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REVIEW ARTICLE

THERAPY-RELATED MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA: ETIOLOGY, PROGNOSIS AND TREATMENT

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SUMMARY

Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are a group of heterogeneous disorders characterized by abnormal hematopoiesis of myeloid cells, uni- or multilineage peripheral cytopenia, marrow dysplasia and cytogenetic abnormalities. Collectively, these disorders are referred to as myeloid neoplasms. When AML or MDS results from prior therapy, usually chemotherapy or radiation, it is referred to as therapy-related MDS/AML, or more generally, therapy-related myeloid neoplasms. Treatments for therapy-related myeloid neoplasms are often ineffective and the prognosis is poor, as evidenced by a median survival of 6-12 months after diagnosis. A number of causative agents, including alkylating agents and topoisomerase inhibitors, are discussed, along with common primary malignancies, the treatment of which increases the risk of developing therapy-related myeloid neoplasms. Investigation of the molecular basis of therapy-related myeloid neoplasms has revealed numerous polymorphisms and cytogenetic abnormalities that are associated with the development and prognosis of these neoplasms. An overview of these polymorphisms and cytogenetic abnormalities is provided, along with current therapeutic strategies.

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INTRODUCTION

Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) constitute a group of heterogeneous disorders characterized by abnormal hematopoiesis of myeloid cells, uni- or multilineage peripheral cytopenia, marrow dysplasia and cytogenetic abnormalities. Collectively, these disorders are referred to as myeloid neoplasms. Both AML and MDS may be classified as primary, or de novo, or secondary. Secondary AML or MDS, when associated with prior exposure to mutagenic agents like chemotherapy or radiation, is termed therapy-related MDS/AML and placed in a distinct subcategory under the diagnosis of AML. This subgroup reflects the belief that therapy-related MDS and AML lie on the same disease continuum, the difference being merely a reflection of marrow blast percentage. Individuals diagnosed with therapy-related MDS have a greater risk and more rapid progression to AML than those with de novo disease. Therapy-related MDS/AML is associated with similar cytogenetic abnormalities, greater resistance to traditional therapies and a more dismal prognosis than de novo MDS or AML (1-4).

In 2008, an estimated 13,000 people were diagnosed with AML and another 10,000 with MDS in the U.S. (5-7). Since some cases of therapy-related MDS/AML may incorrectly be identified as de novo, it is not exactly clear how many cases are therapy-related; however, it has been estimated that 15% of MDS and AML cases are actually therapy-related (8). The incidence of therapy-related MDS/AML continues to increase, particularly in survivors of primary cancers and those exposed to high cumulative doses of chemotherapy, radiation and occupational carcinogens like benzene (9).

The French–American–British (FAB) classification was introduced in the late 1970s as a means of defining hematopoietic malignancies (10, 11). However, it did not distinguish between de novo and therapy-related MDS or AML, and cases of the latter were placed into the best-fitting category at the discretion of the clinician. In the late 1990s, the World Health Organization (WHO) proposed updated

diagnostic classifications, first for AML and then for MDS, that expanded the morphological-based classifications of the FAB group and included cytogenetic and immunophenotypic expression criteria (12, 13). The WHO classification included additional diagnostic categories for MDS, and a distinct category for therapy-related MDS/AML. The category of therapy-related MDS/AML was further divided into two subcategories: alkylating agent/radiation-related type and topoisomerase II inhibitor-related type (13), based on clinical progression and cytogenetic abnormalities associated with prior therapy. The most recent WHO classification in 2008 introduced a change in the nomenclature for myeloproliferative diseases, replacing the term "diseases" with "neoplasms", to more accurately reflect their neoplastic nature (14). The classification keeps therapy-related MDS/AML as a distinct subgroup within AML, but they are now referred to collectively as therapy-related myeloid neoplasms to indicate that they essentially constitute a single biological disease.

The International Prognostic Scoring System (IPSS) was developed in the late 1990s to aid in the evaluation of clinical outcomes in patients presenting with de novo MDS (15). Factors that were determined to increase a patient's risk for progression to AML included the percentage of bone marrow blasts, the number of cytopenias present and cytogenetic abnormalities. In general, however, while the IPSS has been validated for its use in de novo MDS, it has been criticized for its exclusion of therapy-related MDS in the prognostic model. A new risk model that is applicable to all subtypes of MDS, including those excluded from the IPSS model, has identified several additional independent negative prognostic factors, including poor performance, older age, thrombocytopenia, anemia, increased marrow blast count, leukocytosis, abnormalities of chromosome 7 or complex karyotype and prior transfusions (16). The usefulness of karyotype in particular in the prognosis of therapy-related myeloid neoplasms is an intense area of research. For example, Singh and colleagues showed significant survival differences based on the karyotypic complexity (2). The prognostic importance of cytogenetics for predicting response to treatment, relapse and overall survival in patients with de novo or therapy-related disease is unparalleled.

THERAPIES IMPLICATED IN THE DEVELOPMENT OF THERAPY-RELATED MYELOID NEOPLASMS

The majority of cases of therapy-related myeloid neoplasms are associated with exposure to alkylating agents, radiation or topoisomerase inhibitors (17, 18). As overall survival from primary malignancies improves and new therapies are used, new cases of therapy-related myeloid neoplasms emerge. The increased risk and incidence of therapy-related myeloid neoplasms is also associated with the use of granulocyte colony-stimulating factor (G-CSF) and autologous stem cell transplantation (SCT), as well as antimetabolites (i.e., azathioprine). These and other therapies will be discussed in this section.

Alkylating agent-induced therapy-related myeloid neoplasms

Classic alkylating agents such as melphalan, cyclophosphamide and nitrogen mustard have long been associated with the development of therapy-related myeloid neoplasms (19-21). Alkylating agent-induced therapy-related myeloid neoplasms clinically resemble MDS more than AML, with a mean onset of 5-7 years after exposure to alkylating agents, and an increased risk with greater cumulative exposure (22). Therapy with alkylating agents and/or radiation often results in unbalances, losses or deletions of chromosomes 5 and 7 (8, 23-25).

