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Erythroblastic Sarcoma Presenting as Bilateral Ovarian Masses in an Infant with Pure Erythroid Leukemia

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Abstract

Pure erythroid leukemia is a rare subtype of acute erythroid leukemia that is characterized by a predominant erythroid population, and erythroblastic sarcoma has not yet been described in the English literature. Here we report a first case of erythroblastic sarcoma which presented as bilateral ovarian masses in a three and half month old baby girl with pure erythroid leukemia. Bone marrow aspirate and biopsy showed the marrow was completely replaced by large-sized blasts consistent with erythroblasts. Immunophenotypically, both the tumor cells from the ovarian mass and bone marrow blasts were positive for CD117, glycophorin A, and hemoglobin A, demonstrating erythroid differentiation. Reverse transcriptase polymerase chain reaction showed the tumor cells from ovarian mass expressed hemoglobin F and $\alpha 1$ spectrin, confirming their erythroid lineage. Conventional karyotype of the bone marrow aspirates revealed del(6) (q23q25) and trisomy 7 in all 21 cells examined. Fluorescence *in situ* hybridization of the ovarian mass demonstrated loss of C-MYB at 6q23 locus in 41% of the cells, and deletion of chromosome 7 and 7q in 37% and 66% of cells, respectively. Taken together, we showed, for the first time, that pure erythroid leukemia presented as a myeloid sarcoma in the form of ovarian masses.

Keywords

Pure erythroid leukemia; Acute erythroid leukemia; Myeloid sarcoma; C-MYB

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Introduction

Acute erythroid leukemia, a rare subtype of acute myeloid leukemia, accounts for approximately 2-5% of acute leukemia (1). Based on the presence/absence and percentage of myeloid blast component, acute erythroid leukemia is divided into erythroleukemia (erythroid/myeloid) and pure erythroid leukemia (2), the latter of which is composed of more than 80% of immature erythroid elements with undifferentiated or proerythroblastic appearance. The vast majority of acute erythroid leukemia occur either *de novo* or in association with myeloproliferative/myelodysplastic disorders, although rare cases of familial or congenital forms were reported.

Myeloid sarcoma is an extramedullary manifestation as one or more tumor masses consisting of myeloid blasts with or without maturation. Myeloid sarcoma may develop concurrent with, before, or after acute myeloid leukemia, myeloproliferative neoplasm, or myelodysplastic syndrome (3). Cell lineage involved in myeloid sarcoma includes granulocytic, monocytic (4), and occasionally megakaryocytic (5). Myeloid sarcoma derived from pure erythroid leukemia has never been fully reported before, although one case of erythroleukemia presented as a myeloid sarcoma has been reported as an abstract (6). Here we report a first case of erythroblastic sarcoma presented as bilateral ovarian masses in a three and half month old girl diagnosed with pure erythroid leukemia. In addition to the detailed flow cytometric, immunohistochemical, and molecular analysis, we also provided evidence that both the sarcoma and leukemia forms shared deletion of *C-MYB* oncogene at chromosome 6q23 locus.

Clinical History

The patient was born at 30 weeks gestational age, and her neonatal course was reportedly uneventful. At approximately three and half month old, she was admitted to the hospital because of mild intermittent cough, low-grade fever, emesis, poor oral intake, and decrease in her energy level.

Physical examination performed when she was one and half month old by her primary physician revealed asymmetrical enlargement of her left breast, and it was felt at that time that the swelling of her left breast was secondary to hormonal stimulation. Physical examination on admission showed further enlargement of her left breast and a large ovarian mass. Ultrasound examination demonstrated an $8.5 \times 7 \times 4$ cm abdomino-pelvic mass of the left ovary. Laboratory investigation revealed mild leukocytosis, severe anemia and thrombocytopenia with frequent atypical large cells suspicious for blasts. Computerized tomography of the head demonstrated evidence suspicious of extensive skull involvement with soft tissue extension but with spare of brain parenchyma.

Two days after the bone marrow biopsy, prompted by progressive renal function impairment due to abdominal compartment syndrome, the patient underwent exploratory laparotomy. A 144.2 gm, $9.0 \times 7.0 \times 5.0$ cm slightly bosselated tumor bearing remnant left ovary was excised, and a wedge biopsy of the right ovary was also performed.

