**Case 301**

<table>
<thead>
<tr>
<th><strong>Workshop</strong></th>
<th>III Overt MDS</th>
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<tbody>
<tr>
<td><strong>Title</strong></td>
<td>GATA2 familial predisposition syndrome</td>
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**Clinical History**

At ages 5 and 9, the patient developed recurrent group A streptococcal lymphangitis/cellulitis involving the right lower extremity. He also had streptococcal bacteremias coinciding with this. He was subsequently found to have lymphangiectasia in the right thigh region. He underwent immunodeficiency testing and was found to have B lymphopenia without hypogammaglobulinemia. The CD4/CD8 lymphocyte ratio was inverted. Testing for HIV was negative. He has HPV-driven warts, mainly involving the fingers and the toes. He has recurrent scrotal and penile lymphedema.

At the age of 15, CBC revealed a hemoglobin of 14.9, MCV 94.5, WBC 3.1, and a platelet count of 149,000. White blood cell differential %: neutrophils 42; lymphocytes 54; monocytes 3; basophils 1. Bone marrow biopsy performed.

**Morphological findings**

Peripheral blood: No cytologic abnormalities.
Bone marrow aspirate: Hypocellular particles. Erythroid precursors show predominantly normal morphology; rare terminal dyserythropoietic form (nuclear budding). Granulocytic precursors: Slightly left-shifted with prominent primary granulation. No increase in blasts. Scattered histiocytes with no significant hemophagocytosis.

Megakaryocytes: Predominantly normal morphology although rare atypical forms noted (monolobated and widely separated nuclei) (<10%).
Bone marrow core biopsy: Variably cellular, overall hypocellular 10-20%, with panhypoplasia. Occasional monolobated-appearing megakaryocytes. No increase in blasts or histiocytes. No abnormal lymphoid infiltrates.

**Immunophenotype**

Immunophenotype: NA

**Cytogenetics**

Cytogenetics: 46,XY[20]

**Molecular studies**

GATA2 365 base pair deletion mutation detected by targeted gene sequencing on a peripheral blood specimen (no further details available) (resulting in frameshift and premature protein truncation).

ASXL1 mutation c.2638dup; p.Thr880Asnfs*2 (VAF 11%) detected on the bone marrow specimen with NGS testing a panel of mutations.

**Proposed diagnosis**

GATA2 familial predisposition syndrome (haploinsufficiency) with Emberger manifestations. No diagnostic morphologic features of MDS.

**Interesting feature(s) of submitted case**

1) No significant cytopenias aside for the slight neutropenia (ANC 1240) and monocytopenia in a hypocellular bone marrow.
2) Scattered, clearly atypical megakaryocytes: Part of the syndrome or worrisome for (but not diagnostic of) emerging MDS? The 11% ASXL1 mutation suggests syndrome evolution and emerging MDS.
3) This group of entities is now incorporated into the 2016 revised WHO Classification of Haematopoietic
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<td><strong>Panel Diagnosis</strong></td>
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</table>
**Case 302**

**Workshop**
III Overt MDS

**Title**
A case of myelodysplastic syndrome with erythroid-predominance and myelofibrosis

**Clinical History**
A 51-year-old Japanese man presented with fever and general fatigue. Annual check-ups had identified mild leukocytopenia (2,600-2,820/μL) and anemia (hemoglobin (Hb), 12.1-12.8 g/dL) for more than 10 years. However, no further examinations were scheduled. He had been doing well for 7 years until he noticed fever, back and abdominal pain, and general fatigue, because of which he presented to a local clinic. He was found to have severe anemia and thrombocytopenia (peripheral blood: leukocytes, 3,260/μL; blasts, 1%; Hb, 7.0 g/dL; platelets, 80,000/μL). The patient had no previous history of cytotoxic therapy.

**Morphological findings**
The BM was hypercellular due to diffuse proliferation of erythroblasts intermingled with dysplastic megakaryocytes. Myeloblasts were not increased in the marrow. Silver stain demonstrated diffuse reticulin fibrosis without increased collagen fiber or osteosclerosis (MF1/3).

**Immunophenotype**
Flow cytometric results: CD3 2.0%, CD7 42.7%, CD20 4.5%, CD13 78.0%, CD34 84.1%, CD34 55.7%, CD41 23.6%, Glycophorin-A 34.6%, HLA-DR 48.8% Immunohistochemical results: The blastic cells in the marrow expressed CD71, E-cadherin, Glycophorin-A, and Glut1. Sparse immunoreactivity with myeloperoxidase (MPO), c-Kit was observed. Dysplastic megakaryocytes were positive for CD41 and CD61. Nuclear expression of p53 protein was seen in the majority of the nucleated cells.

**Cytogenetics**
43, XY, add(2)(q21), -5, -7, inv (9)(p12q13), -12, -17, add(18)(q21), +der(?) t(?;12)(?;q13) [2] /44, idem, -der(?) t(?;12), +der(?)( t(?;12)(?;q13), +mar1 [4]/46, XY, inv(9)(p12q13) [6]
* inv(9)(p12q13): normal variation

**Molecular studies**
Molecular studies: WT1 mRNA270 copy/μgRNA. JAK2V617F mutation was not detected

**Proposed diagnosis**
Proposed diagnosis: MDS with multilineage dysplasia, with erythroid predominance and myelofibrosis.

**Interesting feature(s) of submitted case**
Interesting feature(s) of submitted case: About 15% of MDS cases show >50% erythroblasts in bone marrow and can be called “MDS with erythroid predominance” (MDS-E). MDS-E has a higher incidence of high-risk cytogenetic abnormalities. On the other hand, about 15% of MDS cases demonstrate reticulin fibrosis on bone marrow biopsy, recognized as MDS with fibrosis or MDS-F, in which the degree of marrow fibrosis has been shown to link to parameters of erythropoietic failure, p53 protein accumulation and WT1 gene expression. Our case may represent an unusual combination of MDS-E and MDS-F, although the two variants of MDS do not represent any distinct subtype in the 2016 revision of the World Health Organization classification.

**Submitted by**
Professor Hidekazu Kayano MD, PhD

**Institute**
Faculty of Health and Medical Care, Faculty of Medicine, Saitama Medical University

**Address**
Saitama
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<th>Country</th>
<th>Japan</th>
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<td>Panel Diagnosis</td>
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</table>
Case 303

Clinical History
3-year old girl was noted to have bruising throughout her body since possibly as early as 3 months of age. She was routinely examined by her pediatrician who believed the bruising was related to learning how to crawl or walk. At the age of 2 years and 1 month, she was referred to a local hematologist, who followed her blood work monthly. She was then referred to our institution at the age of 3 years and 4 months.

Morphological findings
Core biopsy:
Cellularity: Hypocellular for age, ranging from 10 to 30%
Blasts: Not increased
Myeloid lineage: Markedly decreased.
Erythroid lineage: Increased.
Megakaryocytes: Dysplastic, showing widely separated nuclei and occasional forms with hypolobation.

Aspirate smears:
Spicular and hypocellular smears showing dyserythropoiesis (megaloblastoid changes, bi-nucleation, nuclear membrane irregularity) and dysmegakaryopoiesis (nuclear separation, small hypolobated forms). Granulocytes are markedly decreased, and detailed assessment of dysplasia is hard to make; however hypogranulated forms and abnormal nuclear lobation are noted.

Immunophenotype
Abnormal myeloid blast, monocyte and maturing granulocyte populations were detected by flow cytometry.

Flow cytometry identifies an abnormal myeloid blast population having abnormal expression of HLA-DR(bright), CD15 (subset), CD56 (partial), and CD117(dim). Granulocytes show left shift with abnormal expression of CD13(retained). Monocytes show abnormal expression of CD56.

No PNH clone detected on red cells, granulocytes, or monocytes by flow cytometry with peripheral blood.

Cytogenetics
Monosomy 7 detected in 59.3% of the cells by FISH.

Molecular studies
Next generation sequence for specific mutations in 30 genes, NEGATIVE

Gene tested: NPM1, FLT3, CEBPA, JAK2, MPL, KIT, DNMT3A, IDH1, IDH2, ASXL1, JAK1, RUNX1, CBL, SF3B1, JAK3, SH2B3, SUZ12, ETV6, KRAS, TET1, EZH2, TET2, TET3, HRAS, NRAS, TP53, PHF6, TYK2, PTEN, WT1.

Proposed diagnosis
Refractory cytopenia of childhood (RCC)

Interesting feature(s) of submitted case
Bone marrow was markedly hypocellular for age, and showed erythroid predominant hematopoiesis with
trilineage dysplasia. It is difficult to differentiate hypocellular refractory cytopenia of childhood (RCC) from aplastic anemia, inherited bone marrow failure disorders, and paroxysmal nocturnal hemoglobinuria. In this case, the presence of trilineage dysplasia and monosomy 7, and absence of PNH clone supported the diagnosis of RCC.