Cases of alkylating agent-induced therapy-related myeloid neoplasms can roughly be divided into two different cytogenetic groupings (26). The first group includes cases with abnormalities of chromosome 7 (–7/–7q) with normal chromosome 5. This group is often associated with mutations of runt-related transcription factor 1 (*RUNX1* or *AML1*), which encodes a hematopoietic transcription factor implicated in the regulation of thrombopoietin-mediated proliferation (27-29). Similar *RUNX1* point mutations were reported in 7 of 18 patients exposed to ionizing radiation from a nuclear test site (30). The second group includes cases with deletion or loss of the long arm of chromosome 5 with or without normal chromosome 7. Deletions of chromosome 5 or –5q are associated with mutations of the tumor suppressor gene *TP53*, which may promote leukemogenesis (24, 27, 31-32). This group is associated with a complex karyotype and poor prognosis as compared to those with abnormalities of chromosome 7 (27, 28).

Topoisomerase II inhibitor-induced therapy-related myeloid neoplasms

Topoisomerase II inhibitors are a mainstay in numerous cancer treatment protocols. By the 1980s, however, the use of topoisomerase II inhibitors had emerged as a second cause of therapy-related myeloid neoplasms (17, 33). There are two types of topoisomerase enzymes: topoisomerase I and II. Topoisomerase II inhibitors interfere with the re-annealing of DNA strands after tension on the replicating strand is relieved. They can be intercalating (i.e., anthracyclines such as doxorubicin) and nonintercalating (i.e., epipodophyllotoxins such as etoposide). The final result is DNA strand breakage and subsequent cellular apoptosis. Topoisomerase I inhibitors act in a different manner by interfering with DNA replication (17, 23, 34).

Topoisomerase II inhibitor-induced therapy-related myeloid neoplasms have a clinical presentation and cytogenetic pattern that are distinct from alkylating agent-induced therapy-related myeloid neoplasms (Table I). Compared to alkylating agent-induced therapy-induced myeloid neoplasms, topoisomerase II inhibitor-induced therapy-related myeloid neoplasms are more aggressive, as reflected by high blast counts, and are more similar to de novo AML than de novo MDS (22). Cases of therapy-related myeloid neoplasms occurring after exposure to topoisomerase II inhibitors have a relatively short latency period of 1-3 years, compared to the 5- to 7-year latency period observed after exposure to alkylating agents (17). These differences in latency period may reflect the types of cytogenetic abnormalities that are common to each subcategory of therapy-related myeloid neoplasms, with leukemogenesis occurring more rapidly as a result of dominant translocations observed after exposure to topoisomerase II inhibitors as, opposed to deletions and monosomy common after exposure to alkylating agents (22, 35).

Common cytogenetic findings in cases of topoisomerase II inhibitor-induced therapy-related myeloid neoplasms are balanced translocations involving chromosome bands 11q23 (*MLL*) and 21q22 (*RUNX1*), or inversion of chromosome 16 [inv (16)] (36-40). Patients with translocations involving 11q23 in particular were found to have

Table I. *Alkylating agent- vs. topoisomerase II inhibitor-induced therapy-related myeloid neoplasms*

Alkylating agent	Topoisomerase II inhibitor
Resembles MDS > AML	More aggressive, high blast count. More similar to de novo AML > MDS
5-7 years after exposure	1-3 years after exposure
Usually accompanied by chromosomal deletions and monosomy Chromosome 5: mutation in *TP53* Chromosome 7: mutation in *AML1*	Translocations: 11q23 (*MLL*), 21q22 (*AML1*, inv [16])

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.

significantly shorter median survival times than patients with 21q22 translocations or inv(10) and t(15:17) (39). It is postulated that DNA breakage activates nonhomologous end-joining in an attempt to re-anneal the damaged DNA, causing translocations that are responsible for leukemogenesis (17, 41-43).

Recently, Azarova and colleagues used an animal model to study the effects of two topoisomerase II isoenzymes, topoisomerase II α and topoisomerase II β, in etoposide-induced secondary malignancies (44). It was shown that *top2*β knockout mice had a significantly lower rate of etoposide-induced melanoma compared to *top2*β^+ mice. These results suggest that topoisomerase II β inhibition is responsible for the leukemogenic effects of etoposide. DNA translocations and double-strand breaks were shown to be topoisomerase II β-dependent, whereas topoisomerase II α was responsible for cytotoxicity, and therefore the chemotherapeutic activity, of etoposide. Based on the knowledge that topoisomerase II α is preferentially cytotoxic whereas topoisomerase II β is more leukemogenic, a novel topoisomerase II α inhibitor, NK-314, was developed. NK-314 is a synthetic benzo[*c*]phenanthridine alkaloid that inhibits topoisomerase II α activity in vitro and in vivo by inducing double-strand DNA breaks and activation of the G_2 DNA damage checkpoint (45-47). This novel topoisomerase II α inhibitor may prove to be the leader in a new class of chemotherapeutic drugs that offer improved outcomes in terms of less therapy-related myeloid neoplasms.

