

Treatment of refractory anemia with ring sideroblasts associated with marked thrombocytosis with lenalidomide in a patient testing negative for 5q deletion and *JAK2 V617F* and *MPL W515K/L* mutations

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Abstract

Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) is a hematologic malignancy that often results in transfusion dependency and a hypercoagulable state. This rare disease currently lacks formal guidelines for treatment; however, various case reports have demonstrated efficacy in the use of lenalidomide. This immunomodulatory drug has shown promise in patients with 5q deletions, with reports of achieving transfusion independence and normalization of platelet counts. Herein we present the case of a 68-year-old African American woman with RARS-T who tested negative for 5q deletion and *JAK2 V617F* and *MPL W515K/L* mutations. Her treatment with lenalidomide therapy resulted in a five-year durable complete clinical response.

Introduction

RARS-T is a WHO provisional entity that falls under the umbrella classification of myelodysplastic/myeloproliferative neoplasms (MDS/MPN). It is characterized by the findings of persistent thrombocytosis (greater than $450 \times 10^9/L$) and refractory anemia with large, atypical megakaryocytes and ringed sideroblasts ($\geq 15\%$ of erythroid precursors) within the marrow.^{1,2} It is proposed that at least of subset of these cases likely represent previously undiagnosed cases of myeloid neoplasms [such as refractory anemia with ring sideroblasts (RARS)] where disease evolution has occurred. The finding of isolated 5q deletion, t(3;3)(q21;q26) or inv(3)(q21q26) as well as *BCR-ABL1* fusion gene excludes this diagnosis. This rare disorder presents at a median age of 71 to 75 years. Approximately 80% of patients have no detectable clonal cytogenetic

abnormality via karyotype or FISH analysis.^{1,3}

The mutations commonly associated with RARS associated with marked thrombocytosis (RARS-T) can be subdivided into 4 different groups: spliceosome components, signaling pathways, epigenetic regulators and transcription regulators. Of these molecular abnormalities, the *JAK2 V617F* (signaling pathway) and *SF3B1* (spliceosome component) are those most commonly identified with incidences of 57 and 90%, respectively. Approximately 50% of patients studied displayed both *JAK2 V617F* and *SF3B1* mutations.^{1,4} The mutation in *SF3B1* has been strongly associated with the formation of ringed sideroblasts in the bone marrow and it has been proposed that their allele burden often correlates with the percentage of ringed sideroblasts present.^{1,5} *MPL* mutations ($< 5\%$) and *CALR* mutations ($< 5\%$) have also been reported but are less frequent.^{1,6} Treatment of RARS-T is catered towards management of individual disease manifestations as well as identifying risk factors. Individuals are risk stratified based on presence or absence of the following risk factors: age > 60 and prior arterial/venous thrombosis. Patients with zero risk factors are *low risk*, whereas those with one or both risk factors are deemed *high risk*.^{1,7} In 2012, the Revised International Prognostic Scoring System for Myelodysplastic Syndromes was published by Greenberg and colleagues.⁸ This system identifies the prognostic risk for MDS when not treated. Based on cytogenetic abnormalities, bone marrow blast percentage, hemoglobin, platelets, and absolute neutrophil count; patients are risk stratified into categories ranging from very low risk to very high risk. Each category is associated with a median year of survival time and a median time until 25% of patients experience AML evolution.⁸

In the presence of mucocutaneous bleeding and/or extreme thrombocytosis ($> 1000 \times 10^9/L$) with ristocetin cofactor (RC) less than 30%, treatment should include cytoreductive therapy followed by aspirin once RC is $> 30\%$. In patients with RC $> 30\%$ with or without mucocutaneous bleeding, treatment is based on the aforementioned risk status. *High risk* patients are treated with aspirin and cytoreductive therapy with hydroxyurea. If hydroxyurea is not tolerated due to anemia, recommendations are to start an alternative cytoreductive therapy, such as lenalidomide, interferon alpha or anagrelide. If hydroxyurea is not tolerated for reasons other than anemia, it is recommended to treat with alternative cytoreductive therapy such as busulfan, anagrelide or interferon alpha.^{1,7} *Low risk* patients are treated with aspirin or simply observation alone if *JAK2 V617F* mutational analysis is negative.^{1,9}

There have been several case reports identifying the effectiveness of lenalidomide therapy in the treatment of RARS-T. Lenalidomide is a

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Key words: Myelodysplastic-myeloproliferative diseases; Anemia; Refractory; Platelet count; Lenalidomide.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 10 May 2016.