<table>
<thead>
<tr>
<th>Submitted by</th>
<th>Mariko Yabe/Attending hematopathologist</th>
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<tbody>
<tr>
<td>Institute</td>
<td>Memorial Sloan Kettering Cancer Center</td>
</tr>
<tr>
<td>Address</td>
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<td>Country</td>
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**Panel Diagnosis**
**Case 304**

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<th>Workshop</th>
<th>III Overt MDS</th>
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<tbody>
<tr>
<td><strong>Title</strong></td>
<td>Hypocellular MDS with TCF3 rearrangement</td>
</tr>
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</table>

**Clinical History**
The patient is a 54 yr old woman who was in her usual state of health until early 04/2014, when she developed flu-like symptoms with a temperature of 103.5 Fahrenheit. She recovered and went back to work but continued to have decreased energy, dyspnea on exertion and overall pallor. She presented to her primary medical doctor in mid May 2014 and a CBC revealed anemia and thrombocytopenia, for which she was admitted to hospital. Her CBC was: WBC 5.1, HgB 6.9, MCV 97, Retic 2.6% and Plt 32K. The patient's further imaging work up did not show any abnormalities. She was transfused two units of PRBCs and one unit of platelets. She was then evaluated by a hematologist who performed bone marrow tests.

**Morphological findings**
The bone marrow biopsy showed markedly hypocellular marrow with markedly reduced myeloid and megakaryocytic elements and relatively increased erythroid precursors. Reticulin stain highlighted mild to moderate fibrosis.

Aspirate smears were aspicular and inevaluable.

Peripheral blood smear showed pancytopenia, leukoerythroblastic picture, numerous nucleated red blood cells, circulating blasts (3%) and mild basophilia (1.8%).

**Immunophenotype**
Immunostains for CD34 and CD117 highlighted rare immature cells.

Immunophenotyping by flow cytometry showed an atypical blast population (1.1%).

PNH studies using a peripheral blood sample by flow cytometry did not show evidence of PNH clone.

**Cytogenetics**
Conventional karyotyping studies of the marrow aspirate sample showed normal karyotype.

FISH evaluation revealed deletion of 20q12 in 2.7% of the interphase cells examined, which was below the upper level of normal variation for this test.

FISH evaluation did not reveal rearrangement of the EVI1 (3q26) gene, deletion 5q, monosomy 5, deletion 7q, monosomy 7, trisomy 8 or translocation of the MLL (11q23) gene in any of the interphase cells examined.

**Molecular studies**
Next generating sequencing studies of the marrow sample showed a TCF3 BSG-TCF3 rearrangement.

**Proposed diagnosis**
Hypocellular MDS

**Interesting feature(s) of submitted case**
Hypocellular MDS
TCF3 gene rearrangements
### Case 305

**Workshop**  
III Overt MDS

<table>
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<tr>
<td>Myelodysplastic syndrome associated with a rare cytogenetic abnormality associated with aggressive disease course, with early stage disease.</td>
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</table>

**Clinical History**  
The patient is a 76 year-old man with no significant past medical history who presented with symptoms related to macrocytic anemia and thrombocytopenia. CBC: WBC 4.44 K/uL, Hgb 10.6 g/dL, MCV 116 fL, RDW 17.6%, PLT 85.5 K/uL; differential: neutrophils 52%, lymphocytes 32%, monocytes 12%, basophils 3%, myelocytes 1%. A bone marrow biopsy was performed to rule out myelodysplastic syndrome.

<table>
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<tr>
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<tr>
<th>Bone Marrow Aspirate/Biopsy</th>
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<tr>
<td>Bone marrow differential (500-cell/unit prep): neutrophils (segs and bands) 33; metamyelocytes 2; myelocytes 26; eosinophils and precursors 1; blasts 1; normoblasts 23; monocytes 1; lymphocytes 12; plasma cells 1.</td>
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| Cellularity: Markedly hypercellular, 80%. Erythroid precursors: Mild dyserythropoiesis (nuclear budding/irregularity, nuclear cytoplasmic asynchrony) in <10% of the erythroid precursors. Myeloid precursors: Left-shifted maturation. No significant dysplastic changes. No increase in blasts (1%). Megakaryocytes: Normal number. Predominantly normolobated with occasional small hypolobated/monolobated forms. Lymphocytes: No abnormal infiltrate. Plasma cells: Not increased. Iron stain: Markedly decreased stainable iron stores; sideroblasts present; ring sideroblasts not identified. |

<table>
<thead>
<tr>
<th>Immunophenotype</th>
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<tr>
<td>Flow Cytometric Analysis: No increase in blasts. No monotypic B-cell or aberrant T/NK-cell populations. No monotypic plasma cell population.</td>
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| Immunohistochemistry: No increase in CD34 positive blasts. CD34 highlights increased microvessels. CD61 highlights megakaryocytes, including occasional small hypolobated/monolobated forms. CD138 positive plasma cells are not increased. |

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<tr>
<th>Cytogenetics</th>
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<tr>
<td>Karyotype: 46,XY,inv(3)(q21q26.2)[3]/46,XY[17]. The result is abnormal. Of 20 metaphases, 17 metaphases were normal and 3 metaphases had an</td>
</tr>
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</table>
**inv(3)(q21q26.2).**

**FISH analysis:**
FISH analysis using probes against RPN1 (a gene located in the vicinity of the breakpoint cluster on the 3q21 region) and MECOM (a.k.a. EVI1-MDS) detected fusion signal in 1.4% of nuclei, confirming the chromosome study findings.

**Molecular studies**
Not performed.

**Proposed diagnosis**
Myelodysplastic syndrome, unclassified, associated with inv(3)(q21q26.2); GATA2, MECOM.

**Interesting feature(s) of submitted case**
Acute myeloid leukemia (AML) with inv(3)(q21q26.2) or t(3;3)(q21q26.2); GATA2, MECOM represents 1-2% of all AML cases, and is recognized as a distinct subcategory of AML with recurrent genetic abnormalities in the current WHO classification. Recent studies demonstrated that inv(3)(q21q26.2) or t(3;3)(q21q26.2), rather than producing a fusion gene, results in repositioning of a distal GATA2 enhancer to activate MECOM/EVI1 expression and simultaneously confer GATA2 haploinsufficiency, leading to uncontrolled proliferation and impaired differentiation of myeloid cells. In recognition of this ectopic effect of GATA2 on enhancing leukemogenesis, the genes involved in AML with inv(3)/t(3;3) has been revised in the current 2016 WHO classification [formerly RPN1-EVI1].

Myelodysplastic syndrome (MDS) with inv(3)(q21q26.2) or t(3;3)(q21q26.2) is very rare, occurring in <1% of all MDS cases, and may arise de novo or be therapy-related. Regardless of how it arises, MDS with inv(3)(q21q26.2) is associated with a poor prognosis with a high risk of progression to AML. Unlike other "low blast count" AMLs such as those with t(8;21), t(15;17), or inv(16) where a diagnosis of AML can be made with <20% blasts, the current WHO classification mandates that a myeloid neoplasm with inv(3)/t(3;3) and <20% blasts be classified as MDS, but recommends close monitoring for development of AML.

MDS with inv(3)/t(3;3) almost always shows prominent dysmegakaryopoiesis, including frequent small hypolobated/monolobated or bilobated forms. Dysgranulopoiesis and dyserythropoiesis are also very common. Secondary cytogenetic abnormalities are present in over 50% of the cases, the most ones being monosomy 7/7q, monosomy 5/5q, and complex karyotype. The current case is unusual due to the subtle dysplastic features, where dysplastic megakaryocytes are present in a small number, while dyserythropoiesis is mild and no overt features of dysgranulopoiesis are seen. These morphologic features, in conjunction with the low blast percentage (1%) and isolated cytogenetic abnormality with a small clone size detected by karyotype and FISH studies, support the notion that this case represents early disease manifestation of MDS with inv(3)/t(3;3). Two larger studies have demonstrated equally aggressive clinical course and poor survival in MDS vs. AML with inv(3)/t(3;3) (Haematologica 2014;99:821-9; Am J Clin Pathol 2011;136:282-288), while an NGS study found no difference in mutational patterns or gene expression profile between the two (Blood 2015;125:133-9). These findings support that myeloid malignancies with inv(3)/t(3;3) represent a single disease entity with equally aggressive disease course irrespective of blast count, analogous to the case of therapy-related MDS/AML. Early detection of MDS with inv(3)/t(3;3) may lead to early intervention with aggressive therapeutic regimen and improved outcome.

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<th>Submitted by</th>
<th>Dr. April Chiu</th>
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<td><strong>Institute</strong></td>
<td>Mayo Clinic</td>
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<td><strong>Country</strong></td>
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**Panel Diagnosis**
### Case 306

#### Workshop

| III Overt MDS |

#### Title

Erythroid predominant myelodysplasia/ AML - a spectrum

#### Clinical History


#### Morphological findings

- Bone marrow aspirate
- 28% blasts
- Trilineage dysplasia
- Erythroid predominance

#### Immunophenotype

CD34, CD117, E-Cadherin, glycophorinA, LCA, MPO, CD15, CAM5.2, AE1/3, S100, CD61 & p53 were done. These show increased (approximately 12-20%) number of CD34 & CD117 positive blasts. The erythroid cells are identified by glycophorin A and many express E-Cadherin/CD117. There is relative reduction in mature granulocytes on CD15 stain. Almost all of the cells express p53 strongly.