Initial therapy was administrated according to Pediatric Oncology Group protocol 9317 with fractionated cyclophosphamide, vincristine, and doxorubicin, which resulted in marked cytorreduction of the tumor. Subsequently, she received platinum-based regimes (course #2) with cyclophosphamide, carboplatin, and VP-16, followed by another course of Protocol 9317. Her course #4 with cyclophosphamide, carboplatin, and VP-16 resulted in excellent response. After high dose chemotherapy with carboplatin and thiopeta, she underwent autologous peripheral blood stem cell transplant. While there were a few chemotherapy-associated infectious and toxic complications, the patient has been free of disease as a well-

developed well-nourished asymptomatic playful active toddler at the time of this manuscript was prepared at the age of 23 months.

Materials and Methods

Morphology

Bone marrow aspirate smears were stained with Wright-Giemsa. Bone marrow trephine biopsy was fixed in B5 for 2 hours before being fixed in 10% PBS-buffered neutral formalin for overnight. After a $0.5 \times 0.5 \times 0.5$ cm fresh tissue was procured and snap-frozen for molecular studies (see below), the remaining excised ovarian tissue was fixed in 10% PBS-buffered neutral formalin overnight. After brief decalcification of the bone marrow core biopsy, the bone marrow biopsy and ovarian tissue were stained with hematoxylin and eosin (H&E).

Multiparameter multicolor flow cytometry

Single cell suspension from bone marrow aspirate was made and stained with the following antigens: CD1a, CD2, CD3 (both surface and cytoplasm), CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD15, CD16, CD19, CD22(cytoplasm), CD33, CD34, CD36, CD38, CD41, CD45, CD56, CD61, CD64, CD71, CD79a(cytoplasm), CD117, CD123, HLA-DR, myeloperoxidase (cytoplasmic), and TdT (cytoplasmic). The events were acquired using CELLQUEST software and the data were analyzed by Paint-A-Gate software (Beckman Dickson, San Jose, CA).

Molecular studies by reverse transcriptase-polymerase chain reaction

RNA was extracted from the snap-frozen ovary tumor sample using TriZOL reagent (Invitrogen, Carlsbad, CA) and RNA easy kit (Qiagen, Valencia, CA). Total RNA from human cell lines HEK293T and γ 2A (ATCC, Manassas, VA) was extracted using RNA easy kit (Qiagen). The concentration of the RNA was determined by a ND-1000 spectrophotometer (NanoDrop, Wilmington, DE). The cDNA was synthesized using First Strand synthesis kit (Roche, Indianapolis, IN). Polymerase chain reaction conditions were as follows: 94°C for 5 min, 35 cycles of 94°C for 10 s, 50°C for 30 s, and 72°C for 45 s; 1 cycle at 72°C for 7 min. Fetal hemoglobin (HbF) and α 1 spectrin were amplified using the following two pairs of primers - HbF: 5'-AAGGCTCCTGGTTGTCTAC-3' (Forward-F) and 5'-AGGAAGCCTGCACCTCAG-3' (Reverse-R); and α 1 spectrin: 5'-AGCGAGATGCAGATGATCT-3' (F) and 5'-ATGCAGAACTTCGGTGC-3' (R). House-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control, and it was amplified using the following pair of primer: 5'-AGTCCACTGGCGTCTTCA-3' (F) and 5'-TCAGCTCAGGGATGACCT-3' (R).

Immunohistochemistry

The antibodies used for immunohistochemistry on ovarian tissue were as follows: CD31(1:100; Dako, Dakocytomation, Carpinteria, CA), CD34(Predilute; Ventana, Ventana Medical Systems, Tucson, AZ), CD43(1:20; Dako), CD45(1:80; Dako), CD45RO(1:3200; Dako), CD56(1:10; Biocare, BioCare Medical, Concord, CA), CD68(1:200; Dako), CD117(1:200; Dako), calretinin (1:2; Cell Marque, Rocklin, CA), Cam5.2(1:8; Becton Dickinson, Franklin Lakes, NJ), chromogranin (1:1200; Dako), Desmin(1:200;Dako), EMA (1:200; Dako), glycophorin A (1:800; Dako), HbA (Predilute; Biogenex Inc., San Ramon, CA), inhibin (1:40; Dako), lysozyme (1:4; Cell Marque), Mart/Mel A (Predilute; Ventana), myogenin (1:160; Dako), synaptophysin (Predilute; Cell Marque), thyroid transcription factor 1 (TTF1)(1:125; Dako), Wilms tumor 1 protein (WT1)(1:200; Dako), and vimentin (Predilute; Ventana). Antigen retrieval using microwave heating method was applied to all

the antibodies. The immunohistochemistry was carried out using a BioTek Solutions Techmate 1000 automated immunostainer (Ventana) using the Dako Envision + horseradish peroxidase / diaminobenzidine monoclonal detection system (Dako).