Antimetabolites and radiation therapy and therapy-related myeloid neoplasms

The majority of secondary leukemias are associated with prior use of alkylating agents and/or topoisomerase II inhibitors. Less frequently, therapy-related myeloid neoplasms have been described following the use of antimetabolites such as azathioprine, fluorouracil, capecitabine and methotrexate (23). Offman et al. showed that the use of azathioprine immunosuppressive treatment following organ transplantation correlated with the development of therapy-related AML, and the risk of therapy-related AML increased significantly with increasing doses of antimetabolite therapy (P = 0.031 for 1 mg/kg/day versus 2.0-3.0 mg/kg/day at 1 year after transplantation) (48). More recently, a small retrospective analysis of 56 cases of therapy-related myeloid neoplasms subsequent to organ allograft also showed that azathioprine therapy for cytopenia in these patients was associated with chromosomal defects (monosomy 7, deletion of chromosomes 5 and 7, chromosome 11q23 rearrangements) that are hallmarks of therapy-related myeloid neoplasms (49). Cheson et al. analyzed long-term follow-up data for patients with chronic lymphocytic leukemia and hairy cell leukemia treated with antimetabolite chemotherapy (fludarabine, pentostatin and cladribine) and found no increased risk of secondary malignancies (including MDS and leukemia) (50). This is in contrast to the results of several studies of the incidence of therapy-related myeloid neoplasms following radioimmunotherapy for non-Hodgkin's lymphoma (NHL) suggesting an increased risk of therapy-related myeloid neoplasms associated with the use of fludarabine (see below) (51, 52).

Radiation therapy alone for a prior malignancy has not been identified as a risk factor for therapy-related myeloid neoplasms in several studies (53-55). In all of these studies, therapy-related myeloid neoplasms clearly occurred after exposure to radiation therapy alone, but its combination with chemotherapy did not significantly increase the risk of therapy-related myeloid neoplasms. Thus, the specific contribution of radiation therapy alone as a risk factor for therapy-related myeloid neoplasms remains somewhat debatable (4).

HEMATOLOGICAL MALIGNANCIES AND THERAPY-RELATED MYELOID NEOPLASMS

Therapy-related myeloid neoplasms and Hodgkin's lymphoma

Chemotherapy-induced therapy-related myeloid neoplasms were first described in the early 1980s in patients with Hodgkin's lymphoma treated with the MOPP regimen (mechlorethamine, vincristine, prednisone and procarbazine) (56). In fact, therapy-related myeloid neoplasms are the most frequent secondary hematological neoplasm in Hodgkin's lymphoma survivors (57). A diagnosis of therapy-related myeloid neoplasm is particularly devastating in this group of patients, because Hodgkin's lymphoma is highly curable, with a 10-year overall survival rate of 80% (58, 59). Due to the close association of MOPP with the development of therapy-related myeloid neoplasms, less intense radiochemotherapeutic regimens, such as the ABVD regimen (adriamycin, bleomycin, vinblastine and dacarbazine), have become more common. These lower-intensity regimens have been shown to have similar efficacy, while significantly decreasing the risk of developing therapy-related myeloid neoplasms compared to MOPP (60, 61). This is reflected by the progressive increase in overall survival in all age groups between the 1980s and early 2000s (59).

The German Hodgkin's Lymphoma Study Group (GHSG) evaluated data from 9 trials and 5,411 patients diagnosed with Hodgkin's disease to assess the association between treatment regimen for Hodgkin's lymphoma and the incidence of therapy-related myeloid neoplasms (Table II) (62). At a median of 55 months after treatment, 1% of patients had developed therapy-related myeloid neoplasms. Of the 46 patients diagnosed with therapy-related myeloid neoplasms, most received treatment with combined radiochemotherapy (78%), COPP (cyclophosphamide, vincristine, procarbazine and prednisone)/ABVD (52%) and escalated-dose BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone) (19%). The small sample size of each protocol precluded gaining any statistical significance. However, initial treatment with escalated-dose BEACOPP was associated with increased development of therapy-related myeloid neoplasms as

Table II. *Therapy-related myeloid neoplasms in Hodgkin's lymphoma.*

Josting et al. (62)	Retrospective	Data from 9 trials and 5,411 patients with Hodgkin's lymphoma	The small sample size in each protocol precluded the studies from gaining statistical significance. Initial treatment with BEACOPP was associated with increased development of therapy-related myeloid neoplasms compared to radiotherapy or other non-combined chemotherapy regimens. BEACOPP was shown to give overall better survival and complete response rates. Abnormalities on chromosomes 5 and 7 and rearrangements of chromosome 11 occurred in the majority of cases.
Franklin et al. (65)	Meta-analysis	9,312 patients	Therapy related AML occurred significantly more in patients receiving chemoradiation therapy compared to chemotherapy alone.

AML, acute myeloid leukemia; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone.

compared to radiotherapy or other noncombined chemotherapeutic regimens. Further analysis of one of the trials (the H9 trial) showed a statistically significant increase in the incidence of therapy-related myeloid neoplasms associated with dose-escalated BEACOPP regimens as compared to COPP/ABVD, providing support for the association of BEACOPP with therapy-related myeloid neoplasms. BEACOPP, however, was associated with better overall survival and greater complete responses as compared to COPP/ABVD (63, 64). Interestingly, 15 of the 46 therapy-related myeloid neoplasm patients had chromosomal aberrations, with abnormalities on chromosomes 5 and 7 and rearrangements of chromosome 11 present in the majority of cases (12 of 15). Abnormalities of chromosome 5 and 7 point to damage from alkylating agents such as cyclophosphamide, and rearrangement of chromosome 11 is often seen in cases of therapy-related myeloid neoplasms after the use of topoisomerase II inhibitors such as doxorubicin or etoposide (18).

A recent meta-analysis of 9,312 patients from 37 trials was conducted to determine the risk of secondary malignancies in Hodgkin's lymphoma patients (Table II) (65). Of the patients evaluated, 92 developed therapy-related AML. The rate of therapy-related AML was significantly higher in patients who received chemoradiation therapy as compared to chemotherapy alone, regardless of regimen, when data were censored at progression/relapse (odds ratio [OR]: 2.57; $P = 0.02$). These data were concordant with a similar meta-analysis by the International Database on Hodgkin's Disease Overview Study Group (66). One caveat to these results is that significant differences were detected only in censored data.