Accepted for publication: 11 October 2016.

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Hematology Reports 2016; 8:6592
doi:10.4081/hr.2016.6592

drug with immunomodulatory, antiangiogenic and antineoplastic properties. Immunomodulatory effects include the activation of T cells and natural killer cells and inhibition of pro-inflammatory cytokines by monocytes. Antiangiogenic properties occur via an unclear mechanism. The antineoplastic mechanism acts via inhibiting proliferation and inducing apoptosis of malignant hematopoietic tumor cells. Antineoplastic effects have been displayed in MDS with 5q deletions, multiple myeloma, and mantle cell lymphoma. One case report documented a complete molecular remission and the elimination of red blood cell transfusion dependence in a *JAK2 V617F* positive patient with RARS-T.^{1,10}

In a study by Broseus and colleagues, patients with RARS-T demonstrated better overall survival than RARS (76 vs. 63 months), and lesser overall survival than essential thrombocythemia (76 vs. 117 months).^{1,3} Interestingly, survival did not appear to be affected by *JAK2 V617F* status or platelet threshold ($>$ or $<$ $600 \times 10^9/L$). Thrombotic events were noted to occur at a rate of 3.6 events per 100 patient years. The leukemic transformation rate per 100 years was found to be 1.8.^{1,3} In a study conducted by Czibere and colleagues, it was reported that low expression levels of ribosomal protein S14 (RPS14) in MDS patients was associated with a superior median overall survival. Of note, this study was conducted using patients with MDS who tested negative for a 5q deletion.¹¹

Success with lenalidomide has traditionally

been associated with a 5q deletion. We report a case detailing the therapy of a woman presenting with a transfusion dependent RARS-T without a 5q deletion. She achieved a durable complete remission lasting more than five years after initiation of lenalidomide therapy.

Case Report

This 68-year-old African American woman with a past medical history significant for hypertension, hyperlipidemia, Hashimoto thyroiditis (status post thyroidectomy with post-operative hypocalcemia and hyperphosphatemia), and tobacco abuse was referred to our hematology clinic following hospitalization for profound anemia and associated weakness. In May of 2010, the patient had presented to the emergency department with complaints of generalized weakness, fatigue and lightheadedness. A complete blood count revealed hemoglobin of 6.1 g/dL and platelet count of $1257 \times 10^9/L$. A fecal occult blood test was positive. The patient reportedly had undergone an unremarkable screening colonoscopy at an outside facility in December of 2009. The patient was admitted to the internal medicine service for further evaluation.

During her hospitalization, she was evaluated for suspected anemia due to acute blood loss via esophagogastroduodenoscopy and capsule endoscopy. These studies did not show a source of active bleeding. The patient's anemia was treated with transfusions of packed red blood cells and iron supplementation. She improved symptomatically; however, her extreme thrombocytosis persisted. Initial laboratory studies displayed an elevated serum LDH (313 U/L), normal haptoglobin (209 mg/dL), normal uric acid (3.7 mg/dL), and an elevated reticulocyte count (4.7%). Her discharge diagnosis was iron deficiency anemia, based on a serum iron of 10 mcg/dL, total iron binding capacity of 386 mcg/dL, and an iron saturation of 3%. The patient was referred to the Hematology/Oncology Clinic for evaluation of a suspected myelodysplastic or myeloproliferative process.

In the hematology clinic, the patient continued to manifest an elevated platelet count above $1000 \times 10^9/L$. She also had persistent anemia requiring transfusion. A serum protein electrophoresis demonstrated a mild diffuse polyclonal hypergammaglobulinemia. An abdominal ultrasound was performed, and showed a small atrophic spleen and an enlarged fatty liver. A bone marrow biopsy demonstrated hypercellularity with megakaryocytic hyperplasia, dyserythropoiesis and ringed sideroblasts, consistent with a MDS/MPN, unclassifiable. No aberrant immunophenotype was noted on flow cytome-

try. *JAK2* V617F and *MPL* W515K/L mutations were negative. Karyotype was normal. Fluorescence in situ hybridization studies showed no evidence for an abnormal clone with probes specific for recurrent abnormalities in MDS. A diagnosis of RARS-T was established.

