Therapy-Related Myeloid Neoplasms
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Abstract
Session 5 of 2007 Workshop of the Society for Hematopathology/European Association for Haematopathology focused on therapy-related myeloid neoplasms. This report discusses the diversity and relevance of clinical, pathologic, and genetic features and provides an update on the pathogenesis of these disorders. We highlight common diagnostic issues such as the differentiation between therapy-related myelodysplastic syndrome and therapy-related acute erythroid leukemia. As similar therapeutic interventions are frequently considered for patients with either of these diagnoses, in the current World Health Organization classification, regardless of morphologic presentation, therapy-related myeloid neoplasms are considered together as a unique clinicopathologic syndrome of therapy-related myelodysplastic syndrome/acute myeloid leukemia. Nevertheless, recognition of the diverse morphologic features is crucial as bone marrow morphologic examination remains the first and important step of patient evaluation. We also present examples of therapy-related acute myeloid leukemias with recurrent cytogenetic abnormalities. In these cases, the precise classification is clinically important because it is associated with distinct clinical outcome.

Therapy-related myeloid neoplasms (t-MDS [myelodysplastic syndrome]/AMLs [acute myeloid leukemias]) are arguably the most serious unpredictable long-term complication of therapeutic intervention. They represent 10% to 20% of acute leukemias, MDSs, and myelodysplastic/myeloproliferative neoplasms.1 They were first recognized in the 1970s, and, over the years, our knowledge in this field has expanded, providing insight into the diversity of genetic pathways leading to their development. The t-MDS/AMLs were first reported following combination chemotherapy and radiotherapy for malignant lymphoma and multiple myeloma.2-7 Currently, there is an equal distribution between cases arising after treatment of hematologic and nonhematologic malignancies. The latency period and clinical manifestations vary depending on the type of cytotoxic drug, cumulative dose, and dose intensity. Two most common subtypes of t-MDS/AML are characterized by distinct clinicopathologic features and are described in the following section.

Etiology and Clinical Characteristics
A variety of cytotoxic agents have been implicated in the development of t-MDS/AMLs [Table I]. The most common culprits include alkylating agents and topoisomerase II inhibitors, which are responsible for 2 syndromes with distinct clinical manifestations. In addition, therapy with antimetabolites such as methotrexate, 6-mercaptopurine, and fludarabine has been linked to the development of t-MDS/AMLs.

Alkylating agents are often combined with other drugs or with radiotherapy and are also used for myeloablation. t-MDS related to previous exposure to the alkylating agents

[Table I]

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are typically associated with monosomy of chromosomes 5 and 7 or deletions of long arms of these chromosomes. The evolution of the disease is dependent on the cumulative dose of alkylating agent with a latency period of 5 to 10 years (range, 1-20 years), and its incidence increases with patient age.8,9 Initially, patients most commonly have bone marrow failure and morphologic features of MDS.

Another distinct type of therapy-related leukemia occurs after treatment with epipodophyllotoxins and other topoisomerase II inhibitors (Table 1). Approximately 30% of patients with t-MDS/AML present with this disease. The relative risk of leukemia following treatment with topoisomerase II inhibitors is related to the concurrent use of other drugs such as asparaginase and granulocyte colony-stimulating factor.10,11 Patients treated with these drugs frequently develop overt AML with no discernible myelodysplastic phase. These leukemias show features of monocytic differentiation with rearrangements of the MLL gene12 or show other recurrent cytogenetic abnormalities such as t(8;21), t(15;17), and inv(16).13 Other less common, recurrent, balanced cytogenetic abnormalities occurring in myeloid neoplasms associated with previous therapy include t(3;21q26, 11p15, t(9;22) (q34;q11), 12p13, and t(8;16)(p11;p13).14

In addition to the aforementioned classic examples, other drugs, such as platinum-based agents and histone deacetylase inhibitors (valproic acid), have been implicated in the development of t-MDS/AMLs.15 The contribution of hydroxyurea to the development of AML in chronic myeloproliferative neoplasms warrants further investigation.16,17