Therapy-related myeloid neoplasms and NHL

In 2008, an estimated 66,120 new cases of NHL were diagnosed and there were approximately 19,160 associated deaths (6). Compared to Hodgkin's lymphoma, low-grade, indolent or follicular NHL has a lower cure rate and is associated with transient, incomplete response to therapy and multiple relapses, and thus, multiple exposures to cytotoxic regimens. Drug resistance and therapy-related myeloid neoplasms are common consequences of treatment. The median survival after diagnosis of NHL is 9-10 years (67, 68). Research on the incidence and risk of therapy-related myeloid neoplasms in NHL began well after the realization of its link to Hodgkin's lymphoma, possibly due to the lower curative potential of NHL compared to Hodgkin's lymphoma. However, with the advent of new therapeutic regimens such as immunotherapy, the prognosis of NHL has improved, and the focus is turning to secondary malignancies, including therapy-related myeloid neoplasms (69).

Patients with NHL are exposed to various combinations of alkylating agents, radiation and possibly topoisomerase II inhibitors, all of which are linked to therapy-related myeloid neoplasms (Table III). In fact, it has been reported that up to 10% of NHL patients treated with either standard-dose chemotherapy or high-dose chemotherapy plus autologous SCT may develop therapy-related myeloid neoplasms within 10 years of first treatment (70). Population studies have also demonstrated that patients with NHL are at a significantly greater risk for secondary cancers overall, including therapy-related myeloid neoplasms. Chemotherapy and chemoradiation therapy, but not radiation therapy alone, in NHL patients has been shown to increase the risk of secondary hematological malignancies. In addition, younger patients (peak: 25-49 years) have a significantly higher risk of developing a secondary malignancy (71, 72). Since treatment regimens vary based on type, stage and progression of NHL at presentation, this section will focus on large studies of the potential link between chemotherapy and combination chemotherapy plus autologous SCT in NHL and therapy-related myeloid neoplasms.

The British National Lymphoma Investigation (BNLI) group has published data on secondary malignancies after NHL treatment from a long-term cohort study of 2,456 patients (73). Within this cohort, 87% of the patients received either chemotherapy or chemoradiation. The most common chemotherapy regimens were CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) (1980-1994) and chlorambucil (1973-1989). During follow-up, 125 (5%) patients developed a secondary malignancy. The risk of developing secondary leukemia was significantly increased (relative risk [RR]: 8.8; 95% confidence interval [CI]: 5.1-14.1; $P < 0.001$) as compared to the general population. That risk was confined to patients who received chemotherapy or chemoradiation ($P < 0.001$); there were no cases reported for patients who received radiotherapy alone. Patients who received CHOP had a relative risk of 14.2% (95% CI: 6.8-26.2) versus 19.2% (95% CI: 9.6-34.3) for those treated with chlorambucil. The 15-year cumulative risk for the development of secondary leukemia was 1.5% and for overall secondary malignancies 11%, which was in agreement with other studies (72).

A similar cohort study of 748 patients conducted by the European Organization for Research on Treatment of Cancer (EORTC) analyzed the incidence of secondary malignancies in patients with inter-

Table III. *Therapy-related myeloid neoplasms in non-Hodgkin's lymphoma (NHL).*

Mudie et al. (73)	Cohort study	2,456 patients with secondary malignancies after NHL	The risk of developing secondary leukemia was significantly increased in patients who received chemotherapy or chemoradiation, but not radiation alone.
Moser et al. (74)	Cohort study	748 intermediate- and high-grade NHL patients who received CHOP-like chemotherapy and radiotherapy or high-dose chemotherapy	Patients who were < 45 years old had a significantly higher risk of developing therapy-related myeloid neoplasms.
Andre et al. (69)	Retrospective	2,837 patients with aggressive NHL	The standard incidence ratio for the development of therapy-related myeloid neoplasms after ACVBP therapy was significantly higher than the general population (5.6-fold higher for men and 19.9-fold higher for women)
McLaughlin et al. (76)	Retrospective	202 patients with indolent NHL who received therapy consisting of FND regimen plus rituximab and interferon alfa	Increased risk of therapy-related myeloid neoplasms with FND, although some of the patients who developed therapy-related myeloid neoplasms (2 of 8) had been previously treated with CHOP.
Sacchi et al. (79)	Cohort study	563 patients with indolent NHL	Therapy-related myeloid neoplasms were not associated with fludarabine therapy.
Bennett et al. (51)	Prospective	1,071 patients treated with tositumomab and ^{131}I-tositumomab chemotherapy	Patients who were previously treated with fludarabine had a significantly higher risk of developing therapy-related myeloid neoplasms than those who had not. A 4.6-year follow-up of 76 naïve patients who received only RIT showed no incidence of therapy-related myeloid neoplasms.
Macklis et al. (52)	Meta-analysis	746 NHL patients with multiple histologies and numerous prior treatments	The use of ibritumomab tiuxetan RIT did not alter annualized rates of therapy-related myeloid neoplasms expected from extensive prior chemotherapy.
Gill et al. (80)	Cohort study	125 patients treated with the hyper-CVAD regimen	The 4-year cumulative incidence of therapy-related myeloid neoplasms was 4.43%; study was limited by size and short duration of follow-up.

CHOP, cyclophosphamide, doxorubicin, vincristine and prednisolone; ACVBP, adriamycin, cyclophosphamide, vindesine, bleomycin and prednisone; FND, fludarabine, mitoxantrone and dexamethasone; RIT, radioimmunotherapy; hyper-CVAD, hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone.

mediate- and high-grade NHL who received CHOP-like chemotherapy and radiotherapy or high-dose chemotherapy (74). The 15-year cumulative incidence of therapy-related myeloid neoplasms was 3% in this study. Patients < 45 years old had a significantly greater risk of developing therapy-related myeloid neoplasms (hazard ratio [HR]: 3.8; 95% CI: 1.2-5.7), similar to prior studies (73). This may be due to younger patients living long enough for secondary malignancies to emerge.