Conventional cytogenetics

Conventional karyotypic analysis using G-banding on unstimulated cultured cells from bone marrow aspirate was performed according to standard procedure. No tissue was procured for cytogenetic studies from the ovarian mass.

Paraffin-embedded tissue fluorescence *in situ* hybridization (FISH)

The probes used in this study were LSI MYB (6q23) SpectrumAqua probe and LSI D7S486 (7q31) SpectrumOrange / CEP7 SpectrumGreen dual color probe set (Abbott Laboratories, Abbott Park, IL).

Results

Morphologic features

The patient's complete blood count on the day of admission was as follows: WBC of 18,200/ul (normal range: 6,000-17,500/ul), Hgb of 5.7 gm/dl (normal range: 10.4-16 g/dl), and platelet of 28,000/ul (normal range: 150,000-450,000/ul). Review of the peripheral blood smear revealed profound anemia and markedly thrombocytopenia with approximately 20% (based on 200-cell differential count) medium to large sized atypical cells with irregular to oval nuclear contours, slightly condensed chromatin, and deeply basophilic cytoplasm, suspicious for blasts. In addition, there was left-shifted myeloid maturation with occasional pseudo Pelger-Huetoid neutrophils.

The bone marrow aspirate smear contained no spicules; however, the smear was very cellular and composed of predominant population of medium to large-sized blasts (Fig. 1A) with oval to irregular nuclear contours, slightly condensed nuclear chromatin, absence of nucleoli, and scant amount of basophilic cytoplasm with no granules. Some of the blasts exhibited cytoplasmic blebs (Figure 1A) and frequent cytoplasmic and nuclear vacuoles ranging with up to 3-4 vacuoles per cell were also appreciated (Fig. 1A). However, there were no Auer rods seen. A 300-cell differential count was as follows: blast-88%, metamyelocytes-1%, banded neutrophils-2%, segmented neutrophils-2%, and lymphocyte-7%.

Bilateral bone marrow core biopsies were small and subcortical. As shown in Fig. 1B, the marrow was entirely replaced by sheets of medium sized blasts with similar morphologic features to the aspirate. Rare normal marrow elements were noted.

During the exploratory laparotomy, a bosselated mass (Fig. 1C) weighed 144 gm and measured $9.0 \times 7.0 \times 5.0$ cm was excised from the left ovary, together with a wedge biopsy from the right ovary. The surface of the mass was smooth with multifocal dark areas, suggestive of hemorrhage (Fig. 1C). The cut-surface of the left ovarian mass showed a multi-nodular white-tan to orange-red-brown fleshy tissue with central cystic degeneration and multifocal hemorrhage (picture not shown).

Multiple H&E sections showed the mass was composed of sheets of compact small to medium sized cells arranged in a vaguely cord/island structure separated by fine vasculature, mimicking the so-called "small blue cell tumor" with frequent mitoses (Fig. 1D). In addition, areas of increased apoptosis were easily appreciated (not shown). At higher magnification, the cells exhibit irregular nuclear contours, absent to small nucleoli, slightly

condensed chromatin and scant amount of cytoplasm, reminiscent of blasts (Fig. 1E). The wedge biopsy from the right ovary showed identical morphologic features to those of the left (data not shown).

Flow cytometry

Compared to small mature lymphocytes including both B- and T-cells (population in green, Fig. 2A-D, F, G, I-L), monocytes (population in blue, Fig. 2A-B, D, F-I), myeloblasts (population in highlighted yellow, Fig. 2A, G, I), and granulocytes (population in cyan, Fig. 2A-B, D, F-I, L), these blastoid cells (population in red), accounting for 82% of total events, had large cell size (forward scatter property, Fig. 2A) and variable cell complexity (side scatter property, Fig. 2A), and these cells fell into three discrete areas (Fig. 2A). These neoplastic cells were positive for CD71 (Fig. 2B), glycophorin A (Fig. 2B), CD117 (Fig. 2C), CD36 (bright) (Fig. 2D), weakly positive for CD2 (Fig. 2C) and CD64 (Fig. 2D), variably positive for CD56 (Fig. 2E), and weakly and variably positive for CD13 (Fig. 2F); however, these cells were negative for T-cell antigen cytoplasmic CD3 (Fig. 2K), B-cell antigen cytoplasmic CD79a (Fig. 2L), granulocytic antigens CD16 (Fig. 2E), CD15 and CD33 (Fig. 2H), myeloperoxidase (Fig. 2L), monocytic antigen CD14 (Fig. 2G), activation antigen HLA-DR (Fig. 2F) and CD38 (Fig. 2I), immature antigens CD34 (Fig. 2G), CD123 (Fig. 2J), and TdT (Fig. 2K). In addition, these cells were negative for CD45 (Fig. 2I). Furthermore, these cells were negative for CD1a, CD4, CD8, CD5, CD7, CD8, CD10, CD11b, CD19, CD45RO, CD61, and immunoglobulin light chains (data not shown).