Even though initially t-MDS/AMLs have been described following treatment of hematopoietic malignancies and solid tumors, there is an increasing recognition of this malignancy occurring in the course of intensive treatment in patients with nonmalignant diseases9,18-21. The majority of the latter cases involve drugs that are frequently used to treat hematopoietic and solid tumors. There is accumulating evidence that the support of hematopoiesis with granulocyte colony-stimulating factor in patients treated with chemotherapy or in patients with severe congenital neutropenia is also related to an increased risk of therapy-related leukemia.22-24

**Pathogenesis**

During the last few decades, research focused on the genetic events central to the pathogenesis of human leukemias has led to critical advances in the diagnosis and treatment of hematopoietic neoplasms. Two types of genetic lesions have been shown to cooperate in the leukemogenesis of de novo AMLs.25 Class I mutations lead to constitutive activation of receptor tyrosine kinases or the downstream **RAS-BRAF-MEK-ERK** signal transduction pathway with resultant abnormalities in cell proliferation and survival. These are accompanied by class II mutations involving genes encoding for hematopoietic transcription factors that affect cellular differentiation. Similar pathways have been mapped in t-MDS/AMLs.26

Leukemias following alkylating agent therapy, which induces centromeric chromosome breakage, are characterized by complex karyotypes with the loss of entire chromosomes 5 and 7 or the loss of the long arms of these chromosomes. The primary mechanism of action is induction of double-stranded DNA breaks with a propensity to the centromeric and pericentromeric regions of chromosomes 1, 5, 7, 13, 17, 21, and 22.27 Two genetic pathways have been defined for this group of...
Czader and Orazi / Therapy-Related Myeloid Neoplasms

Table 2
Diseases Treated With Drugs Associated With Risk of Development of Therapy-Related Myeloid Neoplasms

<table>
<thead>
<tr>
<th>Group/Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic neoplasms</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>Chronic myeloproliferative neoplasms</td>
</tr>
<tr>
<td>Solid tumors</td>
</tr>
<tr>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
</tr>
<tr>
<td>Lung carcinoma</td>
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<tr>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>Testicular tumors</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
</tr>
<tr>
<td>Primitive neuroectodermal tumor</td>
</tr>
<tr>
<td>Neuroblastoma</td>
</tr>
<tr>
<td>Osteosarcoma</td>
</tr>
<tr>
<td>Wilms tumor</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
</tr>
<tr>
<td>Nonmalignant disorders</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Wegener granulomatosis</td>
</tr>
<tr>
<td>Seizure disorders</td>
</tr>
<tr>
<td>Psoriasis</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>Severe congenital neutropenia</td>
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Genetic Susceptibility to t-MDS/AMLs

In summary, there is accumulating evidence that t-MDS/AMls show similar cooperating genetic events to those seen in de novo AMLs. Thus, the advancement of knowledge regarding the pathogenesis of any of these 2 entities may become helpful to identify urgently needed targeted approaches for t-MDS/AMLs.

Maintenance of Genomic Stability Through Detoxification and DNA Repair

Two major enzyme systems are involved in detoxification: the cytochrome P450 family (phase I) and the metabolizing/conjugating enzymes involved in phase II metabolism. Several polymorphisms of enzymes of the cytochrome P450 system have been implicated in metabolizing cytotoxic drugs and environmental pollutants. CYP11 has a role in bioactivation of polycyclic aromatic polycarbons, and its polymorphism, CYP1A1*2A, is associated with increased enzyme expression. Even though the individual contribution of this polymorphism to the development of t-MDS/AMLs is questionable, in combination with polymorphisms of other enzymes, this variant was associated with an increased risk of t-MDS/AML.