The results of three studies on secondary cancers in NHL patients receiving the ACVBP regimen (adriamycin, cyclophosphamide, vindesine, bleomycin and prednisone) were reported by the Groupe d'Etude des Lymphomes de l'Adults (GELA) (69). The ACVBP regimen, which is more effective than the conventional CHOP regimen, is commonly used in the treatment of NHL. The GELA group reviewed data on 2,837 patients with aggressive NHL and found that the standard incidence ratio for the development of therapy-related myeloid neoplasms after ACVBP therapy was significantly higher in this group compared to the general population. The risk of developing therapy-related myeloid neoplasms was 5.6- and 19.9-fold higher, respectively, in males and females. In multivariate analysis, age was a significant risk factor for the development of therapy-related myeloid neoplasms.

The FND regimen (fludarabine, mitoxantrone and dexamethasone) is a first-line treatment for patients with indolent NHL and has been shown to be superior to CHOP (75). The development of therapy-related myeloid neoplasms was reviewed in 202 patients with indolent NHL who had received therapy with the FND regimen plus rituximab and interferon alfa (76). Eight patients developed therapy-related myeloid neoplasms and the actuarial risk in patients treated with FND was 3% at 4 years. All patients who developed therapy-related myeloid neoplasms had cytogenetic abnormalities commonly seen after alkylating therapy, namely abnormalities of chromosome 5 and 7 (six of eight patients). Fludarabine has been shown to inhibit DNA repair and may contribute to the cytogenetic abnormalities induced by alkylating agents (77). Confounding these results was the fact that of the eight patients who developed thera-

py-related myeloid neoplasms, two had been previously treated with cyclophosphamide. Thus, it is difficult to reach definitive conclusions on the link between fludarabine and therapy-related myeloid neoplasms. The 5-year duration of follow-up was another shortcoming of this study, as therapy-related myeloid neoplasms typically develop at a median of 6 years after primary therapy in NHL patients (78).

Recently, the Gruppo Italiano Studio Linformi (GISL) published the results of a 16-year follow-up study on a cohort of patients with indolent NHL to determine the incidence of secondary cancers (79). Of the 563 patients studied, 39 developed secondary malignancies, 12 of which were therapy-related myeloid neoplasms. The cumulative 12-year incidence of developing a secondary malignancy was 10.5%, and notably, the development of therapy-related myeloid neoplasms was not associated with fludarabine therapy. These results contrast with other studies that have shown some risk associated with this therapy (see below).

Aside from traditional chemotherapy and radiation regimens, radioimmunotherapy (RIT) has also been incorporated into the treatment guidelines for NHL. Bennett et al. assessed the rate of occurrence of therapy-related myeloid neoplasms in 1,071 patients treated with tositumomab and ^{131}I-tositumomab, an alternative therapy for follicular, or low-grade, NHL (51). RIT targets tumor cells that express specific surface receptors and facilitates radiation-induced cytotoxicity. Surrounding healthy cells may also be damaged by this type of therapy. The 5-year cumulative incidence rate of therapy-related myeloid neoplasms was 5%, which was similar to for traditional chemotherapy regimens. Patients who were previously treated with fludarabine had a significantly higher risk than those who were not (RR: 3.08; $P = 0.01$). A subset analysis of 76 chemotherapy-naïve patients who received RIT only revealed no incidence of therapy-related myeloid neoplasms after 4.6 years of follow-up, which suggested that RIT alone does not contribute to the development of therapy-related myeloid neoplasms.

In a separate trial, data from 746 NHL patients with multiple histologies and numerous prior treatments were investigated to determine the rate of occurrence of therapy-related myeloid neoplasms after the use of ibritumomab tiuxetan RIT. Ibritumomab tiuxetan RIT had no effect on the annualized rate of therapy-related myeloid neoplasms expected from extensive prior chemotherapy, with a 5-year cumulative incidence rate for patients who received the therapy of 4.3% (52). An increased risk of therapy-related myeloid neoplasms was significantly associated with prior exposure to nucleoside analogues such as fludarabine ($P = 0.006$). Similar to the tositumomab study, cytogenetic abnormalities in chromosomes 5 and 7 were observed in patients who developed therapy-related myeloid neoplasms, which was consistent with the use of alkylating agents and purine nucleoside analogues. The results of both of these trials suggest that the use of RIT does not increase the incidence of therapy-related myeloid neoplasms, and support an increased risk of therapy-related myeloid neoplasms associated with use of the nucleoside analogue fludarabine.

A recent report by Gill et al. examined the association between the hyperfractionated CVAD regimen (cyclophosphamide, vincristine, doxorubicin and dexamethasone; hyper-CVAD), an effective first-line regimen for mantle cell and Burkitt's lymphoma, and therapy-related myeloid neoplasms in a cohort of 125 patients (80). In addition to persistent cytopenia which occurred in 59% of patients after 3 months, four patients developed therapy-related myeloid neoplasms. The 4-year cumulative incidence of therapy-related myeloid neoplasms was 4.43%. This study was limited by the sample size and short duration of follow-up. However, the results support larger prospective trials with adequate duration of follow-up to accurately assess the leukemogenicity of the hyper-CVAD regimen.