#### Cytogenetics

**1st Clone:** 5/10 cells analysed
56~65,XY,+X,+Y,+1,+2,+3,add(3)(p21),+add(3)(p21),+4,-5,+6,+7,+8,+9,+10,+11,+11,+12,+15,+16,+20,+22,+4mar[cp5]

**2nd Clone:** 3/10 cells analysed
95~99,XXYY,+1,+3,add(3)(p21),add(3)(p21),-5,+6,+11,-13,+14,-17,-20,+5mar[cp3]

FISH for ETV-RUNX1, PML-RARA- negative

#### Molecular studies

Not performed

#### Proposed diagnosis

AML with myelodysplasia related changes; erythroid predominant

#### Interesting feature(s) of submitted case

Difficulty in classifying MDS and AML in the setting of erythroid predominance Complex karyotype not typical of erythroid predominant MDS/AML erythroid-myeloid type Near triploidy/tetraploidy rare in AML/MDS; poor prognosis

#### Submitted by

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<th>Institute</th>
<th>Royal Victoria Hospital</th>
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<td>Address</td>
<td>Belfast</td>
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<tr>
<td>Country</td>
<td>United Kingdom (Northern Ireland)</td>
</tr>
<tr>
<td>Co authors</td>
<td>Dr Lakshmi Venkatraman, Dr Conal McConville, Dr Peter McGrattan, Mr Mervyn Humphreys, Dr Brian Herron</td>
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#### Panel Diagnosis

Workshop III Overt MDS
# Case 307

## Workshop

III Overt MDS

## Title

Myelodysplastic syndrome with excess blasts-2 and WT1 mutation and missense variants in FTL3 and TP53

## Clinical History

46-year old man with a known hemoglobin C trait presented to ED with fatigue and diarrhea following a trip to Mexico and Europe. He experienced weight loss, abdominal pain and fatigue since 2 months following the trip.


Reticulocyte count was 14% and urine analysis showed small blood.

There was high suspicion for a parasitic infection and hemolysis, given travel history and jaundice.

Initial lab data: suggestive of hemolysis, with undetectable haptoglobin, elevated LDH in 500s, and elevated Indirect bilirubin of 2.3.

## Morphological findings

**Aspirate Smear:**
- The aspirate smear is hemodilute with one minute spicule present.
- Variably sized blasts account for 6% of cellularity, with irregular nuclear contours, open chromatin, prominent nucleoli, and scant to moderate amounts of grey-blue cytoplasm with frequent delicate Auer rods and occasional azurophilic granules.
- Myeloid elements are left-shifted with limited maturation and occasional hyposegmented neutrophils.
- Erythroid elements are markedly increased with maturation, and show cytologic atypia (irregular nuclear contours, nuclear budding, megaloblastoid change, binucleation, karyorrhexis, satellite nuclei, and internuclear bridging) in >10% of cells.
- The myeloid to erythroid ratio is 0.3:1.
- Rare megakaryocytes are noted with cytologic atypia, including separated nuclei and hypolobated nuclei.
- Storage iron is present; ringed sideroblasts are not identified.

A differential count of 200 nucleated cells shows the following percentages:
- 6 Blasts
- 2 Promyelocytes
- 2.5 Myelocytes/Metamyelocytes
- 15 Bands/Neutrophils
- 0 Monocytes
- 0 Eosinophils
- 0 Basophils
- 3 Lymphocytes
- 0 Plasma cells
- 71.5 NRBCs

**Aspirate Clot:**
- H&E and PAS stained sections show that the aspirate clot contains scant marrow elements which are morphologically similar to the biopsy.
- Storage iron is present; ringed sideroblasts are not identified.

**Biopsy:**
- H&E and PAS stained sections show trabecular bone with hypercellular marrow for age (>95% cellularity).
- The myeloid to erythroid ratio is 1:3.
- Erythroid elements are increased with left shifted maturation.
Myeloid elements are present with left shifted maturation. Megakaryocytes are decreased with occasional hypolobation. No aggregates of lymphocytes are identified. A reticulin stain shows grade 1 fibrosis (European Consensus System).

CBC:
A recent (12/13/2016) CBC shows: WBC 8.0 (neutrophils 71%, bands 3%, lymphocytes 25%, monocytes 1%, eosinophils 0%, basophils 0%), Hgb 8.6, Plt 59, MCV 99.

***
In summary, this is a hypercellular marrow (>95%) involved by myelodysplastic syndrome (MDS) best classified as MDS with excess blasts-2 (MDS-EB-2, formerly RAEB-2). The combined morphologic and histologic features are most consistent with myelodysplastic syndrome, best classified as myelodysplastic syndrome with excess blasts-2 (MDS-EB-2, formerly RAEB-2).

**Immunophenotype**

**Immunohistochemistry:**
Immunostains performed on the bone marrow core with adequate controls show that CD34+ blasts account for <5% of cellularity. E-cadherin highlights increased early erythroid precursors. CD117(c-kit) highlights increased early myeloid and erythroid precursors. MPO highlights decreased maturing myeloid lineage cells.

**Cytogenetics**

**Karyotype:** normal 46,XY[20] Negative FISH studies for t(8;21) and t(15;17).

**Molecular studies**

*DISEASE ASSOCIATED MUTATIONS* (see interpretation and comments):

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<th>GENE</th>
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<th>cDNA CHANGE</th>
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<tr>
<td>WT1</td>
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*VARIANTS OF UNCERTAIN SIGNIFICANCE* (see interpretation and comments):

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<tr>
<td>TP53</td>
<td>p.G226V</td>
<td>c.677G&gt;T</td>
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**Proposed diagnosis**

myelodysplastic syndrome, best classified as myelodysplastic syndrome with excess blasts-2 (MDS-EB-2, formerly RAEB-2)

**Interesting feature(s) of submitted case**

MDS-EB-2 with blasts with very delicate Auer rods and WT1 mutation, as well as other variants of uncertain significance in FLT3 and TP53

**Submitted by**

Dr. Agata M Bogusz

**Institute**

Pathology and Laboratory Medicine

**Address**

University of Pennsylvania
Philadelphia

**Country**

USA

**Panel Diagnosis**
**Workshop III Overt MDS**

**Case 308**

**Title**
Bone marrow failure with del13q and missense variant in ETV6 gene

**Clinical History**
66-year-old woman presented with anemia and thrombocytopenia
CBC: WBC 3.5 (40.5% neutrophils, 53.1% lymphocytes, 6.0% monocytes, 0.2% eosinophils, 0.2% basophils), Hgb 7.3, MCV 97, Plt 7.
A bone marrow biopsy was performed.

**Morphological findings**

Aspirate smear:
The aspirate smear contains cellular spicules.
The myeloid to erythroid ratio is 0.7:1
Erythroid precursors are increased show orderly maturation with occasional cytologic atypia (karyorrhexis, nuclear budding, irregular nuclear contours).
Myeloid precursors elements are markedly decreased and left shifted without cytologic atypia.
Rare megakaryocytes are seen.
An iron stain is increased. Ringed sideroblasts are not seen.

A differential count of 200 nucleated cells shows the following percentages:
3 Blasts
1 Promyelocytes
8 Myelocytes/Metamyelocytes
15 Bands/Neutrophils
3 Monocytes
4 Eosinophils
3 Mast cells
5 Lymphocytes
2 Plasma cells
56 Nucleated RBCs

Aspirate clot:
H&E and PAS stained sections of the aspirate clot show approximately 40-50% cellular bone marrow. Erythroids are markedly increased and show maturation and mild cytologic atypia. Myeloids are decreased and megakaryocytes are decreased. The M:E ration is approximately 1:5. An iron stain shows storage iron; ringed sideroblasts are not seen.

Biopsy:
H&E and PAS stained sections show suboptimal for evaluation specimen with an extensive aspiration artifact and scant, disrupted bone marrow elements.
The myeloid to erythroid ratio cannot be reliably assessed.
Erythroid precursors appear to be increased and show maturation. Myeloid precursors show full maturation and are relatively decreased.
Megakaryocytes are markedly decreased.
A well-circumscribed lymphoid aggregate composed of small mature lymphocytes is noted.
In small evaluable areas there is mild increase in reticulin fibrosis (grade 1); (European Consensus System).

**Immunophenotype**
Flow cytometry:
Studies performed on the bone marrow aspirate demonstrate an expansion of a non-discrete population of blasts and maturing myelomonocytic cells within the blast gate [7.6% of total events]. There is no overt immunophenotypic evidence of an acute leukemia.

Immunohistochemistry:
Immunostains performed on the biopsy core with adequate controls demonstrate no increase in CD34+ Blasts (<2%). Lymphoid aggregates are composed primarily of CD3+, CD5(dim)+ T cells and few CD20+, PAX5+, CD79a+ B cells. E-cadherin stains early erythroid precursor and glycophorin A intermediate to late erythroid precursors. MPO, cKIT and CD61 stains rare cells.

### Cytogenetics
First biopsy Normal female karyotype: 46,XX[20].
Second biopsy 46,XX,del(13)(q12q22)[2]/46,XX[19]

### Molecular studies
Variant of uncertain significance

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<tr>
<th>GENE</th>
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### Proposed diagnosis
Bone marrow failure vs hypoplastic MDS with del13q and VUS in ETV6 gene.

### Interesting feature(s) of submitted case
Absence of significant dysplasia and acquired del13q that was detected in the second biopsy.

