek
130.236.82.7 - 4/11/2019 11:40:09 AM

ed in MethoCult H4435 methylcellulose medium (Stemcell Technologies), and colony formation was assessed 14 days after plating. Twenty colonies for each condition were plucked and RNA was isolated using TRIzol (Thermo Fisher Scientific). A direct reverse transcription and PCR was performed using the OneStep RT-PCR kit (Qiagen) employing the indicated forward 5′-GAGGCCTACTCATATGAACCAAAT-3′ and reverse 5′-CATGCCAACTGTTTAGCAACTTCA-3′ primers. To determine whether the *JAK2*^{V617F} mutation was carried in a homozygous or heterozygous state, digestion of the PCR products with BsaXI (New England Biolabs) was then performed as the V617F mutation abolishes a motif in wild-type *JAK2* that is recognized by the restriction enzyme. Of the 20 colonies screened from the sample collected at ET diagnosis, 15 (75%) exhibited wild-type *JAK2* and 5 (25%) were heterozygous for *JAK2*^{V617F} (Fig. 3a). Of the 20 colonies derived from the samples collected at CML diagnosis, 4 (20%) exhibited wild-type *JAK2*, 6 (30%) were homozygous for *JAK2*^{V617F} and 10 (50%) were heterozygous for *JAK2*^{V617F} (Fig. 3a, b). Of the 20 colonies derived from the samples gathered after IM treatment, 5 (25%) were wild type for *JAK2*, 6 (30%) were homozygous for *JAK2*^{V617F} and 9 (45%) were heterozygous for *JAK2*^{V617F} (Fig. 3a). Concurrently, expression of the BCR-ABL1 transcripts was analysed by a semi-nested PCR employing the indicated forward 5′-TATGACTGCAAATGGTACATTCC-3′ and reverse 5′-GTTCCAACGAGCGGCTTCACT-3′ primers for the first PCR and the internal forward primer 5′-GTGCAGAGTGGAGGGAGAACA-3′ for the second PCR. In the samples at CML diagnosis, 12 colonies (60%) were BCR-ABL1-positive (5 homozygous and 7 heterozygous for *JAK2*^{V617F}) (Fig. 3b, c). We could not detect BCR-ABL1 transcripts in colonies expressing wild-type *JAK2*. Of the 20 colonies derived from the samples obtained after IM, 17 were BCR-ABL1 negative while 3 colonies displaying the BCR-ABL1 transcript also displayed the *JAK2*^{V617F} mutation in a heterozygous state (Fig. 3c).

Discussion/Conclusion

The association of the *JAK2*^{V617F} mutant and the *BCR-ABL1* fusion gene is infrequent but even more so the occurrence of CML after an ET diagnosis [13]. In this article, we report that cultured colonies derived from a patient diagnosed with ET and then developing CP-CML showed the presence of the *JAK2*^{V617F} mutant alone or in combination with *BCR-ABL1*. We did not detect the *BCR-ABL1* fusion gene alone.

These findings suggest that the *BCR-ABL1* fusion gene was a secondary genetic event in haematopoietic cells that had already acquired the *JAK2*^{V617F} mutation, as previously suggested by Wang et al. [14]. Two additional results further support this hypothesis: (1) the observed increase in the *JAK2*^{V617F} allele burden at the time of CML diagnosis and (2) the lack of a significant reduction in the number of *JAK2*^{V617F}-positive colonies after prolonged IM treatment. Indeed, 36 months of IM induced a considerable decrease in the number of *BCR-ABL1*-positive colonies but did not modify the number of *JAK2*^{V617F}-positive progenitors. Moreover, our data suggest that the genetic event generating the *BCR-ABL1* fusion may have occurred in a heterozygous sub-clone since we found some *JAK2*^{V617F} heterozygous with and without *BCR-ABL1* fusion. The loss of heterozygosity has also happened in 2 independent *JAK2*^{V617F} clones since we detected *JAK2*^{V617F} homozygous clones with and without *BCR-ABL1*. In agreement with what has been previously reported by Cambier et al. [15], IM therapy successfully repressed *BCR-ABL1*-positive progenitors but failed to reduce the percentage of *JAK2*^{V617F}-positive clones as demonstrated by the similar percentages of *JAK2*^{V612F} colonies observed before and after IM treatment. While we cannot exclude that during exposure to the second-generation TKIs NIL and DAS there may have been a reduction in *JAK2*^{V617F}-positive colonies, the lack of samples collected during these treatments prevents us from experimentally testing this hypothesis. These data would have been of great interest as previous reports have suggested that second-generation TKIs, alone [16] or in combination with ruxolitinib [17], repress the proliferative capacity of clones displaying both *JAK2*^{V617F} and *BCR-ABL1*. Indeed, the combination of NIL or DAS with a JAK2 inhibitor would have been a good choice for this disease but could not be offered to the patient as he did not meet the current criteria required for ruxolitinib prescription and reimbursability in Italy. Hence, a second-line therapy with anagrelide was commenced, and the drug considerably decreased the patient's PLTs but not the number of his *JAK2*^{V617F}-positive colonies.