Another enzyme of the cytochrome P450 system, CYP3A4, is involved in metabolism of numerous epipodophyllotoxins, cyclophosphamide, and vinblastine. The CYP3A4-V polymorphism has been suggested to lead to a decreased production and, ultimately, lower levels of the toxic metabolites of these drugs. Studies of pediatric therapy-related AML by Felix et al showed a significant difference in the frequency of this polymorphism between therapy-related and de novo cases. Similar results were obtained in the study by Rund et al. However, the significance of this association requires further study because it has been questioned in other reports.
Phase II drug-detoxifying enzymes are typically responsible for conjugation and enhanced secretion in bile or urine. These include sulfontransferase, glucuronosyltransferase, NAD(P)H:quinone oxidoreductase NQO1, epoxide hydrolase, glutathione S-transferase (GST), and N-acetyltransferases. Polymorphisms of these enzymes have been reported to be associated with the development of de novo and t-MDS/AMLs. Specifically, a higher degree of NQO1*2 polymorphism, in homozygous and heterozygous forms, was seen in patients with t-MDS/AML and was associated with abnormalities of chromosomes 5 and/or 7.40,45,46 Similarly, GSTs metabolize numerous cytotoxic drugs, including alkylators and topoisomerase II inhibitors, through conjugation to reduced glutathione. The main purpose is the prevention of DNA damage following cytotoxic therapy. There is a significant variation in the GSTs across the Caucasian population.57,48 Various GST polymorphisms, including GSTM1, GSTT1, and GSTP1, by themselves or in association with other polymorphisms of metabolic pathways were reported to be overrepresented in people with t-MDS/AMLs.40,49-51

DNA repair safeguards the integrity of the genome and prevents the persistence of potentially carcinogenic mutations and gene rearrangements. Large differences in the capacity to repair DNA damage exist between individuals.52 Double-strand DNA breaks are particularly important for genome integrity because they can result in cell death or gene rearrangements that can contribute to leukemogenesis. A high number of double-strand DNA breaks arises following chemotherapy or exposure to radiation. RAD51 is the central gene involved in the repair of double-strand breaks through adenosine triphosphate–dependent homologous recombination and, as such, is essential in the maintenance of genome stability.53 Polymorphisms in this gene and its paralog, XRCC3, were linked to susceptibility to breast cancer and bladder cancer.54,55 Further studies by Seedhouse et al56 linked RAD51 to an increased risk of developing t-MDS/AML. In addition, strong synergy was observed between RAD51 and XRCC3. Nucleotide excision repair is yet another pathway with a role in DNA repair after environmental exposure or after exposure to chemotherapy. Polymorphisms in one of the components of this pathway, the xeroferma pigmentosum complementation group D (XPD) gene, were reported to be associated with high-risk cytogenetic changes involving chromosomes 5 and 7 in AML arising after chemotherapy.57 Similarly, defects in the DNA mismatch repair genes as evidenced by microsatellite instability were described in t-MDS/AMLs arising in patients with prior malignancy and in organ-transplant recipients treated with azathioprine.58,59

In summary, our understanding of gene polymorphisms and other potential factors predisposing to therapy-related myeloid neoplasms is rapidly expanding. Even though select pathways have been implicated, we are far from being able to identify individuals with a higher risk of developing therapy-related leukemia. It is likely that multiple polymorphisms and lesions contribute to this risk; thus, techniques such as high-throughput gene arrays may be helpful to identify susceptibility phenotypes in a more comprehensive manner. Prototypes of such genome-wide approaches identified novel pathways and patients at high risk for t-MDS/AML.60,61 In addition, these studies discovered novel candidate genes implicated in secondary leukemogenesis.

Discussion of Workshop Cases

The session on t-MDS/AMLs included 13 cases. Originally, 10 cases were submitted to this session. Three additional cases were moved from the categories of myelodysplastic/myeloproliferative disease and mast cell disease owing to their association with previous therapeutic intervention. The workshop review panel agreed with the submitted diagnosis in all cases. In 1 case, a minor modification of the diagnosis was proposed. The following discussion is focused on specific morphologic and genetic categories and on groups of cases linked by a common diagnostic issue.

Morphologic Spectrum From Therapy-Related Myelodysplastic Syndrome to Acute Myeloid Leukemia With Erythroid Differentiation

t-MDS/AMLs have variable morphologic manifestations ranging from MDS and myelodysplastic/myeloproliferative disorder to acute leukemia. Four cases submitted to the workshop represented a morphologic spectrum spanning MDS and acute erythroid leukemia. Cases of t-MDS with significant populations of erythroid precursors (>50%) and cases fulfilling the criteria of acute erythroleukemia and pure erythroid leukemia are discussed.