Molecular studies

As shown in Fig. 3A, compared to negative control cell lines HEK293T (lane 2) and γ 2A (lane 3), a human embryonic kidney cell line and a human fibrosarcoma cell line, respectively, the ovarian mass (lane 1) expressed hemoglobin F and α 1 spectrin, both of which were erythroid lineage specific transcripts.

Immunohistochemistry

A panel of immunohistochemistry was used to confirm the nature of erythroid differentiation among the tumor cells. As shown in Fig. 3B, the tumor cells were diffusely and strongly for hemoglobin A, but only minority of the tumor cells were faintly positive for glycophorin A (Fig. 3C). These tumor cells were weakly and diffusely positive for CD117 (Fig. 3D), partially positive for CD56 (approximately 22%, data not shown). However, the tumor cells were strongly and diffusely positive for CD43 (Fig. 3E).

Other negative markers by immunohistochemistry included epithelium (Cam 5.2, cytokeratin, and epithelial membrane antigen), histiocyte (CD68 and lysozyme), neuroendocrine (chromogranin and synaptophysin), mesothelium (calretinin), skeletal muscle (desmin and myogenin), sex-chord (inhibin), melanocyte (mart/mel A), intermediate filament (vimentin), and immaturity/blast (CD34).

Cytogenetics

Among 21 bone marrow aspirate cells karyotyped, as the ideogram shown in Fig. 4A, all showed deletion of the long arm of chromosome 6 at band q23-q25 and gain of extra chromosome 7, therefore, the karyotype was as follows: 47,XX,del(6)(q23q25),+7[21]. The FISH studies for further characterization of gene(s) deleted at 6q23q25 from the bone marrow were not able to be performed due to lack of cell pellet.

Paraffin-embedded tissue FISH

In a normal cell for *C-MYB* (6q23) probe, two aqua signals are seen. As shown in Fig. 4B, approximately 41% (82/200) of cells showed one aqua signal, indicating loss of *C-MYB* at 6q23 locus. With regard to LSI D7S486 SpectrumOrange/CEP7 SpectrumGreen dual color probe, two orange and two green signal pattern is seen in a normal cell. As shown in Fig. 4C, approximately 33% (66/200) of the cells had only one green signal and approximately 66% (132/200) of the cells had only one orange signal, indicating loss of one chromosome 7 and 7q, respectively. Surprisingly, there was no trisomy 7 seen.

Discussion

In the present study, we present, for the first time to the best of our knowledge, an erythroblastic sarcoma presented as ovarian masses in a three and half month old baby girl. This case expanded our understanding and knowledge that myeloid sarcoma could derive from any myeloid lineage, including granulocytic, monocytic, megakaryocytic, and in this case erythroid.

The neoplastic cells from the ovarian mass, at first glance on H&E stain, might not look like erythroblasts; however, we have showed unequivocal evidence that the neoplastic cells from the ovary were indeed erythroblasts. At the transcription level, the ovarian tissue expressed HbF and $\alpha 1$ spectrin, both of which are erythroid lineage specific transcripts, and exclusively expressed in erythrocytes (7). While HbF and $\alpha 1$ spectrin are erythroid lineage specific, HbF remains relative high (approximately 10% of total hemoglobin) in the first 3 months after birth, and $\alpha 1$ spectrin is present in the red cell membrane throughout the entire life cycle, therefore, the amplified HbF and $\alpha 1$ spectrin from the ovarian mass could be contributed by the contaminated anucleated erythroid elements in the tumor tissue. To rule out this possibility, immunohistochemical stains using erythroid specific markers (HbA and glycophorin A) confirmed the tumor cells were clearly erythroid precursors (Fig. 3B& C).

