Atypical Cytogenetics in Therapy-Related Myelodysplastic Syndrome Secondary to Indolent B-Cell Lymphoma

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ABSTRACT: A case of therapy-related myelodysplastic syndrome (t-MDS) with unusual cytogenetics is presented. While therapy related myeloid neoplasms account for 10-20% of all myeloid neoplasms, 90% of therapy related myelodysplastic syndromes(MDS) present with a del(7q) or a del(5q) and fewer than 1% present with a del(20q). In this case, the common cytogenetic pattern of either del(7q) or del(5q) is absent while presenting with an abnormal del(20q). Also demonstrated is the potently poor prognostic indicator of cytomegalovirus (CMV) seropositivity, even when seropositivity is matched between donor and recipient of hematopoietic stem-cell transplant. The patient also continues to demonstrate the inherent dangers of a stem-cell transplant, presenting with graft-versus-host disease (GvHD) while being a haploidentical 10 out of 10 HLA match to the allogeneic stem cell donor.

INDEX TERMS: therapy-related myeloid neoplasm, therapy related myelodysplastic syndrome(t-MDS), cytomegalovirus(CMV), Graft-versus-host disease (GvHD), deletion of the long arm of chromosome 20(del(20q))

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INTRODUCTION

Treatments for neoplastic disorders can predispose patients to further cytogenetic abnormalities and the development of other cancers. For example, there is a 15% greater likelihood for a survivor of non-Hodgkin lymphoma (NHL) to develop a malignancy than an individual who has not had NHL. While it remains unclear as to the root cause of the elevated risk for the development of new malignancies, it is thought that the cytotoxic effects of alkylating agents and irradiation can further insult the genome.[1] Therapy related myeloid neoplasms account for roughly 10-20% of all myeloid neoplasms, including therapy-related myelodysplastic syndrome (tMDS).[1]

Therapy-related MDS is characterized by similar morphological characteristics to *de novo* MDS. Macrocytic anemia in the presence of normal folate and B12, and multi-lineage dysplasia are both commonly associated with tMDS. The multi-lineage dysplasia is often demonstrated by asynchronous maturation through all three major cell lines. Increased ineffective hematopoiesis is demonstrated in the bone marrow through a variety of different maturation abnormalities.[2] Asynchronous maturation between the cytoplasm and the nucleus is a common occurrence. Patients also typically present with hypocellular bone marrow.

TMDS typically presents with predictable cytogenetic abnormalities. 90% of tMDS cases present with either a del(7q) or del(5q). The other 10% of tMDS cases is made up of a large number of relatively unpredictable cytogenetic abnormalities with no other single genetic abnormality appearing in more than 3% of cases. Prognosis is generally poor, with an overall 5-year survival rate frequently reported at less than 10%, regardless of cytogenetic abnormalities.[2]

CASE PRESENTATION

A 61 year old female originally presented with abdominal symptoms at age 52 and upon biopsy of

enlarged retroperitoneal lymph nodes, was diagnosed with indolent marginal zone B-cell lymphoma. Flow cytometry of a retroperitoneal lymph node demonstrated cells positive for CD19 and CD20, indicative of mature B-cells. Over the next year she underwent 14 rounds of aggressive chemotherapy, including 2 cycles of fludarabine. Her cancer went into remission for the next 5 years. Five years after therapy another bone marrow biopsy was performed which demonstrated similar morphology to the original diagnosis, but cytogenetic analysis demonstrated 3 out of 20 metaphases with a deletion of the long arm of chromosome 20 with no other significant cytogenetic anomaly. A repeat bone marrow biopsy performed two months later was consistent with lymphoma and 2 more cycles of fludarabine therapy were initiated. Two years later, the patient had a PET scan showing persistent uptake in the left supraclavicular area and lung. The patient was then enrolled in a clinical trial of perfosine 207 oral. The lung uptake resolved, but the left supraclavicular uptake did not, and the uptake had spread into the right axilla. She was taken off the clinical trial and began treatment for a presumed myelodysplastic syndrome, consistent with the cytogenetics and peripheral blood smear and cell counts. Nine years after the original diagnosis she was prepared for and received a non-myeloablative stem cell transplant.

The preparation for the bone marrow transplant included another course of chemotherapy including fludarabine and cyclophosphamide. The patient's serologic tests were positive for CMV and negative for hepatitis. She was transplanted with a 10 out of 10 HLA matched donor, also seropositive for CMV. She experienced mild post-transplant reactions, including fever, pleural effusion and hyperbilirubinemia. Prophylactic antimicrobial dosages were lowered as creatinine levels began to rise. She was discharged 14 days post-transplant and followed up in clinic.