Case 14 was t-MDS with a significant erythroid component. The patient was a 54-year-old man originally diagnosed with diffuse large B-cell lymphoma and treated with numerous courses of multiagent chemotherapy and radiation. Twelve years after the initial diagnosis, he developed severe pancytopenia. The first bone marrow sample was hypercellular with marked erythroid hyperplasia (myeloid/erythroid ratio, 0.6) and trilineage dyspoiesis. Image 1A1. Blasts constituted 1% of the differential count. The cytogenetic study showed a complex karyotype with monosomy 5 and 7. The patient was scheduled for a bone marrow transplant. The second bone marrow sample, obtained as a part of the pretransplantation evaluation, was again hypercellular with 66% erythroid precursors, trilineage dyspoiesis, and 24% blasts. When calculated as a percentage of myeloid cells, blasts represented 92% of the differential count. Presentation as MDS and rapid progression of the disease is frequently
seen in patients with complex karyotypes involving abnormalities of chromosomes 5 and 7 and previous treatment with alkylating agents.62

Case 34 demonstrated an evolution of MDS into erythroleukemia in a patient with a history of follicular lymphoma. The patient received several courses of therapy with alkylating agents, anthracyclines, and antimetabolites. Twelve years after the initial diagnosis and 7 years after the first course of chemotherapy, the patient developed pancytopenia. A peripheral blood smear showed pseudo–Pelger-Huët cells and occasional blasts. The bone marrow was markedly hypercellular (95%) with significant erythroid hyperplasia, trilineage dysplasia, and 10% blasts **Image 1B** and **Image 1C**. The reticulin stain showed 2+ reticulin fibrosis, a finding frequently encountered in cases of t-MDS/AML.63 Flow cytometric studies showed a granulocytic population with decreased side scatter consistent with hypogranularity and blasts expressing CD13, CD34, CD117, and CD7. Based on the morphologic features, this case was classified as t-MDS/AML with features of erythroleukemia. Cytogenetic analysis showed a complex karyotype with del(7)(q22), consistent with previous treatment with alkylating agents.
The extreme of the spectrum of t-MDS/AML with erythroid predominance is demonstrated by 2 additional cases of pure erythroid leukemia. Case 183 involved a 62-year-old man in whom pancytopenia developed 18 months after treatment for tonsillar squamous cell carcinoma. The patient received irradiation and chemotherapy, including paclitaxel and carboplatin. The bone marrow was hypercellular with marked erythroid hyperplasia, dyserythropoiesis including numerous vacuolated pronormoblasts, karyorrhexis, nuclear budding, mild myeloid dyspoiesis, and monolobated megakaryocytes. Myeloid blasts were not increased, a finding confirmed by flow cytometric studies and cytochemical staining for myeloperoxidase (<3% myeloperoxidase-positive blasts). Flow cytometric and immunohistochemical studies highlighted marked erythroid hyperplasia with numerous pronormoblasts. Cytogenetic analysis showed a complex karyotype with del(5)(q13q33). The development of secondary hematopoietic neoplasms after treatment with carboplatin and paclitaxel has been reported previously. In addition to cases manifesting as erythroleukemia with abnormalities of chromosomes 5 and/or 7, AML with inversion of chromosome 16 has been reported in this setting.

Case 206 was therapy-related pure erythroid leukemia. A 53-year-old man was originally diagnosed with mediastinal precursor T-lymphoblastic lymphoma. The patient was successfully treated; however, 44 months after the original diagnosis, he developed pancytopenia. The bone marrow showed marked erythroid predominance with sheets of proerythroblasts and was diagnosed as t-MDS with transformation into AML with pure erythroid differentiation. The karyotype was near-triploid with numerous complex abnormalities. Cases of therapy-related pure erythroid leukemia have been reported in association with alkylating agent treatment and, similar to the 2 cases described herein, showed complex karyotypes. These cases are most commonly associated with poor survival.