The case was further complicated by the patient developing a third malignancy, namely a NSCLC of the right lung. An increased rate of secondary malignancies has been reported in patients with haematopoietic malignancies receiving antineoplastic therapies. Furthermore, while the introduction of TKIs for CML treatment has greatly improved the life expectancy of patients diagnosed with this disease [18], it is still controversial if prolonged exposure to these drugs may increase the risk of

Downloaded by:
Linköpings Universitetsbibliotek
130.236.82.7 - 4/11/2019 11:40:09 AM

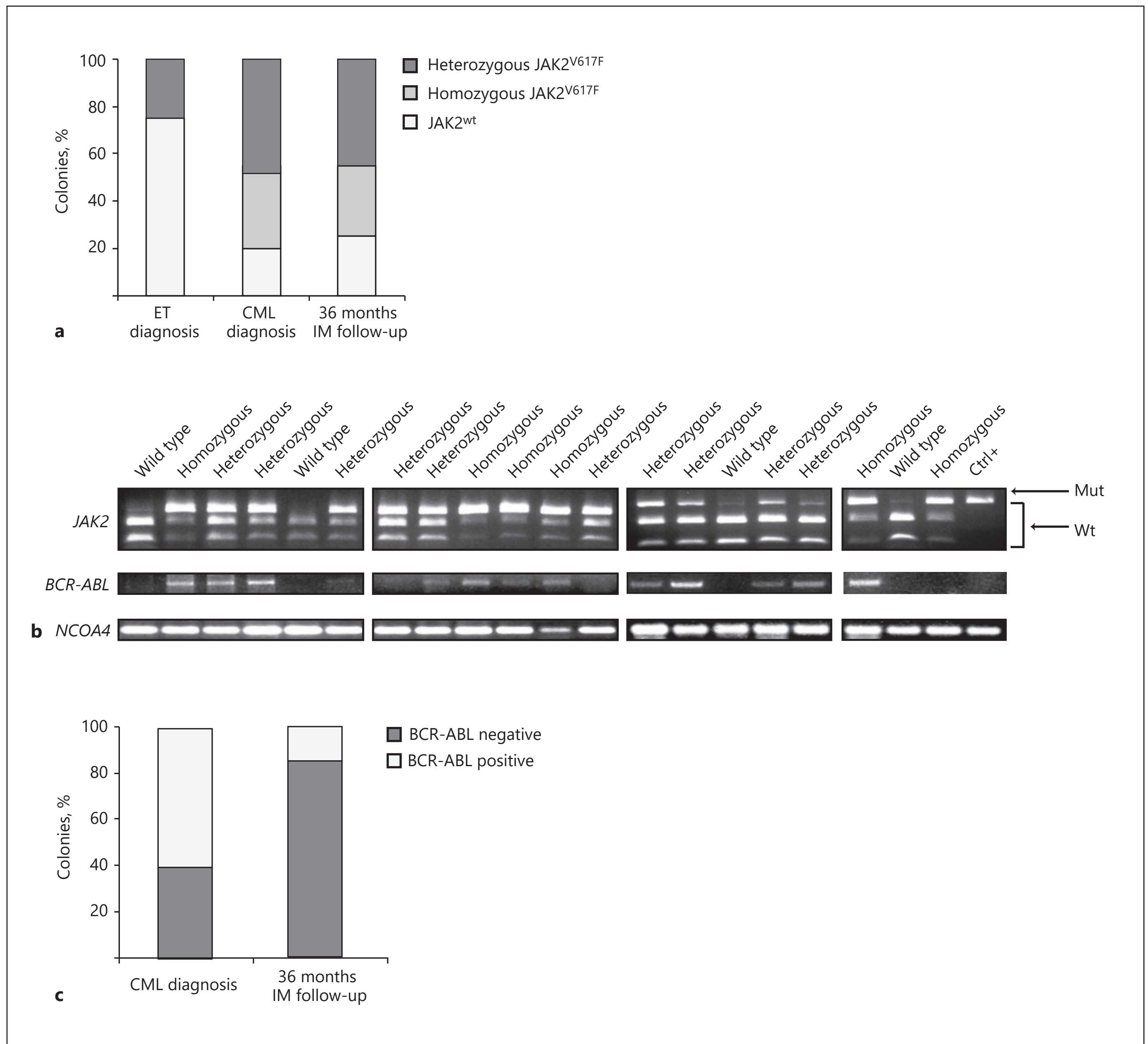

Fig. 3. a Percentage of colonies expressing wild-type or mutated JAK2 at ET and CML diagnosis and after IM follow-up. **b** Expression of the *JAK2*^{V617F} mutant and *BCR-ABL1* oncogene in 20 colonies collected at the time of CML diagnosis. HEL cells were used as a positive control (Ctrl+) for the homozygous *JAK2*^{V617F} mutation. Expression of the housekeeping gene *NCOA4* was used to verify the quality of the extracted RNA. **c** Percentage of BCR-ABL1-positive and -negative colonies at CML diagnosis and after IM follow-up. ET, essential thrombocythaemia; CML, chronic myeloid leukaemia; IM, imatinib.

developing secondary tumours [19–21]. As our patient was also a heavy smoker (41 pack/years), it is difficult to evaluate the contribution that extended exposure to TKIs and anagrelide may have made to the development of his lung cancer.

In summary, we describe the case of a patient with a *JAK2*^{V617F}-positive ET who subsequently acquired, in part of his malignant clones, the *BCR-ABL1* fusion gene while maintaining a number of *JAK2*^{V617F}-positive/*BCR-ABL1*-negative clones. Our findings suggest that the original

Downloaded by:
Linköpings Universitetsbibliotek
130.236.82.7 - 4/11/2019 11:40:09 AM

JAK2^{V617F}-positive stem cell may have given rise to multiple sub-clones (some heterozygous, others homozygous for the *JAK2* mutation) and that – because of an inherent genomic instability [22] or unknown genetic predisposing factors [23] – some of these sub-clones eventually acquired the *BCR-ABL1* oncogene. We hypothesize that the *BCR-ABL1* fusion gene developed in a heterozygous *JAK2*^{V617F}-positive clone that ultimately became homozygous for the *JAK2* mutation. Additional whole-exome sequence studies are currently being performed on the *JAK2*-positive, *JAK2*/*BCR-ABL1*-positive and NSCLC cells of the patient to investigate how a germline predisposition may have influenced the patient's risk of developing further malignancies.