Nine days post discharge she was readmitted with hemorrhagic cystitis. A battery of tests were performed yielding the results found in Table 1. The patient's urine also tested positive for BK virus by immunostain. BK virus is a human polyomavirus that is often associated with hemorrhagic cystitis in solid organ and bone marrow transplant patients, specifically in graft versus host disease.[3] While hospitalized, she developed diarrhea secondary to CMV colitis, due to reactivation of latent CMV positivity. Hematoxylin and eosin stains of the duodenal mucosa demonstrated CMV inclusions and immunostain positivity for CMV. Furthmore, quantitative polymerase chain reaction (PCR) was positive for CMV. Upon examination of intestinal mucosa, graft vs. host disease (GvHD) was also demonstrated. The GvHD was graded at stage four. The colitis and accompanying diarrhea worsened, culminating with fluid loss in excess of 8 liters per day. Fluids were bolused in an attempt to maintain blood pressure, but lack of renal perfusion led to worsening kidney function. She was started on a nasogastric tube, with significant production. Her oxygen requirements also increased, demonstrating multi organ system involvement. A repeat bone marrow biopsy 6 days prior to the cessation of respiration demonstrated no improvement in the underlying lymphoma and myelodysplastic syndromes. To further complicate matters, the myelodysplastic syndrome had developed into a full pancytopenia including a significant thrombocytopenia. Three days post bone marrow biopsy, another complete metabolic panel was performed, yielding the results in Table 2 which demonstrate significant renal dysfunction. She ultimately succumbed to her multiple systemic diseases.

The case raises several questions. What caused the development of tMDS? Was the cytogenetic abnormality significant? Did the patient's CMV seropositivity affect her outcome?

DISCUSSION

Therapy related myelodysplastic syndrome has a myriad number of causes, but only limited numbers of typical cytogenetic presentation. The likely cause of this patient's t-MDS development comes from the use of fludarabine to treat the original non-Hodgkin lymphoma, though previous documented cases of conversion were demonstrated with more extensive use of fludarabine.[1,5] While the patient's therapy was not as extensive as other recorded conversions to t-MDS, flu darabine has been recognized as one of the key contributing factors leading to the development of t-MDS.[1] Another study did demonstrate that fludarabine

Table 1. Laboratory values from 12/20

Analyte	Conventional Units	SI units	Reference range (conventional)	Reference range (SI)
BUN	29 mg/dL	10.353 mmol/L	5-20 mg/dL	1.79-7.1 mmol/L
Creatinine	1.9 mg/dL	168 µmol/L	0.6-1.4 mg/dL	53-123.8 µmol/L
Total protein	5.4 mg/dL	54 g/L	6.7-8.6 mg/dL	67-86 g/L
Albumin	2.7 mg/dL	27 g/L	3.5-5.0 mg/dL	35-50 g/L
WBC count	14.1 x 10^3/mm^3	14.1 x 10^9/L	4.5-11.5 x 10^3/mm^3	4.5-11.5 x 10^9/L

Table 2. Values from 01/22

Analyte	Conventional Units	SI units	Reference Range (conventional)	Reference Range (SI)
BUN	99 mg/dL	35.35 mmol/L	5-20 mg/dL	1.79-7.1 mmol/L
Creatinine	2.1 mg/dL	186 µmol/L	0.6-1.4 mg/dL	53-123.8 µmol/L
Total protein	2.9 mg/dL	29 g/L	6.7-8.6 mg/dL	67-86 g/L
Albumin	1.9 mg/dL	19 g/L	3.5-5.0 mg/dL	35-50 g/L
WBC count	0.3 x 10^3/mm^3	0.3 x 10^9	4.5-11.5 x 10^3/mm^3	4.5-11.5 x 10^9/L

therapy was more likely to lead to t-MDS than radiation therapy, though this study encompassed post stem-cell transplant and the patient did not receive a stem-cell transplant until after the diagnosis of t-MDS.[6]

While development of t-MDS occurred in a relatively predictable manner, the cytogenetic presentation of the disease did not. Greater than 90% of therapy-related myelodysplastic syndromes present with a del(7q) or a del(5q).[7] The cytogenetics in this case did not present with a del(7q) or a del(5q), but instead with a del(20q) as discovered through the use of fluorescent in situ hybridization(FISH). This cytogenetic abnormality occurs in 5-8% of *de novo* myelodysplastic syndromes(MDS) but accounts for less than 1% of t-MDS cases.[1] Del(20q) cytogenetic abnormalities in *de novo* MDS appears to be a favorable prognostic indicator;[8] however, therapy related myeloid malignancies often do not respond in the same manner as *de novo* malignancies, with five year survival rates of less than 10%.[1] The prognosis appears to be particularly poor with a del(7q) or a del(5q), and while the patient did not have either of these cytogenetic abnormalities, her course did not progress well. As typical for recurrent therapy related hematopoietic malignancies, the patient's t-MDS did not respond well to further chemotherapies.[1] The next therapeutic choice was a non-myeloablative stem-cell transplant. Most leukemias are treated using myeloablative stem-cell transplants, where the treating physicians attempt to eradicate the patient's bone marrow with radiation before replacing it with matched, healthy bone marrow. This eradication is intended to destroy all of the cells with the cancerous mutations, allowing the transplant to start fresh. A non-myeloablative stem cell transplant is performed without the usual eradication of the recipient's bone marrow. Non-myeloablative stem cell transplants are more often used in non-malignant conditions. Continued chemotherapies and the nature of the MDS did drop the peripheral blood white counts, and the bone marrow was already significantly hypocellular. This may have led to the decision not to eradicate the patient's remaining bone marrow with total body irradiation. It may also be that the patient's treatment was an attempt to elicit the graft-versus-malignancy phenomenon.[9] In light of the fact that the patient's donor was haploidentical, a perfect 10 out of 10 HLA match; it has also been shown that non-myeloablative therapies have shown decreased transplant-related mortality.[10]

