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CASE REPORT

AML IN REMISSION, ORIGINATING FROM MDS-RARS-T, EXPANDS THE UNDERLYING JAK2 V617F MUTATED CLONE

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Abstract: A mutation in the JAK2 gene is commonly found in patients with MPN, which can sometimes lead to secondary AML. In this case study, we are reporting on an interesting case of secondary AML originating from MDS-RARS-T. The patient had no gross chromosomal changes, and we found that he was JAK2 V617F-mutated. His BM showed 53% of myeloid blasts. After the induction of combined therapy of Venetoclax and Azacytidine, a complete remission of the disease was achieved. However, instead of the expected decrease in the mutated JAK2 alleles, we documented a rise from the initial 55% to 79% of mutated alleles. This can be explained by the fact that treatment for AML targets only one subclone.

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INTRODUCTION

A somatic mutation in the Janus kinase 2 (JAK2) gene is commonly found in patients with myeloproliferative neoplasms (MPN). These patients have a proliferation of one or more of the myeloid cell lineages in bone marrow (BM) and immature cells in the peripheral blood (PB). The mutation is found in almost 95% of polycythemia vera (PV), approximately half (50-60%) of essential thrombocythemia (ET) and half (50-60%) of primary myelofibrosis (PMF) patients. The JAK2 V617F mutation is caused by a G-to-T transversion at nucleotide 1849 in exon 14 of the JAK2 gene, resulting in a valine-to-phenylalanine amino acid substitution at codon 617.[1] Normally, the binding of JAK kinase to the associated cytokine receptor results in a conformational change in the cytokine receptor and phosphorylation, as well as activation of the JAK kinase. Phosphorylated tyrosine residues in JAKs act as binding sites for the SH2 domains in signaling molecules. This leads to the activation of signal transducers and activators of transcription (STATs), which then dimerize and enter the nucleus to regulate gene transcription.[2] The JAK2 V617F mutation constitutively activates the JAK2-STAT signaling pathway, which further influences cell proliferation, differentiation, migration, and apoptosis.[3] It is well known that MPN can lead to fibrosis or leukemic transformation.[4] In 2005, Levine et al. identified 4 out of 222 acute myeloid leukemia (AML) patients with a JAK2 V617F mutation, 3 of whom had a preceding MPN.[5]

In this paper, we report on an interesting case of secondary AML which had originated from a myelodysplastic syndrome including refractory anemia with ring sideroblasts and thrombocytosis (MDS-RARS-T). The patient, a 73-year-old male, first visited the department of hematology in another hospital in 2009. After establishing a diagnosis of refractory anemia with ring sideroblasts (RARS), the patient was treated with Litalir. Ten years later he developed AML. MDS-RARS-T was established based on the findings

of refractory anemia with ring sideroblasts and thrombocytosis with no gross chromosomal changes. In 2018, his BM showed no signs of blasts, but in 2019 the percentage of myeloid blasts rose to 53%. Blasts contain immature chromatin, one or more prominent nucleoli and a scant cytoplasm. Among other findings, conserved erythropoiesis and polymorphic megakaryocytes were observed. After the induction of therapy, a complete remission of the disease in the BM was achieved. However, instead of an expected decrease in the mutated JAK2 alleles, we documented a rise in the percentage of mutation.

MATERIAL AND METHODS

Sample collection

Over 13 months (from May 2018), 1 bone marrow and 3 peripheral blood samples in ETDA were collected.

DNA extraction

DNA extraction from peripheral blood and bone marrow samples was carried out using the Quick-DNA Miniprep Plus Kit (Zymo Research; cat no. D4069), according to the manufacturer's protocols. Each DNA sample was quantified using a BioSpec-nano UV-VIS spectrophotometer (Shimadzu). Optimal samples had a DNA concentration of over 20 ng/µl and an A260/280 value between 1.8 and 2.0.

Real-time quantitative PCR (qPCR)

Two qPCR assays were performed using primer and probe sequences designed by Larsen et al. The sequences are as follows: common forward primer 5'-CTT TCT TTG AAG CAG CAA GTA TGA-3', wild type-specific reverse primer 5'-GTA GTT TTA CTT ACT CTC GTC TCC ACA TAC-3', JAK2 V617F mutation-specific reverse primer 5'-GTA GTT TTA CTT ACT CTC GTC TCC ACA TAA-3' and common genomic probe 5'- (6-FAM) TGA GCA AGC TTT CTC ACA AGC ATT TGG TTT (TAMRA)-3'. The reverse primers contain an intended mismatch at the 3'-minus 2-position.[6, 7]
The final reaction mixtures contained: 12.5µl 2X Brilliant II QPCR High Rox Master Mix (Agilent, cat no. 600805-51), 1.5µl 15µM common forward primer, 1.5µl 15µM either WT or MUT reverse primer, 0.5µl 10µM probe, 25ng DNA and PCR-grade water to a total of 25µl. The PCR thermocycling conditions were as follows: 50°C for 2 min; 95°C for 10 min; 50 cycles at 95°C for 15 sec and 60°C for 1 min. All qPCR assays were performed in duplicate on a 7300 Real-Time PCR System (Applied Biosystems, Thermofisher Scientific). JAK2 V617F was quantified using the ΔCt method (threshold 0.2) in comparison to a fivefold dilution series of homozygous JAK2 V617F mutated DNA into donor wild type DNA.