Therapy-Related Myelodysplastic/Myeloproliferative Neoplasms

Three cases submitted to the workshop fulfilled the morphologic criteria of myelodysplastic/myeloproliferative neoplasm. According to the literature, fewer than 5% of patients present with therapy-related myeloid neoplasms other than MDS or AML. Philadelphia chromosome–positive chronic myelogenous leukemia and various myelodysplastic/myeloproliferative diseases were previously reported in adult and pediatric patients. Various agents have been implicated, including alkylating agents, topoisomerase inhibitors, and platinum-based therapies.

Case 40 involved a 58-year-old man with a history of AML with inv(16)(p13q22) who, 6 years after induction therapy and autologous stem cell transplantation, presented with declining peripheral blood counts and monocytosis (WBC count, 3,500/μL [3.5 × 10^9/L] with 35% monocytes). Within a year, the patient developed microcytosis. Peripheral blood review demonstrated marked anisocytosis with a subpopulation of microcytic hypochromic RBCs and hemoglobin H inclusions on brilliant cresyl blue stain. The bone marrow was hypercellular with erythroid hyperplasia,
mild megaloblastoid change, and megakaryocytic hyperplasia with dysmegakaryopoiesis. The cyogenetic study showed monosomy 7. The workshop review panel agreed with the diagnosis of therapy-related myeloid neoplasm with acquired α-thalassemia and highlighted features of chronic myelomonocytic leukemia. The presence of α-thalassemia clones has been reported in approximately 8% of cases of de novo and t-MDS and 2.5% of chronic myeloproliferative disorders.72 Somatic mutations in α-thalassemia/mental retardation X-linked gene transactivating α-globin expression, and loss of the terminal portion of 16p containing α-globin genes have been implicated.73

Case 156 illustrates therapy-related myelodysplastic/myeloproliferative neoplasm following treatment of a non-neoplastic disease. A 49-year-old woman with a long-standing history of Wegener granulomatosis, previously treated with methotrexate and cyclophosphamide, had progressive anemia and thrombocytopenia. The WBC count was 10,700/μL (10.7 × 10⁹/L) with an absolute monocyte count of 5,200/μL (5.2 × 10⁹/L). The most striking features of the peripheral...
blood smear were monocytosis and dysgranulopoiesis. The bone marrow was hypercellular with 10% blasts, granulocytic hyperplasia with a left shift, and trilineage dyspoiesis. CD34 immunostain highlighted abnormal localization of immature precursor–like aggregates. Flow cytometric immunophenotyping demonstrated 8% myeloid blasts, 54% monocyes with partial coexpression of CD2, and hypogranulation of granulocytic series. Cyto genetic studies demonstrated monosomy 7 and trisomy 21.

The occurrence of t-MDS/AMLs in patients with nonneoplastic disorders treated with chemotherapeutic agents is a well-known phenomenon. A recent report by Faurschou et al described an increased incidence of AML in patients with Wegener granulomatosis treated with high doses of cyclophosphamide. t-MDS/AMLs have been previously reported in patients with rheumatoid arthritis, Behçet syndrome, polymyositis, idiopathic thrombocytopenic purpura, and renal allografts. The patients are more likely to present with t-MDS vs AML. They also show longer latency periods and frequent involvement of chromosomes 5 and 7, similar to other t-MDS/AML cases associated with treatment with alkylating agents. The prognosis is not significantly different from t-MDS/AMLs arising in patients treated for malignant diseases.

Finally, case 32 illustrates an interesting progression of a therapy-related myeloid neoplasm in a patient previously treated for Hodgkin lymphoma and subsequent diffuse large B-cell lymphoma. Twelve years after the original diagnosis, the patient presented with anemia and thrombocytopenia and with bone marrow morphologic features consistent with t-MDS. This initial marrow sample showed dyserythropoiesis and dysmegakaryopoiesis, along with deletion of 5q and abnormalities of the long arm of chromosome 7. A subsequent bone marrow sample demonstrated persistent erythroid and megakaryocytic dyspoiesis and a new finding of myeloid hyperplasia with eosinophilia and basophilia. These characteristics were reflected in the peripheral blood counts showing neutrophilia, marked eosinophilia, basophilia, and mild monocytosis. At that time, the cytogenetic study demonstrated additional karyotypic abnormalities consistent with clonal evolution. BCR-ABL rearrangement and JAK2 mutation were not detected. Thus, in the course of the disease, morphologic features of this case changed from those of an MDS to those seen in myelodysplastic/myeloproliferative neoplasms. This change was accompanied by a clonal evolution as evidenced by the emergence of new chromosomal abnormalities.