Statement of Ethics

The patient was followed at the Division of Hematology of the A.O.U. "Policlinico-Vittorio Emanuele" and signed an informed consent releasing anonymously his sample for research purposes in accordance with the Declaration of Helsinki.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

The authors received no specific funding for this work.

Author Contributions

E.T., S.S. and M.M. designed and performed the experiments; E.T., S.S., M.M., C.R., M.S.P., S.R.V., S.D.G. and A.P. analysed and interpreted the data; E.T. wrote the paper; F.S. and V.Z. made a critical revision of the paper and managed the patient; F.D.R. helped supervise the project; L.M. conceived the original idea and supervised the project.

References

1 Barbui T, Thiele J, Gisslinger H, Kvasnicka HM, Vannucchi AM, Guglielmelli P, et al. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. Blood Cancer J. 2018 Feb;8(2):15.

2 Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017 Feb;129(6):667–79.

3 Stagno F, Vigneri P, Del Fabro V, Stella S, Cupri A, Massimino M, et al. Influence of complex variant chromosomal translocations in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors. Acta Oncol. 2010 May;49(4):506–8.

4 Giallongo C, Parrinello N, Tibullo D, La Cava P, Romano A, Chiarenza A, et al. Myeloid derived suppressor cells (MDSCs) are increased and exert immunosuppressive activity together with polymorphonuclear leukocytes (PMNs) in chronic myeloid leukemia patients. PLoS One. 2014 Jul;9(7):e101848.

5 Massimino M, Consoli ML, Mesuraca M, Stagno F, Tirrò E, Stella S, et al. IRF5 is a target of BCR-ABL kinase activity and reduces CML cell proliferation. Carcinogenesis. 2014 May;35(5):1132–43.

6 Preyer M, Vigneri P, Wang JY. Interplay between kinase domain autophosphorylation and F-actin binding domain in regulating imatinib sensitivity and nuclear import of BCR-ABL. PLoS One. 2011 Feb;6(2):e17020.