### Submitted by
Dr. Agata M Bogusz

### Institute
Pathology and Laboratory Medicine

### Address
University of Pennsylvania, Philadelphia

### Country
USA

### Panel Diagnosis
**Case 309**

**Workshop**

III Overt MDS

**Title**

MDS with myeloid maturation arrest, clonal hematopoiesis, and associated autoimmune cytopenias

**Clinical History**

73 year-old woman with history of renal cell carcinoma (right kidney, unknown date of diagnosis) treated only with total nephrectomy, invasive ductal breast carcinoma (low grade, ER/PR+, Her2-, stage 1) diagnosed in 2009 and treated with lumpectomy, and hormonal (2010-2015) and radiation (2010) therapy. At the time of diagnosis of breast cancer in 2009, the patient was found to have leukopenia, thrombocytopenia and borderline low hemoglobin. She continued to develop worsening pancytopenia during the next years. Bone marrow biopsy done in 2012 showed a mildly hypercellular marrow with maturing trilineage hematopoiesis and no morphologic evidence of overt MDS. The cytogenetic studies showed a normal female karyotype (46, XX). The etiology of mild pancytopenia was thought to be autoimmune given positive direct antiglobulin test, normal B12/folate, normal TSH (on replacement) and a spleen of normal size. The patient was treated with trial of steroids and the neutrophil and platelet counts improved transiently. However, she had to receive several RBC transfusions.

In 2016 she was found to have a more pronounced pancytopenia with recent worsening of CBC and only mild response to steroids. A bone marrow was performed and herein we describe the findings.

Social history: Never smoked, never drinks alcohol, never took illegal drugs

Medications: Cetirizine, Cholecalciferol, Ezetimibe, Fenoxifanadine, Levothyroxine Sodium, Multiple vitamins pills, Prednisone, Sertraline

**Morphological findings**

**Bone Marrow Biopsy:**

Variably cellular marrow, hypercellular overall for age (50% cellularity) Dyspoietic megakaryocytes (abnormally spaced nuclear lobes, multinucleation, micromegakaryocytes) in at least 40% of megakaryocytes Myeloid elements are left-shifted with very limited maturation Erythroid elements show full maturation Scattered small lymphocytes and plasma cells account for <5% of cellularity. No lymphoid aggregates are noted Storage iron is present; ring sideroblasts are not identified PAS stain highlights lack of mature neutrophils A reticulin stain shows grade 0 fibrosis (European Consensus System)

**Bone Marrow Aspirate Smears:**

Hypercellular spicules Myeloid elements are left-shifted with markedly limited maturation and nucleocytoplasmic dyssynchrony (immature granulocytes morphologically compatible with dyspoietic myelocytes and promyelocytes with prominent nucleoli, fine to somewhat clumped chromatin, and irregularly distributed secondary granules) Erythroid elements show full maturation without significant dyspoiesis The myeloid to erythroid ratio is 3.6:1 Megakaryocytes are present with frequent dyspoiesis (atypical and irregular nuclear lobation, separated nuclear lobes) Storage iron is present; ring sideroblasts are not identified

A differential count showed the following percentages: 5% Blasts, 4% Metamyelocytes, 58% Myelocytes/Promyelocytes, 2% Bands/Neutrophils, 1% Monocytes, 0% Eosinophils, 0% Basophils, 10% Lymphocytes, 1% Plasma cells, 19% NRBCs

**Immunophenotype**

Flow cytometry:
Data were acquired by gating on events with the CD45 vs. side scatter characteristics of blasts (up to 14% of total events). Within this region there is a non-discrete population of CD34+ blasts (8% of total events) with increased expression of CD117.

The granulocytes (~50% of total) are markedly left shifted with limited maturation and express low density partial CD34, partial CD117 and low density CD45, which are findings compatible with immunophenotypic dyspoiesis.

Granulocytes do not show significant expression of CD10, CD16 or CD11b, indicating dysmaturation.

### Cytogenetics

| Karyotype: 46,XX[20] |

### Molecular studies

| Next Generation Sequencing: Mutations in SRSF2, ASXL1, and two TET2 frameshift mutations. |

### Proposed diagnosis

Myelodysplastic syndrome with multilineage dysplasia.

Given that the cytopenias were present before the treatment with radiation and that the cytogenetic evaluation showed a normal karyotype on both bone marrow biopsies, a therapy-related MDS appears less likely but should be considered.

An associated autoimmune component is also a contributing factor for the development of cytopenias in this patient.

### Interesting feature(s) of submitted case

1) Unusual myeloid maturation arrest that mimics a medication effect or the marrow morphology sometimes observed following administration of granulocyte colony stimulation factor (GCSF). Although in this case severe congenital neutropenia/Kostmann disease are not considered, this finding may also be observed in these conditions.

2) Only a few cases of MDS associated with autoimmune cytopenias but without a known established diagnosis of autoimmune disease (i.e. rheumatoid arthritis) are rarely described in the literature and are likely under reported.

3) Molecular evidence of clonal hematopoiesis was identified, with mutations in genes that are members of the spliceosome machinery (SRSF2), chromatin modifiers (ASXL1) and regulators of DNA methylation (TET2).

### Submitted by

| Dr. Sharon Song |

### Institute

| Hospital of the University of Pennsylvania |

### Address

| Pathology and Laboratory Medicine – Hematopathology |
| Philadelphia, PA 19104 |

### Country

| United States of America |

### Panel Diagnosis
**Case 310**

**Workshop**  
III Overt MDS

**Title**  
MDS/MPN U with t(9;22) and komplex karyotype

**Clinical History**  
66 year old male with a few months of fatigue and anemia. No B-symptoms, no lymphadenopathy nor splenomegaly. Hb 100 g/L, LKP: 2x10^9/L, Thr 500x10^9

**Morphological findings**  
Blood smear: Anisopoikilocytosis, thrombocytosis, dysplastic granulocytes (pseudopelger and hypgranulation, a few blasts.  
Bone marrow smear: Dysplastic megas including hypolobulated and hyperlobulated forms. Dysplastic granulopoiesis with 6% blasts, scant erythropoiesis.  

**Immunophenotype**  
Flow: 11% myeloid blasts CD34+/CD117-/DR+/CD13+/CD33-/MPO-/TdT-

**Cytogenetics**  
Karyotype:  
45,XY,der(1)t(1;16)(p36;p11),der(1)inv(1)(p22p36)t(1;22)(p36;q11),der(9)t(1;9)(?p36;q34),der(9)t(9;12)(q 12:?p11),-12,der(16)t(12;16)(q13;p11),der(22)t(9;22)(q34;q11)[25]

**Molecular studies**  
PCR: p210 fusion transcript t(9;22)

**Proposed diagnosis**  
MDS/MPN U

**Interesting feature(s) of submitted case**  
p210 fusion transcript and komplex karyotype. Progression of CML or de novo disease?

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<tr>
<th>Title</th>
<th>Mats O Ehinger Associate professor</th>
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<td>Clinical Sciences</td>
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**Panel Diagnosis**
**Case 311**

**Workshop**

III Overt MDS

**Title**

MDS associated with trisomy 8 and SRSF2 and IDH2 mutations

**Clinical History**

The patient is a 67-year-old male who presents with anemia and thrombocytopenia. History of rheumatoid arthritis on steroids and methotrexate. History of marginal zone lymphoma of the conjunctiva left eye in 2005, stage IE, he required localized low-dose radiation therapy and no chemotherapy.

CBC at presentation: WBC 14.1 K/uL (3.8-10.6); Absolute neutrophil count 11.8 K/uL; Absolute monocyte count 0.8 K/uL; Absolute lymphocyte count 1.4 K/uL; HGB 10.2 g/dL (13.0-18.0); MCV 84 fl (80.0-100.0); PLT 113 K/uL(150-440)

**Morphological findings**

Aspirate: Maturing trilineage hematopoiesis is evident. Erythroid maturation is megaloblastoid with occasional karyorrhexis, irregular nuclei, and binucleation (dysplasia does not exceed 10% of total erythroid precursors). Complete myeloid maturation to neutrophils is evident. Giant and vacuolated forms are noted. Occasional maturing granulocytes with engulfed cells are also seen. Megakaryocytes are adequate in number and highly variable in appearance. A 500-cell differential cell count shows: 2% blasts, 45% erythroid precursors, 48% maturing myeloid precursors, <1% eosinophils, <1% monocytes, 3% lymphocytes and <1% plasma cells. The M:E ratio is 1.1:1.0. Iron stain: Reticuloendothelial storage iron is not identified. Rare ring sideroblasts are identified.

Biopsy: The marrow is hypercellular for age (60-80% ratio of hematopoietic cells to fat). Maturing trilineage hematopoiesis is present. Interspersed immature-appearing mononuclear cells are noted. Megakaryocytes appear increased in number and dysplastic forms including hyperlobated, bulbous nuclei, hypolobated nuclei, or bare/pyknotic nuclei are seen. No lymphoid aggregates are identified. Variably thickened bone trabeculae and occasional dilated sinuses are noted. Reticulin: Dense network of reticulin with many intersections (grade MF-1 to MF-2).

**Immunophenotype**

By Immunohistochemistry a single lymphoid aggregate comprised of a mixture of CD3+ T-cells and CD20+ B-cells is identified. No increase of CD34+ blasts. CD117 highlights occasional, scattered mononuclear cells.

By flow cytometry there is a mixed population of maturing myeloid cells and lymphocytes. No increase in CD34+ blasts is seen. The lymphoid population is comprised of 74% T-cells, 2% B-cells and 24% NK-cells. The CD4:CD8 ratio is approximately 3.8:1.0. The B-cells consists of a mixture of kappa and lambda light chain bearing cells. No B-cell light chain restriction is identified. A distinct population of normal B-cell precursors (hematogones) is not identified.

**Cytogenetics**

An abnormal male karyotype with trisomy 8 was detected in fourteen (14) cells. No other chromosome abnormality was reported (total of 20 cells examined).