The appreciation of the variability in the morphologic features of t-MDS/AMLs is critical for the appropriate diagnosis of these disorders. However, there is accumulating evidence that their precise morphologic subclassification may be of little clinical significance. The degree of dysplasia and even blast percentage did not add any significant prognostic information in a cohort reviewed in this study. In addition, only a borderline difference in overall survival was noted between cases of therapy-related MDS and therapy-related AML, questioning the importance of classifying these diseases based on a 20% blast cutoff point. The most important prognostic information was derived from the cytogenetic data. Cases with complex karyotypes (3 or more chromosomal abnormalities) had significantly shorter survival. Similarly, the poor-risk group, as defined by International Prognostic Scoring System, showed shorter survival compared with the low- and intermediate-risk groups. These data led to the merger of the t-MDS/AMLs under one category in the current World Health Organization classification of hematopoietic and lymphoid neoplasms.

**t-MDS/AMLs With Recurrent Cytogenetic Abnormalities**

The presence of balanced cytogenetic abnormalities in therapy-related acute leukemias and their favorable impact on patient prognosis has long been recognized. The number of therapy-related acute leukemias with recurrent cytogenetic abnormalities is growing owing to their close association with topoisomerase II inhibitors, which are currently commonly used in the treatment protocols. These cases represent approximately 10% of all t-MDS/AMLs and include cases with the abnormalities of 11q23, t(8;21), inv(16), t(15;17), t(9;22), and 11p15 (NUP98).

Several instructive cases of therapy-related acute leukemias with recurrent cytogenetic abnormalities were submitted to our workshop. Case 188 involved a 63-year-old man treated with radiation, ifosfamide, doxorubicin, and eteineascidin 743 (ET-743) for metastatic malignant fibrous histiocytoma. Four years after the start of therapy, the patient developed marked pancytopenia. There were no blasts in the peripheral blood smear. The bone marrow was hypercellular with 6% blasts and 46% promyelocytes. Flow cytometric studies showed a population of promyelocytes that expressed CD33, CD31, and CD117 and were negative for CD34, HLA-DR, and CD15. Karyotypic analysis confirmed the presence of t(15;17)(q22;q21). Numerous cases of therapy-related acute promyelocytic leukemia (t-APL) have been reported in the literature. The t-APL represents approximately 20% of all cases of acute promyelocytic leukemia. It most commonly follows treatment of breast carcinoma, presumably owing to a higher number of patients receiving anthracycline-based therapies. Mistry et al established the relationship between topoisomerase II inhibitor treatment and t-APL through the demonstration of breakpoint clustering in intron 6 of the PML and in RARA genes after exposure to mitoxantrone. As illustrated by case 188, the majority of cases develop fewer than 3 years after the primary therapy. There is no preleukemic phase, and the presentation and outcome are generally similar to cases of de novo APL.
Case 89 illustrated an interesting association of therapy-related AML with t(8;21)(q22;q22) and systemic mastocytosis (systemic mastocytosis and associated hematologic non–mast cell lineage disease). The patient was a 54-year-old woman treated with doxorubicin, paclitaxel, anastrozole, and involved-field radiation for breast carcinoma. One year after therapy, the patient developed AML with t(8;21)(q22;q22). At that time, flow cytometric studies showed expression of dim CD33, CD34, dim CD117, and HLA-DR. The patient underwent induction with daunorubicin and cytarabine and consolidation with 3 cycles of high-dose cytarabine. The following bone marrow examination showed persistent AML and numerous mast cells in perivascular and peritrabecular aggregates coexpressing CD25. The KIT D816V mutation was detected in the postconsolidation bone marrow sample. The presence of mast cell aggregates composed of spindle-shaped mast cells displaying aberrant coexpression of CD25 supported the diagnosis of systemic mastocytosis. The KIT D816V mutation was detected in the whole bone marrow preparation.