The patient was serotyped positive for CMV and so was the allogeneic donor. This combined with the immunosuppressive therapy and the cytopenias associated with the t-MDS are the most likely causes of the reactivated CMV infection. A study involving antithymocyte globulin demonstrated that CMV

seropositivity is a significant predictor of patient mortality post allogeneic stem cell transplantation regardless of reactivation status.[9] Seropositivity; however, is not the only way to detect if CMV infection is present. Seropositivity occurs post infection, regardless of reactivation. Polymerase chain reaction assays have been developed and can quantify a viral load to determine whether or not a patient is having an acute infection.[10] While this patient's hospital course does not appear to contain antithymocyte globulin therapy, it does appear to support that CMV positivity can directly affect prognosis. The patient's renal failure is most likely directly related to the CMV colitis and decreased renal perfusion due to an inability to keep the patient hydrated through such voluminous fluid loss.

The reactivation of CMV in cases such as this is somewhat predictable due to the pathogenic nature of tMDS. TMDS often presents with multiple peripheral blood cytopenias. While tMDS often affects the granulocytic series, it can also affect platelets, monocyte and erythrocyte lines. The cytopenias are accompanied by dyshematopoiesis. The dyshematopoiesis presents with bizarre morphologies both in the peripheral blood and bone marrow. The cells circulating in the peripheral blood can be characterized by nuclear-cytoplasmic asynchrony, visualized most often by a persistence of basophilia in the cytoplasm of cells with nuclei that appear to be more mature. The cells can also experience abnormal nuclear lobulation, displaying either hyposegmentation or hypersegmentation. Another key characteristic of MDS is the presence of micro-megakaryocytes. In the bone marrow, nuclear-cytoplasmic asynchrony can also be seen. One of the key morphological presentations is in the erythroid series, when erythroid precursors demonstrate nuclear bridging, or a failure of the daughter nuclei to fully separate during mitosis. These morphologic characteristics are just a few of the ones that can be seen with tMDS. Because of the broad scope of the disease, a myriad number of other changes can be seen throughout the hematopoietic lines.[2]

Post engraftment, the patient's white count showed improvement as evidenced by Table 3; however, as the disease progressed, her differential showed an increasing number of granulocytes with a decreasing number of lymphocytes, the opposite of what would be expected in an acute viral infection but are more consistent with an inflammatory response, such as GvHD. As time progressed the white count plummeted. A bone marrow biopsy performed on January 19 demonstrated 10% cellular marrow, staining negative for reticulin. Such hypocellularity is consistent with end stage myelodysplastic syndrome.

While CMV reactivation was a significant cause of gastrointestinal dysfunction, the patient's work-up also demonstrated intestinal GvHD. The patient's timeline shows an acute GvHD reaction, developing in the short span of just over a month. This demonstrates the continued risk of allogeneic stem cell transplantation, as the patient in question was a 10 out of 10 HLA match, was on appropriate immunosuppressive therapies to prevent such a reaction and still developed significant GvHD complications in less than one month.

CONCLUSION

Described here is an unusual case of t-MDS, presenting with abnormal cytogenetic variations. Specific abnormal

Table 3. White blood count values from 11-26 to 1-22

Date	White count (x 10^3 cells/mm^3)	White count (x 10^9 cells/L)	Reference range (conventional units)	Reference range (SI units)
11-26	0.4	0.4	4.5-11.5x10^3/mm^3	4.5-11.5x10^9/L
11-30	1.2	1.2		
12-4	3.0	3.0		
12-8	4.1	4.1		
12-11	4.7	4.7		
12-18	9.8	9.8		
12-22	11.3	11.3		
1-22	0.3	0.3		

cytogenetics includes the lack of del(7q) and del(5q) alterations typically shown in t-MDS. This case also shows that the del(20q) which has been shown to be a good prognostic indicator in *de novo* MDS did not prove to demonstrate a good prognosis in this case of t-MDS. This case has also demonstrated that fludarabine therapy alone, with as few as 2 cycles, continues to demonstrate a strong correlation with therapy related myeloid malignancies.

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