**Molecular studies**

No BCR/ABL rearrangement identified by PCR analysis. Mutation Panel by Next Generation Sequencing performed at outside reference laboratory showed:

- Tier 1 Variants (Variants of known significance in myeloid malignancies): SRSF2 c.284C>A, p.Pro95His (NM_003016.4) Variant Frequency: 47.2%; and IDH2 c.419G>A, p.Arg140Gln (NM_002168.3) Variant Frequency: 46.0%.

- Tier 2 Variants (Variants of unknown significance in myeloid malignancies): None Detected.
**Proposed diagnosis**

HYPERCELLULAR BONE MARROW WITH VARIABLE DYSPLASIA, TRISOMY 8 AND SRSF2 AND IDH2 MUTATIONS; FAVOR MDS, UNCLASSIFIABLE

**Interesting feature(s) of submitted case**

Based on the persistent cytopenias, dysplastic changes in <10% of cells on one or more myeloid lineages, and clonal cytogenetic and molecular findings (Trisomy 8 and SRSF2 and IDH2 mutations), further classification as a myelodysplastic syndrome, unclassifiable, is favored.

The sustained neutrophilia may represent chronic steroid therapy effect.

The clinical history also raises the possibility of therapy-related myeloid neoplasm. Radiation therapy received in 2005 was low-dose and localized. Risk of secondary myeloid neoplasm with low-dose radiation is rare but possible. This patient did not receive large field radiation therapy including active bone marrow. It is unclear if low-dose methotrexate for RA is considered a possible risk for therapy-related myeloid neoplasm. Myelodysplastic/myeloproliferative neoplasm, unclassifiable, and primary myelofibrosis were also considered in the differential diagnosis. JAK2, MPL, and Calreticulin mutations were not detected.

Trisomy 8 as a sole abnormality is common in MDS (15-20%), AML (10-15%) and myeloproliferative neoplasms (10% of CML, 20% of P Vera). Possible genes involved in trisomy 8 are unknown. Myeloid neoplasms with trisomy 8 are heterogeneous with different clinical and pathologic presentations. Trisomy 8 may be a secondary event. Of note, trisomy 8 as the sole cytogenetic abnormality in the absence of morphologic criteria is not considered definitive evidence of MDS. Also, individuals with constitutional trisomy 8 are at increased risk of developing a myeloid neoplasm.

Mutations in genes of the splicing machinery have been described recently in myelodysplastic syndromes (MDS). SRSF2 is found in 28-47% of patients with CMML, 10-15% in patients with MDS, and in 3% of patients with MPN. SRSF2 mutations appear to have a negative prognostic impact in MDS and may become a useful biomarker for risk stratification.

Mutations in genes involved in DNA methylation also occur in MDS. Mutations in the genes encoding the isocitrate dehydrogenase (IDH) enzymes, IDH1 and IDH2, are reportedly found in approximately 5% of patients with MDS. IDH2 is also found in 9% of MDS/MPN neoplasms including 6% of patients with CMML. IDH mutations has been reported to be associated with SRSF2 and may represent a poor prognostic factor in patients with low-risk MDS. IDH mutations may be a useful biomarker for risk stratification.

Follow-up: Blood counts have remained stable. The patient is on active surveillance at this time (follow-up every 3 months). He has not required any transfusions or growth factor therapy. His chief complaint continues to be his rheumatoid arthritis. The patient continues on prednisone and also takes 1 mL methotrexate injection weekly.

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<th>Submitted by</th>
<th>Enrique Ballesteros M.D.</th>
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<td>University of Connecticut</td>
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<td>Address</td>
<td>Department of Pathology and Laboratory Medicine UCONN Health Farmington, Connecticut</td>
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**Panel Diagnosis**
**Case 312**

**Workshop**

III Overt MDS

**Title**

MDS/MPN-U with rapid evolution to acute myeloid leukemia

**Clinical History**


March/April 2016: macrocytic, non-regenerative anemia with thrombocytosis. Mild hyperosinophilia. No monocytosis and myelemia. No splenomegaly nor chronic inflammatory conditions. Mild fatty liver without hepatomegaly. No recent history of cytotoxic or growth factor therapy. BM karyotype was normal; the search for JAK2 (V617F and Exon 12), MPL, CalR mutations and PDGFRα/β and BCR-ABL genes rearrangements were negative but SRSF2 (P95H) gene mutation was found suggesting a clonal hematopoiesis. BM aspirate: absence of myelodysplastic changes without blasts cells. On bone marrow trephine biopsy it was found hypercellularity with increased M:E ratio, marked granulocytic hyperplasia and mild dysmegakaryopoiesis dysplasia without clustering. No fibrosis (MF0). A diagnosis of MPN/MDS-U was considered. Erythropoietin treatment was started with good response.

September/October 2016: increasing anemia and thrombocytopenia with blasts cells in PB (6%). In suspicion of leukemic transformation: another workup was ordered. On BM aspirate, there was granulocytic dysplasia with 9% of blasts cells; further evaluation was hampered by difficult aspiration and analysis of erythroid and megakaryocytic lineages was impossible. On trephine biopsy, hypercellularity (80%) but slightly reduced compared to the previous BM biopsy (04/2016). Marked megakaryocytic hyperplasia (either unlobed or bi-/monolobed nuclei) with clustering and dysplastic changes. Focal bone sclerosis. Increased number of blasts cells averaging 20-25%, with clustering and evidence of myelofibrosis (MF2).

Retrospective molecular study on bone marrow samples drawn in March 2016 did not show mutation of ASXL1 in exon 12 which was screened during classical analyses. However, NGS analysis revealed a nonsens mutation of ASXL1 (exon 11).

After leukemic evolution (10/2016), SRSF2 (P95H) gene mutation was also highlighted, in the absence of NPM, Flt3, IDH1 and 2, CEBP mutations on conventional methods. Analysis of ASXL1 gene after leukemic evolution is ongoing. In October, chest CT scan identified a pulmonary nodule in the left upper lobe with intense FDG uptake on PET-CT. The nodule was surgically excised, revealing squamous cell carcinoma on histology, without node metastasis (AJCC 2017: pT1aN0Mx). Induction therapy for AML was performed in December 2016.

**Peripheral blood counts (at onset)**

- February 2016: PLT 665 G/L, Hb 9.5g/dl, WBC 5G/L PMN 3200/mm3, eosinophilis 1.2G/L, monocyes 1.01G/L ; basophilis 0% ; no dacryocytes ; myelemia 0%, blastes 0%.

- September 2016: PLT 119 G/l, Hb 7.5g/dl, WBC 4.38 G/l, PNN 2650/mm3, eosinophilis 0.35G/L, monocyes 0.04G/L ; basophilis 0% ; no dacryocytes ; myelemia 0% ; blastes 6%

**Details of microscopic pathology**

Myelogram (7/03/2016) : quite hypercellular marrow in the absence of significant myelodysplastic changes in all hematopoietic lineages without blasts cells.

Bone marrow trephine biopsy (27/04/2016) : hypercellularity (90%) with granulocytic hyperplasia (no dysplasia). No eosinophilic proliferation. M/E 10:1. No dyserythropoiesis. Megacaryocytes: increased in number, with dysmegakaryopoiesis and small megacaryocytes (with bi- or monolobed nuclei or non-
lobed) no clusters. Blasts CD34+ <5% without clustering. No myelofibrosis (MF0).

Myelogram 10/10/2016: difficult aspiration and partial hemodilution of bone marrow with failure of karyotype. Mild hypercellular marrow with granulocytic dysplasia (nuclear hypo-segmentation and neutrophil hypogranulation). Because of hemodilution, analysis of erythroid and megakaryocytic lineages was impossible. 9% of blasts was found.

Bone marrow threphine biopsy (18/10/2016): BM threphine biopsy showed hypercellularity (80%) but quite inferior at the April BM biopsy with marked megakaryocytic hyperplasia (either unlobed or bi-/monolobed nuclei and small cells) with clustering and dysplastic changes. It persisted mild granulocytic hyperplasia with an erythroid hyperplasia (erythropoietin treatment) without evident dysplastic changes. No eosinophilic proliferation. It was observed an increased number of blasts cells averaging 20-25% CD34 and CD117 positives, with clustering and evidence of myelofibrosis (MF2). Focal bone sclerosis.

### Immunophenotype

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<td>(flow cytometry / immunohistochemistry)</td>
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<td>07/03/16: Ogata score: 2; redscore no available</td>
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<td>10/10/2016 and 06/10/2016: Flow cytometry not contributive because of difficulty at bone marrow aspiration</td>
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### Cytogenetics

- 07/03/2016 (bone marrow): 46,XY[20].nuc ish(EGR1,D5S721-D5S23)x2[100]- normal karyotype without EGR1 deletion by FISH
- 06/10/2016 (bone marrow): no available karyotype (difficulty at aspiration)
- 10/10/2016 (bone marrow): no available karyotype (difficulty at aspiration)
- 18/10/2016 (peripheral blood): no available karyotype

### Molecular studies

- 09/03/2016: on PB negative for mutations of Jak2 (V617F and exon 12), MPL, CalR, PDGFRA/PDGFRB and BCR-ABL.
- 07/03/2016: Retrospective analysis bone marrow showed presence of SRSF2 (P95H) gene mutation and no mutation of ASXL1 (Exon 12). By NGS analysis we found a nonsens ASXL1 gene mutation in exon 11 (R404X a 40%) and confirming SRSF2 (P95H) gene mutation (allele burden 49%).
- 10/10/2016: After leukemia transformation, analysis bone marrow showed presence of SRSF2 (P95H) gene mutation and no mutations of NPM, Flt3, IDH1 and 2, CEBPa were found (conventional methods). Analysis of ASXL1 gene after leukemia transformation is ongoing.