The association of systemic mastocytosis with MDSs and myeloproliferative neoplasms is well recognized. Among AMLs, AML with t(8;21)(q22;q22) has been reported to be associated with systemic mastocytosis more frequently than other types of AML. Anecdotally, these cases are frequently associated with a worse prognosis in comparison with cases of AML with AML1-ETO without associated systemic mastocytosis (verbal communication, Russell K. Brynes, MD, University of Southern California, Los Angeles). Mast cell aggregates may be detected in the bone marrow at the time of initial diagnosis of AML or later in the course of the disease. Frequently, the mast cell population persists after induction.
chemotherapy and throughout the course of the treatment, at which point it may be more apparent morphologically. Close attention to morphologic detail is critical. Addition of immunohistochemical stains is essential to recognize the mast cell aggregates and spindle-shaped mast cells and confirm their aberrant immunophenotype. Unfortunately, activating mutations at codon 816 can be detected in approximately 10% to 30% of patients with AML with \textit{AML1-ETO}; thus, the presence of a \textit{KIT} mutation can only be considered supportive of the diagnosis of systemic mastocytosis when mutational analysis is performed on the isolated population of mast cells. The mechanism underlying the association between AML with \textit{AML1-ETO} and the treatment with topoisomerase II inhibitors has been elegantly described by Zhang et al,\textsuperscript{32} who mapped genomic DNA breakpoints in \textit{AML1} and \textit{ETO} to topoisomerase II DNA cleavage and DNase I hypersensitive sites.

Another entity frequently associated with topoisomerase II inhibitor therapy is AML with 11q23 abnormalities.\textsuperscript{12} This is the most frequent entity in the group of therapy-related leukemias with balanced chromosomal aberrations. The most common translocation partners include 9p22, 19p13.3, 19p13.1, 4q21, and 6q27. Case 86 is a classic example of this entity. The patient was a 22-year-old woman originally given a diagnosis of Ewing sarcoma and treated with combination chemotherapy, including vincristine, cyclophosphamide, and doxorubicin. She remained in complete remission for 2 years and at relapse had disseminated disease, which was treated with cyclophosphamide, etoposide, and combination high-dose idarubicin, cyclophosphamide, and etoposide chemotherapy. Five years after the original diagnosis, the patient presented with pancytopenia and 48% monocytes. The bone marrow was normocellular with trilineage hematopoiesis, numerous monoblasts and promonocytes representing more than 20% of marrow cellularity, and focal aggregates of metastatic Ewing sarcoma. Image 4A and Image 4B. Cytogenetic analysis showed involvement of the \textit{MLL} gene [46,XY,t(9;11)(p22;q23)]. In addition, 2 cells showed t(6;11;22)(q15;q24;q12), consistent with involvement by metastatic Ewing sarcoma.

In previous studies, patients developing t-MDS/AMLs with rearrangement of 11q23 more commonly presented with overt AML. Less frequently, this entity can present as MDS or acute lymphoblastic leukemia. Despite frequent bone marrow transplantation, t-AML with involvement of 11q23 showed shorter survival compared with other therapy-related acute leukemias with recurrent cytogenetic abnormalities.\textsuperscript{12}

Another rare example of therapy-related AML with a recurrent cytogenetic abnormality was case 176. The patient was a 43-year-old man originally diagnosed with AML, not otherwise specified (French-American-British classification, AML-M4) in 2003. At that time, cytogenetic analysis showed isolated trisomy 21. The patient received induction chemotherapy and autologous bone marrow transplant after conditioning with high-dose busulfan and etoposide. One year after transplantation, anemia and thrombocytopenia developed. Rare blasts with Auer rods and accompanying dysplastic granulocytes were seen on the peripheral blood smear. The
bone marrow was hypercellular with striking megakaryocytic hyperplasia, dysmegakaryopoiesis, prominent erythroid dysplasia, and 9% blasts [Image 4C] and [Image 4D]. The karyotype was complex with t(9;22)(q34;q11.2) seen in 15 metaphases. Two weeks after bone marrow biopsy, the patient had worsening fatigue and 48% blasts on peripheral blood smear. The patient died early in the course of reinduction and treatment with imatinib mesylate.