RESULTS

In May 2018, a 73-year-old male patient came to us in the transformation to secondary AML, which had originated from MDS-RARS-T, which he had controlled earlier in another hospital. Cytogenetics saw no gross chromosomal changes, and we found that he was JAK2 V617F-mutated. When he first came to us, his BM showed no signs of blasts, but in 2019 the percentage of myeloid blasts rose to 53%. The nucleus of the blasts contained immature chromatin with 2-3 prominent nucleoli, while the cytoplasm was scant with a greyish-blue hue. Conserved erythropoiesis and polymorphic megakaryocytes were also observed. A combined protocol of Venetoclax and Azacitidine was started. After 6 cycles (approximately 4-5 months, 5 cycles of treatment) the BM cleared of blasts and moderate cytopenia in the blood was established. Unexpectedly, instead of a decrease in the mutated JAK2 alleles, we documented a rise from the initial 55% to 79% of mutated alleles. The therapy with Venetoclax (anti-BCL2 agent + Azacytidine) was continued in monthly cycles. His JAK2 V617F clone was monitored. It continued to rise to 88% at month 6 and 85% at month 9 of treatment (Figure 1). The patient is now at month 13 (after cycle 12) and in hematological remission with moderate leukopenia (3.1x10/9/L) and no immature cells in his blood differential. Anemia of 80-90 g/L is also present, but with normal hemoglobin and platelet counts.

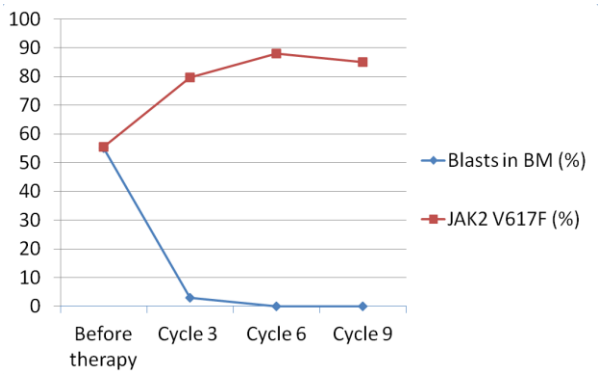

Figure 1. The percentage of the JAK2 V617F mutation in response to therapy. Even though a complete remission was achieved with combined therapy of Ventoclax and Azacytidine, the patient displayed an increase in the percentage of the JAK2 V617F mutation.

DISCUSSION

Changes in the degrees of apoptosis (programmed cell death) are involved in the regulation of blood cell numbers.[8, 9, 10, 11, 12] It has been hypothesized that

progression to AML in MDS may be due to a reduction in apoptosis in the patients' hematopoietic precursors, resulting in their accumulation, as well as the potential for additional differentiation abnormalities.[13] Such a mechanism of tumorigenesis has been demonstrated in certain neoplasms, e.g. follicular lymphomas. Recent reports have shown decreased apoptosis in blasts of AML and advanced MDS, providing support for this mechanism in leukemogenesis. In their research on in-situ labeling of DNA strand breaks to detect apoptosis in BM biopsy sections, Raza et al. reported low labeling in AML blasts and blast clusters of advanced MDS. [14, 15, 16] Rajapaksa et al. used fluorometry and flow cytometry to measure DNA fragmentation as evidence of apoptosis in immature CD34-positive ($CD34^{pos}$) cells from BM aspirates. They found significantly lower values for advanced MDS (RAEB and RAEB-T) and AML patients in comparison to healthy and early MDS patients (RA and RARS).[17] This led to the suggestion of an immature hematopoietic cell population with decreased apoptosis arising from a background of increased apoptosis in early MDS.

Bcl-2 oncoprotein expression protects normal[18], neoplastic[19, 20], and gene-manipulated cells of several lineages against apoptosis.[21, 22] Data strongly suggests that over-expression of Bcl-2 plays a central role in the pathogenesis of most cases of follicular lymphomas. The over-expression of Bcl-2 leads to prolonged survival, decreased apoptosis and accumulation of neoplastic cells, although Bcl-2 is expressed in normal lymphoid cells as well.[19, 23, 24] Similarly, Bcl-2 is over-expressed in blasts in most cases of AML and, in the small number of MDS patients studied, associated with a worse prognosis.[25, 26, 27] Bcl-2 is also expressed by normal myeloid precursors, in levels which decrease as myeloid cells mature. Increased blast accumulation is positively correlated with Bcl-2 expression, which is shown by in vitro studies of AML blasts.[26, 27, 28] Other in vitro studies of various CD34pos myeloid cells (blasts from normal BM, BCL-2-positive myeloid leukemic cell lines, and cells from AML patients) report that exposure to Bcl-2 antisense oligonucleotides reduces the expression of Bcl-2, which is accompanied by decreases in both cell growth and resistance to chemotherapeutic agents.[29] The Bcl-2 oncoprotein also prolongs cellular survival in cytokine-deprived hematopoietic cells by blocking apoptosis.[21, 30, 31] With regard to the combined therapy of Venetoclax and Azacytidine, which targets cells with Bcl-2 expression and acts as a demethylating agent[32], the percentage of JAK2 V617F can be explained by an untouched cell subclone. Furthermore, checking the degree of methylation of the JAK2 gene might give us some insight into understanding the increased percentage of the JAK2 V617F mutation.

Although it was presumed that the chronic pre-leukemic clone would regress with the treatment, as was observed with the leukemic clone, the observed findings can be explained by the fact that treatment for AML resets the clone to an earlier stage, but does not eradicate it, or it targets only one subclone. It is not always clear what this means clinically, as is described for DNMT3A, TET2 and ASXL1 persistence after chemotherapy for (*de novo*) AML and this does not necessarily predict relapse.[33] One of the scenarios for the patient is that he will go back to his RARS-T/MDS for a period, which may be why his JAK2 V617F is rising and that he might eventually re-develop his AML.

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