### Proposed diagnosis

MDS/MPN-U, featuring <5% blasts with SRSF2 (Exon 1 P95H) gene and ASXL1 (Exon 11) gene mutations, with rapid evolution to acute myeloid leukemia.

### Interesting feature(s) of submitted case

Difficulty at classifying the disease both clinically and morphologically, given the rapid evolution to AML. Interesting molecular biology with mutation in SRSF2 often associated with short OS and AML transformation, according to current data.

### Comments

Analysis of ASXL1 gene and other genes after leukemia transformation will be available for the meeting.

| Submitted by | Barbara Burroni |
| Institute | Pathology Department Cochin hospital |
| Paris | |

Workshop III Overt MDS
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<td>Co-authors</td>
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Panel Diagnosis
Case 313

Workshop

III Overt MDS

Title

An unexpected cause of aplastic anaemia

Clinical History

A 28 year old woman presented in January 2011 with vomiting and a petechial rash and was found to have pancytopenia requiring blood and platelet transfusion. A bone marrow examination showed features consistent with aplastic anaemia. In June 2011 she was found collapsed with 2 bottles of Melphalan (which she had obtained via the internet), with 30 tablets missing. She admitted to taking Melphalan prior to the previous admission. She had persistent thrombocytopenia and a bone marrow biopsy in April 2012 appeared normocellular with reduced megakaryocyte frequency. Her peripheral blood counts continued to fall and in November 2012 repeat bone marrow examination showed features of t-MDS and an abnormal karyotype. She was being considered for allogeneic stem cell transplant when a further bone marrow biopsy in April 2013 showed progression to Acute myeloid leukaemia (t-AML). The leukaemia was refractory to chemotherapy and she died in July 2013 following an episode of pneumocystis pneumonia.

Morphological findings

Bone marrow from January 2011 showed aplasia.
Bone marrow from November 2012 was hypercellular with decreased megakaryocyte frequency and dysplastic features in the erythroid and granulocyte lineages (reported in the aspirate).

Immunophenotype

CD34 and CD117 positive blast cells were <5%, however, non-paratrabecular clusters of CD117 positive blasts were present which were mast cell tryptase negative.

Cytogenetics

November 2012
KARYOTYPE: 46,XX,der(6)t(3;6)(q2?5;p23~24)[10]
Karyotype analysis of this cultured bone marrow has shown an abnormal female karyotype in all ten cells examined. All cells show a derivative chromosome 6, from an unbalanced rearrangement between the long arm of chromosome 3 and the short arm of chromosome 6. This has resulted in partial trisomy for 3q and partial monosomy for the terminal region of 6p.

Molecular studies

FISH NEGATIVE for deletions of chromosomes 5q, 7q and 20q, and trisomy 8.
FISH analysis using the MECOM (EVI1) probe has shown three copies of MECOM, with NO Rearrangement of MECOM confirming the presence of the additional 3q material on 6p.

Proposed diagnosis

Aplasia progressing to myelodysplasia with multilineage dysplasia secondary to self administered Melphalan.

Interesting feature(s) of submitted case

Documented time course and evolution of bone marrow findings following self administration of Melphalan resulting in marrow aplasia, persistently reduced megakaryocytes and progression to myelodysplasia and acute myeloid leukaemia.

Submitted by

Dr Penny A Wright

Institute

Cambridge University Hospitals NHS Trust
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<tr>
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<th>Histopathology Department</th>
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<td>Addenbrookes Hospital,</td>
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<td>Cambridge University Hospitals NHS Trust, Cambridge</td>
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<td>Panel Diagnosis</td>
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<td><strong>Case 314</strong></td>
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<td><strong>Workshop</strong></td>
<td>III Overt MDS</td>
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<tr>
<td><strong>Title</strong></td>
<td>The more you look the more you find.... Cytogenetic findings in an MDS/MPN overlap</td>
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<tr>
<td><strong>Clinical History</strong></td>
<td>73 year old female patient, presented with fatigue and a cluster of nosebleeds</td>
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<tr>
<td><strong>Morphological findings</strong></td>
<td>Dyserythropoiesis Pleomorphic megakaryocytes with both proloferative and dysplastic features Ring sideroblasts</td>
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<tr>
<td><strong>Immunophenotype</strong></td>
<td>N/A</td>
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<td><strong>Cytogenetics</strong></td>
<td>Normal female karyotype by G-banding Five deletions on SNP array</td>
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<tr>
<td><strong>Molecular studies</strong></td>
<td>JAK2 V617F positive SF3B1 R625L mutation detected</td>
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<tr>
<td><strong>Proposed diagnosis</strong></td>
<td>MDS/MPN-RS-T</td>
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<tr>
<td><strong>Interesting feature(s) of submitted case</strong></td>
<td>1. Typical morphological features for this new full entity 2. Complex cytogenetic alteration shown in SNP array despite normal karyotype</td>
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**Submitted by** | Dr Livia Raso-Barnett |
**Institute** | Haematopathology and Oncology Diagnostic Service (HODS), Cambridge University Hospital NHS Trust |
**Address** | Addenbrooke’s Hospital, Cambridge |
**Country** | United Kingdom |

**Panel Diagnosis** |
### Case 315

**Workshop**
III Overt MDS

**Title**
Won the battle, lost the war....

**Clinical History**
80 year old patient, presented with mild anaemia and thrombocytopenia.

**Morphological findings**
Trilineage dysplasia with improving blast count (15% to 5%) yet marked worsening of dyserythropoietic features over the course of disease.

**Immunophenotype**
Myeloid blasts CD34+.

**Cytogenetics**
- 48% positive for deletion of chromosome 5q.
- 43% positive for monosomy of chromosome 7.
- 52% of cells show three copies of MECOM, suggesting partial or complete trisomy of chromosome 3.

**Molecular studies**
- 

**Proposed diagnosis**
MDS-EB2 at diagnosis

**Interesting feature(s) of submitted case**
Interesting morphology of the second biopsy (slides) with almost anaplastic erythopoiesis. The decreasing blast count indicates good treatment response with parallel worsening of cytomorphology. 5q- megakaryocyte morphology.

Poor risk cytogenetic features including 5q-, but additional gains and losses therefore not MDS with 5q-.

**Submitted by**
Dr Livia Raso-Barnett

**Institute**
Haematopathology and Oncology Diagnostic Service (HODS), Cambridge University Hospital NHS Trust

**Address**
Addenbrooke's Hospital, Cambridge

**Country**
United Kingdom

**Panel Diagnosis**
# Case 316

**Workshop**

III Overt MDS

**Title**

MDS associated with del(12p)

## Clinical History

The patient is a 64-year-old male who presented with fatigue and weight loss. Found to have pancytopenia including severe anemia (initial Hgb at outside hospital of 5.1). History of Hodgkin lymphoma in 1998, status-post surgical resection and local radiation to the parotid gland area (no chemotherapy received).

## Morphological findings

### Peripheral Blood

Pancytopenia is evident. Occasional nucleated red cells and few blasts are noted. Red cell morphology shows anisopoikilocytosis including macroovalocytes and teardrop forms. Occasional hypersegmented and hypogranular neutrophils are noted. Platelets are evenly dispersed and no platelet clumps are identified.

### Aspirate

The aspirate is cellular with adequate spicules. An increased proportion of medium-sized to large blasts with prominent nucleoli and scant to moderate cytoplasm are noted. Erythroid maturation shows overt dyserythropoiesis. Myeloid maturation is left-shifted and few neutrophils are seen. Dysgranulopoiesis is evident. Megakaryocytes are decreased in number and dysplastic forms with hypolobulated or disjointed nuclei are seen. A 500-cell differential cell count shows: 6% blasts (range 5-10%), 48% erythroid precursors, 24% maturing myeloid precursors, 3% eosinophils, <1% monocytes, 18% lymphocytes and <1% plasma cells. The M:E ratio is 0.6:1.0. Iron stain: Reticuloendothelial storage iron is scant. Rare ring sideroblasts are identified.

### Biopsy

The marrow is mildly hypercellular for age (50-70% ratio of hematopoietic cells to fat). Myeloid maturation is shifted towards immature forms. Megakaryocytes with hypo-, hyperlobulated, or bare nuclei are seen. No lymphoid aggregates are identified. Reticulin: Slightly increased loose network of fine reticulin fibers.

## Immunophenotype

Increased number of single, dispersed CD34+ blasts, representing approximately 10% of total cellularity. No sheets or large clusters of CD34+ blasts are seen.

Flow cytometry: 15% blasts positive for CD34, HLA-DR, CD38, CD33 and CD117, and partial CD13

## Cytogenetics

Abnormal male karyotype: 17/20 metaphases showed an interstitial deletion in the short arm of one chromosome 12 (bands p11.2 to p13). No other chromosome abnormality was identified.

FISH: 3.5% of cells suggesting 7q- abnormality (slightly exceeds laboratory cutoff of 2.3%); No evidence of monosomy 5 or deletion 5q, trisomy 8, or deletion 20q

## Molecular studies

Not applicable

## Proposed diagnosis

MDS-EB (associated with del[12p])

## Interesting feature(s) of submitted case
The interstitial deletion in the short arm of the chromosome 12 [del(12p)] is a well-known, recurrent chromosome abnormality in MDS, detected in about 1-3% of cases. The frequency of del(12p) may be underestimated in MDS. Association with a complex karyotype is more common than del(12p) as a sole abnormality.