Rare cases of therapy-related AML, ALL, and chronic myelogenous leukemia with Philadelphia chromosome have been reported following DNA topoisomerase II inhibitors.\textsuperscript{14,86-88} Cases with the original leukemic clone and an acquisition of the Philadelphia chromosome as a secondary change and cases with new clones with distinctly different karyotypes that included t(9;22) were reported.

Another recurrent cytogenetic abnormality commonly occurring in t-MDS/AMLs is involvement of chromosome 3q21q26/\textit{EVI1}. This abnormality was first recognized in de novo AMLs and MDSs and later reported in acute leukemias arising in the context of MDS, blast crisis of chronic myelogenous leukemia, and t-MDS.\textsuperscript{89-93} The 3q21q26/\textit{EVI1} syndrome is associated with normal to elevated platelet counts, rapid

\textbf{Image 4I} (cont) \textbf{C} and \textbf{D}, Case 176, Therapy-related myelodysplastic syndrome/acute myeloid leukemia (AML) with Philadelphia chromosome showing hypercellular bone marrow with megakaryocytic hyperplasia, dysmegakaryopoiesis, prominent erythroid dysplasia, and 9% blasts (\textbf{C}, H&E; \textbf{D}, Wright-Giemsa). Contributed by C.P. Soupir and R.P. Hasserjian. \textbf{E}, Case 144, Therapy-related AML with t(3;3)(q21;q26) accompanied by persistent chronic lymphocytic leukemia; peripheral blood with lymphocytosis and smudge cells (Wright). \textbf{F}, Case 144, Dysplastic megakaryocytes with hypolobation and separated nuclear lobes typical for 3q21q26/\textit{EVI1} syndrome seen in a bone marrow aspirate smear (Wright-Giemsa). Contributed by C.C. Massey and colleagues.
Case 57 involved a 39-year-old woman with pancytopenia. The patient initially sought care at 34 years of age with fatigue, weight loss, severe hypertension, and headaches. The presence of Bence Jones protein and renal failure due to light chain nephropathy led to bone marrow examination and diagnosis of plasma cell myeloma. The patient underwent intensive treatment with dexamethasone, thalidomide, and cyclophosphamide followed by nonmyeloablative bone marrow transplantation, with the patient’s brother being the bone marrow donor. Plasma cell myeloma persisted, and further therapy with bortezomib, dexamethasone, melphalan, and doxorubicin was given. Four years after initial diagnosis, the bone marrow examination showed MDS (refractory anemia with excess blasts 2) and persistent plasma cell myeloma. The patient underwent further treatment with lenalidomide and azacitidine. Progressive pancytopenia was noted with an increase in peripheral blood blasts to 10%. A subsequent bone marrow biopsy sample was markedly hypercellular with numerous blasts (59%) and large atypical plasma cells with \( \lambda \) light chain restriction. Fluorescence in situ hybridization and conventional karyotyping showed the presence of XY chromosomes consistent with donor-origin of therapy-related AML.

Various hypotheses regarding leukemic transformation occurring in bone marrow donor cells have been proposed, including increased donor susceptibility to chemotherapy if given in the posttransplantation course. However, in a proportion of donor-derived leukemias, no additional cytotoxic therapy was given. In those cases, the impact of the bone marrow microenvironment and defective immune surveillance
may play a role. In the current case, the exposure of donor marrow to an alkylating agent and anthracycline supports the diagnosis of a therapy-related myeloid neoplasm. Of note, the bone marrow donor in the current case remains healthy and free of any detectable hematologic abnormalities.

Conclusions

The cases submitted to the session on therapy-related myeloid neoplasms represented a full spectrum of clinicopathologic manifestations of these disorders. Review of these cases further defined the role of morphologic examination and cytogenetics in the diagnosis and illustrated the diagnostic approach recommended by the most recent World Health Organization classification of hematopoietic neoplasms published in September 2008. In addition, many rare and unique entities were discussed showing the substantial diversity of therapy-related myeloid neoplasms.

References


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Czader and Orazi / Therapy-Related Myeloid Neoplasms