THERAPY-RELATED MYELOID NEOPLASMS AND AUTOLOGOUS SCT

High-dose chemotherapy and autologous SCT in appropriate patients is becoming increasingly common in the treatment of NHL, Hodgkin's lymphoma and other hematological malignancies. High-dose chemotherapy followed by autologous SCT is associated with limited mortality (approximately 5%) and prolonged disease-free or progression-free survival (81-86). It is also widely recognized, however, that this therapy is associated with an increased risk of developing therapy-related myeloid neoplasms (81-82, 84, 87-96). Although some of the risk can be attributed to the transplant procedure itself, most is believed to arise from pretransplant conditioning. Studies have also shown that increased risk of therapy-related myeloid neoplasms after autologous SCT is related to prior exposure to radiation and the cumulative dose of prior chemotherapy, particularly alkylating agents (81, 88-93). Total body irradiation (TBI) has been shown to be a significant risk factor in some studies, but this remains a topic of debate (94, 97-99). There is some evidence that the use of fludarabine in autologous SCT is associated with an increased risk of therapy-related myeloid neoplasms (76, 95, 100, 101). Age is also a risk factor, with younger patients having a higher risk than older patients (84, 90, 102, 103), which may again be due to the fact that younger patients typically live long enough for therapy-related myeloid neoplasms to emerge. Although the risk of developing therapy-related myeloid neoplasms after high-dose chemotherapy and autologous SCT is increased as compared to the general population, it has been shown to be the same as with traditional chemotherapy regimens (70, 103, 104).

A recent meta-analysis of randomized, controlled trials comparing the efficacy of high-dose chemotherapy followed by autologous SCT with traditional chemotherapy in patients with aggressive NHL showed that both approaches result in similar treatment-related mortality, overall survival and event-free survival. However, treatment with high-dose chemotherapy followed by transplantation resulted in a significantly higher complete response rate (105). These results suggest that autologous SCT and traditional chemotherapy may be equally effective and carry a similar risk of developing therapy-related myeloid neoplasms. Differences in risk and rate of occurrence of therapy-related myeloid neoplasms reported in different studies may reflect differences in mobilization and conditioning protocols prior to transplantation, as well as differences in prior chemotherapy regimens and radiation. Clinicians must make treatment decisions based on individual risk factors.

Howe et al. investigated the incidence and risk factors associated with the development of therapy-related myeloid neoplasms in 230 patients (Hodgkin's lymphoma, n = 64; NHL, n = 166) who had received autologous SCT (106). Of the 230 patients, 10 (4.3%) developed therapy-related myeloid neoplasms. The 5-year cumulative

risk was 4.2%. Pre- and post-transplantation cytogenetics were available for five of the patients with therapy-related myeloid neoplasms. Development of therapy-related myeloid neoplasms in all five cases correlated with transformation from a normal to an abnormal cytogenetic profile. Significant risk factors for developing therapy-related myeloid neoplasms were male gender (P = 0.01) and number of previous treatment regimens (P = 0.04). The study also looked at the use of alkylating agents and etoposide, and found that the number of cycles of mechlorethamine and cyclophosphamide in particular was significantly higher for patients who developed therapy-related myeloid neoplasms (P = 0.001 and P = 0.05, respectively). The main shortcomings of this study were the inclusion of multiple disease states, the retrospective design and short-term follow-up, making it difficult to draw sound conclusions on the origins and incidence of therapy-related myeloid neoplasms in these particular patients.

Lenz et al. conducted a prospective, randomized trial to investigate the incidence of therapy-related myeloid neoplasms after autologous SCT in 440 patients with low-grade NHL (107). All patients received "CHOP-like" induction therapy and were then randomly assigned to receive either SCT or interferon alfa therapy. Patients who received SCT had a longer progression-free survival than those who received interferon alfa (P < 0.0001). Five of the patients who received SCT developed therapy-related myeloid neoplasms as compared to none in the interferon alfa cohort. The cumulative 5-year incidence rate after autologous SCT was 3.5%, which was significantly higher than in the interferon alfa group (P = 0.02). Although the increased incidence of therapy-related myeloid neoplasms may be related to the transplantation procedure itself, patients in the SCT cohort also received high-dose chemotherapy and TBI for mobilization and conditioning before transplant. The role of TBI in the development of therapy-related myeloid neoplasms is somewhat controversial (70, 93), and the results of this study were not sufficient to distinguish between the effect of TBI or high-dose chemotherapy conditioning and the transplant procedure itself.

A separate prospective study investigated the risk of secondary malignancies in 605 NHL patients undergoing uniform conditioning treatment with cyclophosphamide and TBI before receiving autologous SCT (84). The median follow-up period was 9.5 years, which was likely sufficient to capture additional cases of therapy-related myeloid neoplasms after SCT. At a median follow-up of 9.5 years, 68 (11%) cases of therapy-related myeloid neoplasms were diagnosed. The cumulative 10- and 15-year incidences of therapy-related myeloid neoplasms after SCT were 11% and 14.2%, respectively. Risk factors for the development of therapy-related myeloid neoplasms were age ≥ 44 years at the time of transplantation (P = 0.00007) and previous exposure to radiation therapy (P = 0.03). TBI was not identified by this study as a risk factor for therapy-related myeloid neoplasms because all patients received TBI as part of their conditioning regimen.

Kalaycio and colleagues explored the relationship between difficult-to-harvest stem cells and the incidence of therapy-related myeloid neoplasms after autologous SCT in 526 patients (95). Risk factors associated with difficult-to-harvest stem cells and therapy-related myeloid neoplasms are similar, namely prior cytotoxic therapy, increased number of chemotherapy regimens and radiation therapy (108, 109). In the Kalaycio study, patients were given either G-CSF alone, etoposide plus G-CSF or cyclophosphamide plus G-CSF with or without etoposide to mobilize stem cells. Difficult-to-harvest stem cells were defined as those that required more than 5 days for collection and repeated use of etoposide and/or cyclophosphamide. After mobilization, all patients were administered high-dose chemotherapy. Twenty patients developed therapy-related myeloid neoplasms, resulting in a 10-year actuarial incidence of 6.8%. Of these 20 patients, 15 had cytogenetic data before and after SCT. Prior to transplantation, no cytogenetic abnormalities were found in any of the 15 patients, whereas 9 patients had monosomy of chromosome 7 at diagnosis of therapy-related myeloid neoplasms. Patients treated with prior cyclophosphamide and fludarabine had a significantly higher risk of developing therapy-related myeloid neoplasms, similar to in other studies (76, 100, 101). Notably, patients with difficult-to-harvest stem cells had a higher rate of therapy-related myeloid neoplasms (P < 0.001). Multivariate analysis revealed that prior exposure to radiation therapy (P < 0.006), four or more prior chemotherapy regimens (P < 0.003) and difficult-to-harvest stem cells (P < 0.001) were independent risk factors for the development of therapy-related myeloid neoplasms.