Genes in this deleted area include ETV and KRAS, which are known recurrent mutations in MDS. Of note, ETV6 deletion is a common additional abnormality in patients with MDS and monosomy 7. A subsequent karyotype in this patient confirmed monosomy 7.

Given the reported history of Hodgkin lymphoma treated with local radiation therapy, therapy-related MDS was also considered. It is unlikely this case represents therapy related MDS, but this possibility cannot be unequivocally excluded. Radiation therapy for Hodgkin lymphoma has changed over the years and the risk of second cancers with radiation therapy has been reduced.

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<tr>
<th>Submitted by</th>
<th>Enrique Ballesteros M.D.</th>
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<tbody>
<tr>
<td>Institute</td>
<td>University of Connecticut</td>
</tr>
<tr>
<td>Address</td>
<td>Department of Pathology and Laboratory Medicine UCONN Health Farmington, Connecticut</td>
</tr>
<tr>
<td>Country</td>
<td>United States</td>
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Panel Diagnosis
Case 317

**Workshop**

III Overt MDS

**Title**

MDS with excess of blasts-2 (RAEB2 WHO 2008) with fibrosis and p53 overexpression

**Clinical History**

70-year old previously healthy man presented with dyspnea in October 2016. Lab values showed severe pancytopenia: Hb 49 g/L, MCV 103 fL, TPK 6 x 10(9)/L, LPK 1,3 x 10(9)/L.

**Morphological findings**

Morphological findings: bone marrow trephine biopsy showed increased cellularity 50-80% and fibrosis grade 1 to 2. Megakaryocytes were unevenly distributed and dysplastic (mainly small and hypolobated). Granulopoiesis was well represented, showed maturation but also dysplastic forms and increase of blasts distributed diffusely and in small groups. Erythropoiesis was scarce, dysplastic and abnormally located. Smears were hemodiluted and showed 3% blasts, imprints 12% blasts. Three lineage dysplasia was observed.

**Immunophenotype**

Immunohistochemistry showed 15% blasts positive for CD117 and CD34 (subpopulation). Strong overexpression of p53 protein was seen in all the three lineages.

Flow cytometry of the bone marrow showed hemodiluted material with only 2% blasts positive for CD34, CD33, CD45dim with aberrant expression of CD56. Only short panel was performed due to limited material. Ogata score was 2.

**Cytogenetics**

Complex karyotype with loss of chromosome 17.

**Molecular studies**

NGS pending, complementary report will follow.

**Proposed diagnosis**

MDS with excess of blasts-2 (RAEB2 WHO 2008) with fibrosis and p53 overexpression.

**Interesting feature(s) of submitted case**

This case illustrates the importance of bone marrow biopsy in MDS with fibrosis where smears and flow cytometry underestimate blast frequency. Moreover p53 overexpression by IHC heralds already at early stage of the work-up the possibility of aberrancies of chromosome 17 and/or TP53 mutation.

**Submitted by**

Dr. Anna M Kwiecinska

**Institute**

Klinisk Patologi

**Address**

Skåne University Hospital Lund

**Country**

Sweden

**Co-Authors**

Anna Kwiecinska, Mats Ehinger, Vladimir Lazarovic, Anna Porwit.

**Panel Diagnosis**
Case 318

Workshop III Overt MDS

Title
Hypocellular myelodysplastic syndrome with multilineage dysplasia (associated with del 7q, ASXL1, U2AF1 and ATRX pathogenic mutations, red cell abnormalities, consistent with acquired alpha thalassemia, and clonal T cell expansion).

Clinical History
The patient is a 58 year old male who was found to have acquired microcytic anemia in Sept 2015. Hgb 8.4, MCV 81, RDW 42, platelets 178, WBC 4.3. Review of prior CBCs includes CBC performed at the University of Chicago in 11/93: Hgb 13.2, MCV 99.5, and at the OSH in 10/03: Hgb 14.1, MCV 99.7. The patient was treated with IV iron infusions. A Hgb electrophoresis showed Hgb A 87.4, A2 1.7, and a variant Hgb of 9.9% possibly representing Hgb H. Heinz body supravital staining of peripheral blood performed at the time of bone marrow biopsy (2/16) showed numerous HbH inclusions with characteristic “golf ball” configuration (20% of red cells). BM bx (2/16) showed hypocellular myelodysplastic syndrome with multilineage dysplasia associated with del(7)q(q22q36), ASXL1, U2AF1 and ATRX pathogenic mutations and red cell abnormalities, consistent with acquired alpha thalassemia. Follow up BM bx (5/16) showed similar morphologic findings but decrease in the marrow cellularity to ~ 10%. Additional molecular analysis performed at that time demonstrated monoclonal T cell expansion in a polyclonal background. The patient underwent MRD stem cell transplant with Flu/ Bu/Campath conditioning in 06/16. Six month BM bx revealed cytogenic relapse positive for (-7) and decreased chimerism but no clear morphologic relapse. The patient is currently on the DLI protocol.

Morphological findings
Diagnostic bone marrow biopsy from February 2016:

CBC at the time of BM bx (02/16) showed: WBC 2.9 K/ul, HgB 9.6 g/dL, MCV 88 fL (the patient was transfused), RDW markedly increased not reported, platelets 64 K/ul.

The peripheral blood smear shows leukopenia with low normal neutrophil count (ANC 1.76 K/ul). Some neutrophils show toxic granulations and occasional nuclear excrescences, however there is no significant dysgranulopoiesis. Red cells are normocytic, consistent with recent RBC transfusion, but show marked anisopoikilocytois, including a population of microcytic hypochromic red cells with numerous target cells, many red cell fragments, elliptocytes and occasional dacryocytes. Platelets are moderately to markedly reduced in number but show normal granulation. Some large platelets are noted.

The bone marrow biopsy is adequate in length for evaluation. The bone marrow is variably, overall mildly hypocellular (20-30% cellular). There is no appreciable increase in blasts. There is relative predominance of erythropoiesis and reduced granulopoiesis. Erythroid maturation is shifted towards immaturity with megaloblastic features and evidence of dyserythropoiesis Granulocytic maturation is also shifted towards immaturity but progressive to segmented neutrophils. Megakaryocytes are increased in number and dyspoietic, mostly small hypolobated forms and micromegakaryocytes. In addition, scattered interstitially small lymphocytes and several small lymphoid aggregates are noted. Reticulin stain shows mild (grade 1 of 3) increase in reticulin fibrosis.

The bone marrow aspirate smears reveal hypocellular spicules which essentially reflect findings of the bone marrow biopsy. Blasts are not increased (0.6% of the marrow cells on differential count). Erythroid precursors are relatively increased in number with maturation shifted towards immaturity, megaloblastoid features and evidence of dyserythropoiesis in form of nuclear budding, binucleation, cytoplasmic vacuolization and abnormal hemoglobinization. Granulopoiesis is reduced with maturation shifted towards immaturity but does show progressive maturation to segmented neutrophils. Some mature neutrophils show mild dyspoietic features in forms of nuclear excrescences or prominent primary granules; however there is no significant dysgranulopoiesis. Megakaryocytes are increased and
Dysplastic, mostly small hypolobated. Numerous micromegakaryocytes are noted. An iron stain (Prussian blue) shows increased storage iron. Occasional ring sideroblasts are identified (<7% of the erythroid precursors; 500 erythroid precursors counted).

### Immunophenotype

**Flow cytometry analysis:**

Not performed.

**Immunohistochemistry:**

Immunostains performed on BM core biopsy for immunophenotypic analysis included CD34, Glyco C, MPO, CD61 CD3, CD8, CD4, TIA-1, CD57, CD20, and CD138. CD34 shows no increase in blasts and demonstrates occasional scattered singly CD34+ cells, accounting for ~1% of the marrow cells. GlycoC demonstrates relatively increased erythropoiesis accounting for 40-60% of the marrow cellularity. MPO demonstrates reduced granulopoiesis accounting for 10-20% of the marrow cellularity. CD61 demonstrates markedly increased small dyspoietic megakaryocytes and numerous micromegakaryocytes. CD3 demonstrates numerous small T cells dispersed interstitially and some in intrasinusoidal distribution, (15-20% of the marrow cellularity). CD20+ demonstrates scattered small B cells (5-10% of the marrow cells). In addition, CD3 and CD20 demonstrate that the lymphoid aggregates are comprised of a mixture of CD3+ T cells and CD20+ B cells. Further immunophenotypic analysis of T cells demonstrates that T cells are comprised predominantly CD8+, variably TIA-1+ T cells (~15% of the marrow cells), whereas lymphoid aggregates are comprised predominantly of CD4+ T cells. CD57 demonstrate positivity with occasional lymphocytes. CD138 demonstrates scattered small plasma cells (<5% of the marrow cells) frequently seen in perivascular distribution consistent with their reactive nature.

### Cytogenetics

Demonstrated abnormal mosaic male karyotype: Normal: 46,XY [90%], Clone 1: 46,XY, del(7)(q22q36)[10%]. Total cells examined: 20, imaged and analyzed: 20.