We also provided convincing evidences that the pure erythroid leukemia of the bone marrow and the ovarian tumor were two faces of the same disease at two different levels. At the level of immunophenotype, the pure erythroid leukemia of the bone marrow and ovarian tumor shared almost identical immunophenotypic profile with minor difference being manifested as weak expression of CD117 (Fig. 3D), partial and weak expression of glycophorin A (Fig. 3C) and CD56 (data not shown) in tumor cells of the ovary, all of which could be attributable to the lower sensitivity of immunohistochemistry compared to flow cytometry. While CD36, CD71 and CD117 were expressed individually in many other cell types, the combined expression of CD71 (Fig. 2B), glycophorin A (Fig. 2B), CD117 (Fig. 2C), and CD36 (Fig. 2D) was diagnostic of erythroid lineage. The negative CD11b, CD33, CD34, HLA-DR, and MPO among the leukemic cells supported the diagnosis of pure erythroid leukemia (8). It was of interest to point out that the leukemic cells fell into three discrete regions with regard to their scatter properties (Fig. 2A). While the exact underlying mechanism for such distribution was not known, one possible explanation could be due to the fact that the majority of the leukemic cells contained cytoplasmic vacuoles, which were reported to affect the side/orthogonal scatter properties (9). CD56, also known as a neural cell adhesion molecule, was expressed in approximately 22% of patients with acute leukemia (10). However, CD56 was expressed in all other subtypes of acute myeloid leukemia from M0 to M7 of French-American-British (FAB) classification, except M6 (11), or rarely expressed in M6 (12), hence, more cases are needed to assess the clinical significance, if any, of CD56 expression among patients with M6. Besides similar expression of glycophorin A, CD117 and CD56 to their marrow counterpart, the tumor cells of the ovary also expressed CD43, a sensitive but not so specific marker for T cells, aberrant

B-cells, and leukemic blasts (13), therefore, it was not surprising that CD43 was positive in all 28 cases of myeloid sarcoma, sometime the only one (14).

At the cytogenetic and molecular cytogenetic levels, the pure erythroid leukemia of bone marrow and the erythroblastic sarcoma of the ovary harbored similar aberrancy but exhibited difference, namely, both showed deletion of C-MYB at 6q23, but the pure erythroid leukemia of the bone marrow had trisomy 7 and the tumor cells of the ovary had monosomy 7 and deletion of the long arm of chromosome 7. Due to the limitation of low resolution of conventional karyotype, sub-microscopic deletion of chromosome 7 in the leukemic cells from the bone marrow could not be ruled out. The difference of chromosomal 7 abnormalities between the bone marrow and ovary was most likely due to genetic evolution and disease progression. Although there were no non-random recurrent and specific genetic aberrancies associated with acute erythroid leukemia, large scale studies revealed chromosome 7 was the second most commonly involved chromosome after chromosome 5, but deletion of chromosome 6q23 was not reported (15). C-MYB, located on 6q23 locus, was a cellular oncogene involved in the proliferation, survival and differentiation of hematopoietic cells (16). C-MYB was normally expressed in the erythroid progenitors, and its over-expression inhibited the differentiation of erythroid cell lines (17). Loss of C-MYB was not reported in acute erythroid leukemia including pure erythroid leukemia before this report; however, C-MYB abnormalities including translocation, duplication and rearrangement were reported in T-acute lymphoblastic leukemia (18) and non-Hodgkin lymphoma (19). Furthermore, the effects of deletion of other genes, especially tumor suppressor genes in the 6q23q25 region, if any, could not be assessed and was beyond the scope of this study.

Pediatric Oncology Group Protocol 9317 was a phase III study designed to evaluate the efficacy of intensification chemotherapy with etoposide/ifosfamide vs. no intensification for patients with stage III/IV diffuse undifferentiated non-Hodgkin lymphoma or advanced B-cell acute lymphoblastic leukemia and compare the toxicity and efficacy of high-dose cytarabine administered $q12 \times 4$ vs. by 48-hour infusion in the induction regime (20). Due to the difficulty of the initial diagnosis of this case, the patient was treated with Protocol 9317, she has achieved complete clinical response, and has remained disease free at the time we prepared for this manuscript, 23 months after the initial diagnosis. Analysis of clinical outcomes from 91 patients with acute erythroid leukemia treated with different regimens by Santos and associates revealed that the diagnosis of acute erythroid leukemia did not imply by itself a worse prognosis; rather, the treatment decisions should be guided by well known prognostic factors of acute myeloid leukemia (21).