Although autologous SCT is used most commonly in the treatment of NHL and Hodgkin's lymphoma, it has also been used to treat solid tumors. There is evidence of an increased incidence of therapy-related myeloid neoplasms after SCT in patients treated for breast cancer, but this phenomenon is much less common (110, 111).

BREAST CANCER AND THERAPY-RELATED MYELOID NEOPLASMS

Adjunctive breast cancer therapy has evolved over the past 30 years, from 12-month CMF regimens (cyclophosphamide, methotrexate and fluorouracil) to the current recommended anthracycline-based CMF hybrid regimens, such as CEF (cyclophosphamide, epirubicin, fluorouracil) (112) and AC (doxorubicin plus cyclophosphamide). Studies of early breast cancer patients treated using standard-dose CMF regimens have demonstrated a 15-year cumulative risk of developing therapy-related myeloid neoplasms of only 0.23% (113). The results of a large, retrospective study of the impact of multiple risk factors involved in the treatment of breast cancer, including adjunctive chemotherapy, radiation therapy and growth factors (i.e., G-CSF), showed that patients who received a high-intensity cyclophosphamide AC regimen had a significantly higher risk of developing therapy-related myeloid neoplasms compared to standard AC (114). An increased risk was also associated with use of G-CSF and radiotherapy, and the combination of a high-intensity cyclophosphamide AC regimen and G-CSF had the highest risk. However, it should be noted that the leukemogenic high-intensity cyclophosphamide AC regimen was more effective, especially epirubicin-based regimens, as compared to doxorubicin (114-118). That being said, the use of epirubicin has been linked to cytogenetic abnormalities, namely translocations, that may contribute to the development of therapy-related myeloid neoplasms.

In a prospective, randomized trial involving 3,121 patients, the addition of paclitaxel to standard-dose AC regimen had no effect on the

overall incidence of therapy-related myeloid neoplasms. In contrast, the addition of topoisomerase II inhibitors increased the leukemogenic potential of adjuvant therapy. For example, in a recent study that analyzed the risk of therapy-related myeloid neoplasms for different classes of topoisomerase II inhibitors, mitoxantrone-based regimens had a significantly higher relative risk than anthracycline-based regimens (RR: 15.6 vs. 2.7) (119).

Praga and colleagues investigated the association between the use of epirubicin in early breast cancer and the incidence and risk of therapy-related myeloid neoplasms (118). Follow-up data on 7,110 patients (from 19 randomized trials) on adjuvant epirubicin therapy were analyzed. Most patients also received cyclophosphamide (92%). Twenty-eight (0.394%) patients developed therapy-related myeloid neoplasms, and the results indicated that the risk of developing therapy-related myeloid neoplasms increased with planned epirubicin dose, intensity and cumulative dose of epirubicin and cyclophosphamide. Patients who received "standard"-dose epirubicin and cyclophosphamide had a significantly lower risk of developing therapy-related myeloid neoplasms compared to those receiving higher cumulative doses (0.37% and 4.97%, respectively). The use of G-CSF in 796 patients on intensive epirubicin and cyclophosphamide was significantly associated with the development of therapy-related myeloid neoplasms (P = 0.0001 compared to no G-CSF). These results indicate that epirubicin at higher doses and when combined with cyclophosphamide or G-CSF may increase the risk of developing therapy-related myeloid neoplasms.

Again, one must view these results in the context of overall outcomes. Some argue that the benefit of improved disease-free survival or overall survival observed after the addition of epirubicin outweighs the risk of developing therapy-related myeloid neoplasms (120-125). Data from trials conducted by the French Adjunct Study group on 2,603 patients demonstrated that at standard doses of epirubicin the cumulative 9-year risk of developing therapy-related myeloid neoplasms was 0.34%, and in patients who received CMF regimens the cumulative 15-year rate was 0.23% (113). These results should encourage clinicians to consider all patient-specific risk factors, such as combined treatment modalities, dose intensity and topoisomerase II inhibitor-based regimens in the treatment of breast cancer, particularly early-stage breast cancer.

Administration of G-CSF with adjuvant chemotherapy in breast cancer patients allows the use of increased doses of chemotherapy due to the beneficial effects of G-CSF on hematological toxicity (126). A few large-scale studies have shown an increased risk of developing therapy-related myeloid neoplasms associated with the use of G-CSF as adjunctive therapy for the treatment of breast cancer (127, 128). The evidence is limited for the most part to retrospective studies. Furthermore, it is difficult in many cases to distinguish between therapy-related myeloid neoplasms induced by increased intensity of chemotherapy or the use of G-CSF (129). Smith et al. carried out a retrospective analysis of the incidence of therapy-related myeloid neoplasms after AC in six adjuvant National Surgical Adjuvant Breast and Bowel Project (NSABP) trials (127). They found that the incidence of therapy-related myeloid neoplasms was significantly higher in patients who received G-CSF in addition to two or four cycles of AC with high doses of cyclophosphamide. Several other studies have shown that a minimum of two doses of G-CSF may be enough to improve hematological toxicity and prevent anemia after breast cancer chemotherapy (130, 131), providing additional support for the most conservative use of G-CSF during cancer treatment.