The patient was started on lenalidomide 10 mg daily in September of 2010 due to persistent thrombocytosis, transfusion dependent anemia and the risk of thrombotic-related complications. She returned to the hematology clinic two weeks following the initiation of therapy and complained of severe nausea and anorexia. She was administered intravenous fluids for dehydration. Her platelet count had improved to $798,000 \times 10^9/L$.

She continued to experience nausea and her symptoms worsened, prompting a visit to the emergency department. She was treated with fluids and ondansetron with improvement. It was determined that the patient should stop the lenalidomide due to unacceptable side effects in October of 2010. She was followed biweekly for close monitoring of platelet count and recovered from her toxicity within 2 weeks. Briefly, the patient continued to have downward trending platelet counts to as low as $497,000 \times 10^9/L$ in November of 2010. This effect was short-lived, however, and platelets slowly began to rise. In January of 2011, the platelet count was found to be further elevated at $856,000 \times 10^9/L$.

On January 13th, 2011 lenalidomide was restarted at a dosage of 5 mg every other day. The patient suffered no toxicity from this dosage and had a notable response. In February of 2011, the dosage was increased to 5 mg daily. By July of 2011, the patient had a normalized platelet count of $351,000 \times 10^9/L$ and a normalized hemoglobin/hematocrit of 13.3 g/dL/37.9%. Her platelet count fluctuated on this regimen between $\sim 300,000 \times 10^9/L$ and $\sim 500,000 \times 10^9/L$ until August of 2013. At that time, the patient had issues with compliance due to financial restrictions. Successful efforts were made to ensure the patient obtained her medication. Her platelet counts continued to increase as high as $724,000 \times 10^9/L$ and hemoglobin dropped to 10.6 g/dL despite her reported compliance. In late September of 2013, her lenalidomide was increased to 10 mg daily.

Following the increase in dosage, the patient had excellent response of her platelet count and did not report any significant adverse effects. She has since maintained her platelet counts between $\sim 200,000 \times 10^9/L$ and $\sim 400,000 \times 10^9/L$. Her lowest hemoglobin since the increased dose was 11.4 g/dL. She continued to tolerate the drug well.

More recently, the patient underwent further testing which revealed a *CALR* mutation as well as a *SF3B1* mutation via next generation sequencing panel (Mayo laboratories);

these mutations were not previously evaluated for in this patient. The *CALR* mutation is present in 13% of cases of RARS-T, and is believed to promote thrombocytosis. The *CALR* mutation has no known impact on efficacy of therapy. *SF3B1* mutations has been noted to be very common in RARS-T with an incidence of >80%. The impact of the *SF3B1* mutation on the prognosis or treatment of RARS-T remains unknown.

In early 2016, the patient started demonstrating increased levels of forgetfulness. As a result, she had difficulty maintaining her compliance with treatment. Severe anemia (7.1 g/dL), requiring transfusion recurred along with a resurging thrombocytosis ($831 \times 10^9/L$). With social support to aid in her compliance, her blood counts have begun to improve significantly. Currently, we are awaiting RPS14 levels.

Discussion

This case demonstrates the potential efficacy of lenalidomide therapy in a patient with RARS-T. Although initial tolerance was poor, resuming this drug with gradual escalation to attain desired effect has proven most effective for this particular patient. Of note, *JAK2* V617F and *MPL* W515K/L mutational analysis were both negative. Furthermore, the patient was found to lack the 5q deletion so commonly associated with the effectiveness of lenalidomide therapy. Our observations imply that it may not be entirely necessary to have a 5q deletion or a *JAK2* mutation to demonstrate efficacy with lenalidomide in patients with RARS-T. Our patient achieved transfusion independence rapidly following initiation of lenalidomide in 2010. Although her disease has recently relapsed after five years, it is likely that non-compliance was the major contributing factor to apparent treatment failure.

Conclusions

Lenalidomide therapy in RARS-T has been shown to be an effective therapy in multiple case studies in the recent past. This case exhibits an example of effective treatment of RARS-T with lenalidomide in a patient without 5q deletion and *JAK2* V617F and *MPL* W515K/L mutations. While compliant, the patient experienced a complete durable response for over 5 years following initiation of treatment. Lenalidomide is a promising treatment option for those affected by RARS-T and warrants further investigation in larger clinical trials.

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