7 Giallongo C, Tibullo D, La Cava P, Branca A, Parrinello N, Spina P, et al. BRIT1/MCPH1 expression in chronic myeloid leukemia and its regulation of the G2/M checkpoint. Acta Haematol. 2011;126(4):205–10.

8 Manzella L, Tirrò E, Pennisi MS, Massimino M, Stella S, Romano C, et al. Roles of Interferon Regulatory Factors in Chronic Myeloid Leukemia. Curr Cancer Drug Targets. 2016;16(7):594–605.

9 Castagnetti F, Gugliotta G, Baccarani M, Breccia M, Specchia G, Levato L, et al.; GIMEMA CML Working Party. Differences among young adults, adults and elderly chronic myeloid leukemia patients. Ann Oncol. 2015 Jan;26(1):185–92.

10 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 May;31(5):690–704.

11 Yamada O, Mahfoudhi E, Plo I, Ozaki K, Nakatake M, Akiyama M, et al. Emergence of a BCR-ABL translocation in a patient with the JAK2V617F mutation: evidence for secondary acquisition of BCR-ABL in the JAK2V617F clone. J Clin Oncol. 2014 Jul;32(21):e76–9.

12 Vigneri P, Stagno F, Stella S, Cupri A, Forte S, Massimino M, et al. High BCR-ABL/GUSIS Levels at Diagnosis of Chronic Phase CML Are Associated with Unfavorable Responses to Standard-Dose Imatinib. Clin Cancer Res. 2017 Dec;23(23):7189–98.

13 Weng WH, Shih LY. Occurrence of BCR-ABL1-positive chronic myeloid leukemia following essential thrombocythemia. Acta Haematol. 2011;126(4):220–3.

14 Wang X, Tripodi J, Kremyanskaya M, Blouin A, Roda P, Hoffman R, et al. BCR-ABL1 is a secondary event after JAK2V617F in patients with polycythemia vera who develop chronic myeloid leukemia. Blood. 2013 Feb;121(7):1238–9.

15 Cambier N, Renneville A, Cazaentre T, Soenen V, Cossement C, Giraudier S, et al. JAK2V617F-positive polycythemia vera and Philadelphia chromosome-positive chronic myeloid leukemia: one patient with two distinct myeloproliferative disorders. Leukemia. 2008 Jul;22(7):1454–5.

16 Inokuchi K, Yamaguchi H, Tamai H, Dan K. Disappearance of both the BCR/ABL1 fusion gene and the JAK2V617F mutation with dasatinib therapy in a patient with imatinib-resistant chronic myelogenous leukemia. J Clin Exp Hematop. 2012;52(2):145–7.

17 Massimino M, Stella S, Tirrò E, Romano C, Pennisi MS, Puma A, et al. Non ABL-directed inhibitors as alternative treatment strategies for chronic myeloid leukemia. Mol Cancer. 2018 Feb;17(1):56.

Downloaded by:
Linköpings Universitetsbibliotek
130.236.82.7 - 4/11/2019 11:40:09 AM

18 Stagno F, Vigneri P, Stagno F, Vigneri P, Del Fabro V, Stella S, et al. Uncommon long-term survival in a patient with chronic myeloid leukemia. Acta Oncol. 2009;48(8):1215–6.

19 Gambacorti-Passerini C, Antolini L, Mahon FX, Guilhot F, Deininger M, Fava C, et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. J Natl Cancer Inst. 2011 Apr;103(7):553–61.

20 Stagno F, Stella S, Spitaleri A, Pennisi MS, Di Raimondo F, Vigneri P. Imatinib mesylate in chronic myeloid leukemia: frontline treatment and long-term outcomes. Expert Rev Anticancer Ther. 2016;16(3):273–8.

21 Stagno F, Vigneri P, Del Fabro V, Stella S, Restuccia N, Giallongo C, et al. Concomitant and feasible treatment with dasatinib and the anti-EGFR antibody cetuximab plus radiotherapy in a CML patient with multiple squamous neoplasias. Acta Oncol. 2010;49(1):109–10.

22 Scott LM, Rebel VI. JAK2 and genomic instability in the myeloproliferative neoplasms: a case of the chicken or the egg? Am J Hematol. 2012 Nov;87(11):1028–36.

23 Rumi E, Cazzola M. Advances in understanding the pathogenesis of familial myeloproliferative neoplasms. Br J Haematol. 2017 Sep;178(5):689–98.

Downloaded by:
Linköpings Universitetsbibliotek
130.236.82.7 - 4/11/2019 11:40:09 AM