### Molecular studies

Onco Heme panel NGS molecular analysis demonstrated

Pathogenic Variant mutations:

- **ASXL1** c.2338C>T, p.Q780* (NM_015338.5),
- **U2AF1** c.470A>C, p.Q157P (NM_001025203.1),
- **ATRX** c.7016 del, p.T2339Kfs*4 (NM_000489.4)

Variants of Uncertain Clinical Significance:

- **MPL** c.1774C>T, p.A592* (NM_005373.2),
- **CBL** c.2269G>A, p.A757T (NM_005188.3),
- **RUNX1** c.512A>T, p.K1711 (NM_001754.4),
- **PHF6** c.418+G>A (NM_001015877.1).

Molecular analysis of TCRG and TCRB gene rearrangement with BIOMED-2 assay performed on subsequent follow up bone marrow biops in 05/2016 showed monoclonal TCRG and monoclonal TCRB peaks on a polyclonal background.

### Proposed diagnosis

Hypocellular myelodysplastic syndrome with multilineage dysplasia (associated with del 7q, ASXL1, U2AF1 and ATRX pathogenic mutations, red cell abnormalities, consistent with acquired alpha thalassemia, and clonal T cell expansion).

### Interesting feature(s) of submitted case

- This is a unique case showing combination of hypocellular myelodysplastic syndrome with
multilineage dysplasia with no increase of blasts, associated with del 7q, ASXL1, U2F1 and ATRX mutations, red cell abnormalities with hemoglobin H inclusions, consistent with acquired alpha thalassemia, and clonal cytotoxic T cell expansion.

- ATRX gene mutations are very rare in MDS and this case underscores awareness of an association between acquired microcytic anemia, characteristic red cell abnormalities with hemoglobin H inclusions, ATRX gene mutation and MDS (Ref 1-2).

- ATRX (alpha-thalassemia mental retardation X-linked protein) is a transcriptional regulator which belongs to the SWI/SNF family of chromatin remodeling proteins. ATRX is required for deposition of the histone variant H3.3 at telomeres and other genomic repeats. These interactions are important for maintaining silencing at these sites. In addition, ATRX undergoes cell cycle-dependent phosphorylation, which regulates its nuclear matrix and chromatin association, and suggests its involvement in the gene regulation at interphase and chromosomal segregation in mitosis (Ref 3-5).

- Spontaneous mutations in the ATRX gene induce a complex neurological syndrome that includes mild Alpha-Thalassemia, mental Retardation with facial anomalies, X-linked (ATRX syndrome) (Ref 6-7) Therefore, ATRX was initially described as a critical transcriptional regulator of globin gene expression.

- However, ATRX has recently emerged as a major epigenetic regulator of chromatin structure and function during mitosis and meiosis with important clinical implications for chromosome stability during development as well as neoplastic transformation. Hence, ATRX mutations associated with MDS may have important implications not only for abnormal regulation of alpha-globin gene expression but also for pathogenesis of associated MDS (Ref 8-9).

This case may also represent the neoplastic end of the grey zone between hypoplastic MDS with MDS associated genetic alterations, and clonal T cell expansion with immune mediated BM injury and aplastic anemia (AA) with immune mediated clonal evolution of MDS-associated cytogenetic abnormalities (del7q) and unfavorable MDS/AML associated gene mutations, recently identified in AA patients by next generation deep sequencing (ASXL1, less frequently U2F1 and in rare cases ATRX (Ref 10-12).

References
3. Wong LH et al. ATRX interacts with H3.3 in maintaining telomere structure integrity in pluripotent embryonic stem cells. Genome Research 2010; 20(3)L 351-60.


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<tr>
<th>Submitted by</th>
<th>Elizabeth M Hyjek MD, PhD</th>
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<tr>
<td>Institute</td>
<td>University of Chicago</td>
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<td>Address</td>
<td>Department of Pathology,</td>
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<td>Hematopathology Section</td>
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Panel Diagnosis
Case 319

Workshop III Overt MDS

Title: Atrophic gastritis with macrocytic anemia and MDS with isolated del (5q)

Clinical History
A 75-year-old man with hypothyreosis (on levothyroxine substitution); initially no hematological and neurological symptoms. Macrocytic anemia and leukopenia were diagnosed incidentally during an examination before an orthopaedic operation (at the end of 2015; normal blood count in 2011); subsequently, atrophic gastritis was diagnosed, and parenteral vitamin B12 substitution followed (initial serum value: 75 ng/l).

Blood count: leu 2,2 x 10^9/l; ery 2,49 x 10^12/l; Hb 92g/l; V(ery) 110 fl; HTC 0,273, plt 346 x 10^9/l; normal leukocyte differential count and serum folate; high ferritin 521 g/l, low transferrin 1,72 g/l; high erythropoetin (>200U/l)

After several months, the diagnosis of MDS with isolated del (5q) was established on BM morphological and cytogenetic examination; IPSS intermediate – 1 risk

Follow-up: The patient gradually developed fatigue, dyspnea, and became transfusion-dependent. Blood count remained similar to the initial values. Platelets increased over 500 (but after an operation). Treatment with lenalidomide has been planned.

Past history: herpes zoster; chlamydia pneumonia; diphtheria (1943). Numerous operations: cholecystectomy, tonsillectomy, cataracta bilat., varices of lower extremities; several subsequent orthopedic operations - for degenerative changes and/or trauma: both elbows (entesiopathy), right knee –meniscus, both knees - total endoprosthesis, both carpal tunnels, hernias of intervertebral discs (3x), right shoulder

Morphological findings
Bone marrow aspirate biopsy (May 2016; after vitamin B12 substitution - high vitamin B12 serum value: 1252 ng/l):
slightly hypercellular BM; dominant features: marked erythroid hyperplasia (normoblastic, and megaloblastic) and an increase in the number of megakaryocytes (often monolobate or hypolobate; size: smaller or normal). Granulocytic series not increased, maturation retained. Irregular architecture. Increased hemosiderin; no fibrosis (grade 0); benign lymphoid aggregates

BM trephine biopsy (September 2016, vitamin B12 serum value: 198 ng/l)
similar to the aspirate (from May 2016); no lymphoid aggregates

BM cytology (Sept. 2016):
erythropoiesis - megaloid (but not typical of pernicious anemia) and dysplastic (nucleocytoplasmic asynchrony, atypical nuclei, disorderly hemoglobinization)
granulopoiesis: maturation retained, mild dysplastic changes: hypogranulation, N/C asynchrony, hyposegmentation of some neutrophils; blasts not increased
numerous dysplastic megakaryocytes hypolobate round or oval nucleus, smaller blasts not increased (below 1%)

Immunophenotype: immunohistochemistry (aspirate and trephine):
glycophorin C: increased erythropoiesis; myeloperoxidase: granulocytic series; CD42b and CD61: numerous hypolobate megakaryocytes; CD34: positive only in the endothelium and rather scarce blasts; p53 strongly positive in approx. >1% of BM cells (aspirate May 2016)
benign lymphoid aggregates in the aspirate - a mix of B- and T- mature cells (CD20, CD79a; CD3, CD5; negative: cyclin D1, TdT; a small follicular dendritic cell meshwork - CD23; Ki-67 < 5%)
### Cytogenetics

**G-banding:** interstitial deletion of 5q  
**FISH:** pathological clone with EGR1 gene deletion (5q31).

- Karyotype: 46,XY,del(5)(q13q32-34)[13]/46,XY[7]  
- FISH ish del(5)(D5S23/D5S721+,EGR1-) [15/20]  
- nuc ish 5p15.2(D5S23/D5S721x2),5q31(EGR1x1)[54/100]

### Molecular studies

under investigation

### Proposed diagnosis:

MDS with isolated del(5q)

### Interesting feature(s) of submitted case

Mixed clinical and pathological features: simultaneous occurrence of hypothyreosis, atrophic gastritis with vitamin B12 deficiency, and macrocytic anemia (suggestive of pernicious anemia), and MDS with isolated del(5q).

- Atrophic gastritis with vitamin B12 deficiency may be considered the cause of macrocytic anemia clinically and might delay the dg. of MDS del(5q). Megaloblastic anemia caused by vitamin B12 deficiency is an important pathological differential diagnosis of MDS, too.
- MDS with isolated del(5q) is a discrete subtype of MDS. Generally, it shows a favorable prognosis and a good response to lenalidomide. A small number of cases may behave more aggressively and progress to AML. TP53 mutation (identified by immunohistochemistry or by sequencing) is present in up to 20% of cases and is associated with a greater risk of leukemic progression and worse lenalidomide response.
- MDS with isolated del(5q) has a female predominance (our patient is male). It typically shows macrocytic anemia (erythroid hypoplasia, megaloblastoid erythropoiesis described in the BM), normal or increased platelets (with increased and prominently mononuclear megakaryocytes in the BM), as seen in our case; but normal granulopoiesis and neutrophil count. Erythroid and granulocytic dysplasia is uncommon (in contrast to leukopenia and some dysplastic changes in the erythroid and granulocytic series in our case). There is no excess of blasts in the BM or blood. Aggregates of lymphocytes may be present (seen in our case).

### Submitted by

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<tr>
<th>Ludmila Boudova M. D., Ph. D., Assoc. Professor</th>
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<th>Institute</th>
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<tr>
<td>Department of Pathology</td>
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<td>University Hospital, Medical Faculty, Charles University</td>
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**Message:** results of BM molecular genetic studies (FFPE) and peripheral blood for a part of molecular genetic studies will be obtained in February before the panel meeting

### Panel Diagnosis