In conclusion, we reported the first case of erythroblastic sarcoma as a presentation of pure erythroid leukemia from a three and half month old girl. Immunophenotypically, the leukemia and sarcoma cells were positive for glycophorin A, hemoglobin F, CD36, CD71, CD117, but negative for CD34, CD45, HLA-DR, TdT, myeloperoxidase, myeloid (CD11c, CD16, CD33), monocytic (CD4, CD14, CD68, lysozyme), B-cell (CD10, CD19, CD20, cytoplasmic CD22, and cytoplasmic CD79a), T-cell (CD3, CD4, CD5, CD7, and CD8), epithelial, and mesenchymal associated antigens. Genetically, both the leukemia and sarcoma cells possessed 6q23 deletion involving C-MYB oncogene. At transcriptional level, the sarcoma cells expressed erythroid lineage specific transcripts (HbF and $\alpha 1$ spectrin).

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Figure 1. Cytohistomorphology of blasts from bone marrow and macroscopic as well as microscopic features of an ovarian mass

A. Numerous large-sized blasts were easily appreciated on the bone marrow aspirate. These blasts showed oval, irregular, to indented nuclear contours, fine nuclear chromatin, small to absent nucleoli, variable amount of basophilic cytoplasm with sparse vacuoles. Occasional cytoplasmic blebs and frequent cytoplasmic vacuoles were also noted; however, no Auer rods are seen. (Wright-Giemsa, original magnification 1000×). **B.** Bone marrow core biopsy showed a near 100% cellular marrow composed of sheets of blasts with similar morphologic features to those of the aspirate (H&E, original magnification 400×). **C.** An ovarian mass measuring 9.0 cm in greatest diameter showed bossolated surface appearance. Areas of hemorrhage (top) with focal white appearance (left lower corner) were present. **D.** At low magnification, the mass was composed of sheets of atypical cells separated into islands or cords by narrow-lumened blood vessels. (H&E, original magnification 200×). **E.** At higher magnification, the neoplastic cells exhibited “blastic” appearance including slightly condensed nuclear chromatin. The variable amounts of cytoplasm appeared to be slightly eosinophilic (H&E, original magnification 500×).

Figure 2. Immunophenotypic profile of the blasts from the bone marrow

Compared to the small mature lymphocytes (population in green), myeloid blasts (population in highlighted yellow), monocytes (population in blue), and granulocytes (population in cyan), the blasts (population in red) were medium sized to large cells with variable side scatter properties (A). The blasts were positive for CD71(B), glycophorin A(B), CD117(C), weakly positive for CD2(C), bright positive for CD36(D), weakly positive for CD64(D), variably positive for CD56(E), and weakly and variably positive for CD13(F), but negative for CD16(E), HLA-DR(F), CD14(G), CD34(G), CD15(H), CD33(H), CD38(I), CD45(I), CD41(J), CD123(J), cytoplasmic CD3(K), TdT(K), cytoplasmic CD79a(L), and myeloperoxidase(L).

Figure 3. Molecular and immunohistochemical characterization of the ovarian mass

A. Cells (lane 1) from the ovarian tumor expressed hemoglobin F and $\alpha 1$ spectrin, both of which were erythroid lineage specific transcripts. Lane 2: HEK293T - human embryonic kidney cell line; Lane 3: $\gamma 2A$ - human sarcoma cell line that is deficient in JAK2 expression; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. **B.** The tumor cells were strongly and diffusely for hemoglobin A (original magnification 400 $\times$); **C.** Compared to the positive internal control (dark staining of anucleated red blood cells), tumor cells were partially (~20%) and weakly positive for glycophorin A (original magnification 400 $\times$); **D.** The tumor cells were weakly and diffusely positive for CD117 (original magnification 400 $\times$); **E.** Tumor cells were strongly and diffusely positive for CD43 (original magnification 400 $\times$).

Figure 4. The neoplastic cells possessed genetic aberrancies

A. Among 21 cells from bone marrow aspirate analyzed, all showed deletion of 6q23q25 and extra chromosome 7 as depicted in this ideogram; **B.** LSI C-MYB aqua probe revealed only one signal in ~41% of the cells from the ovarian tumor, consistent with deletion of C-MYB at 6q23 locus; **C.** The LSI D7S486 (SpectrumOrange)/CEP7 (SpectrumGreen) dual color probe set revealed deletion of 7q31 (only one orange signal) and deletion of chromosome 7 (only one green signal) in ~66% and ~37% of tumor cells from the ovary, respectively, consistent with deletion of 7q31 and monosomy 7.