Two reports have investigated the use of patient data contained in the NCI's Surveillance Epidemiology and End Result (SEER) Medicare database to determine whether there is an association between therapy-related myeloid neoplasms and prior use of G-CSF with chemotherapy in breast cancer patients (128, 132). In the first study, data from 5,510 patients aged 65 years and older with type I-III breast cancer were analyzed (128). Of the patients studied, 64 (1.14%) developed therapy-related myeloid neoplasms. Of the 906 who were treated with G-CSF, 1.77% developed therapy-related myeloid neoplasms compared to 1.04% of patients who did not receive G-CSF. The risk of developing therapy-related myeloid neoplasms was twice as high in patients treated with G-CSF as compared to those who were not (HR: 2.24). A second study analyzed data on 64,715 patients (≥ 65 years) with nonmetastatic breast cancer (132). In patients who received G-CSF within the first year of diagnosis, there was no significant increase in the risk of developing therapy-resistant AML. The only significant risk factor was treatment with adjunct chemotherapy, regardless of regimen (taxane, anthracycline or both), which increased risk by 50%. Limitations of these studies are that they were retrospective and the treatment and diagnostic information was obtained from a database comprised of hospital registries and Medicare reimbursement data. Thus, data entry and searches, particularly for therapy-related myeloid neoplasms, which is often coded incorrectly, may have underestimated the true incidence.

Le Deley et al. conducted a retrospective case-control study of 182 therapy-related myeloid neoplasm patients and 534 matched controls to determine risk factors associated with the development of therapy-related myeloid neoplasms after breast cancer treatment (119). The use of G-CSF was associated with an increased risk of therapy-related myeloid neoplasms (RR: 6.3) even after controlling for radiation and chemotherapy. Confounding these results, however, was that G-CSF was often used for patients with poor hematological tolerance to therapy, which may reflect inherently poor or abnormal bone marrow.

Cancer and Leukemia Group B (CALGB) investigators recently published the results of a prospective, randomized clinical trial of dose-intensified AC followed by paclitaxel with C-GSF support for the treatment of early-stage, node-positive breast cancer (133). One hundred and seventy-two patients were enrolled and randomized to receive two different doses of G-CSF. The study found that G-CSF did not improve the tolerability of the treatment regimen, and therapy-related myeloid neoplasms occurred in 2% of the treated patients, which is typical for high-dose chemotherapy regimens with G-CSF support.

A summary of clinical studies that have examined the association between an increased risk of therapy-related myeloid neoplasms and the use of G-CSF in breast cancer treatment is presented in Table IV. Overall, there is support for an increased risk of subsequent therapy-related myeloid neoplasms with the use of G-CSF as an adjunct to breast cancer therapy. Randomized, prospective studies would offer more conclusive evidence of this relationship, particularly in patients with breast cancer who are receiving chemotherapy.

Table IV. *Granulocyte colony-stimulating factor (G-CSF) and therapy-related myeloid neoplasms.*

Smith et al. (127)	Retrospective in six adjuvant NSABP trials	2,063 patients with breast cancer, 34 of whom developed MDS/AML	Patients receiving higher-intensity cyclophosphamide in AC regimens as compared to standard AC regimens had a significantly higher risk of developing therapy-related myeloid neoplasms. Those treated with G-CSF and radiotherapy also had an increased risk. The combination of high-intensity cyclophosphamide AC regimens and G-CSF had an even greater risk. Other risk factors for MDS/AML following breast cancer were older age, other cancers and multiple first-degree relatives with cancer.
Hershman et al. (128)	Retrospective analysis of patient data from the NCI SEER-Medicare database	5,510 patients > 65 years with type I-III breast cancer	Risk of developing therapy-related myeloid neoplasms twice as high in patients treated with G-CSF compared to those who were not.
Patt et al. (132)		64,715 patients > 65 years with nonmetastatic breast cancer	Patients administered G-CSF within the first year of diagnosis did not show a significant increase in the risk of developing therapy-related AML. The only significant risk factor was treatment with adjunct chemotherapy, regardless of the regimen.
Le Deley et al. (119)	Retrospective case-control study	182 patients with therapy-related myeloid neoplasms and 534 matched controls	G-CSF was associated with an increased risk of therapy-related myeloid neoplasms, even after radiation and chemotherapy were controlled for.
Liu et al. (133)	Prospective, randomized pilot clinical trial	172 patients with early-stage, node-positive breast cancer	G-CSF did not improve tolerability of the regimen and therapy-related myeloid neoplasms occurred in 2% of the treated patients.
Praga et al. (118)	Retrospective	Follow-up data on 7,110 patients from 19 randomized trials on adjuvant epirubicin therapy	The risk of developing therapy-related myeloid neoplasms increased according to planned epirubicin dose, intensity and cumulative dose of epirubicin and cyclophosphamide. Also, there was a significant association in patients who were on intensive epirubicin and cyclophosphamide who took G-CSF with the development of therapy-related myeloid neoplasms compared to patients not on G-CSF.

NSABP, National Surgical Adjuvant Breast and Bowel Project; AC, doxorubicin and cyclophosphamide; NCI SEER, National Cancer Society's Surveillance Epidemiology and End Result database; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia.

ROLE OF POLYMORPHISMS IN THERAPY-RELATED MYELOID NEOPLASMS

The search for a genetic predisposition to therapy-related myeloid neoplasms has led to the identification of a number of genetic polymorphisms associated with the disease. These polymorphisms may affect the metabolism of chemotherapeutic agents or the function of detoxification enzymes and DNA repair mechanisms. Cytochrome P450 (CYP) enzymes are responsible for the metabolism of a variety of exogenous substances, including chemotherapeutic agents. CYP3A4 metabolizes epipodophyllotoxins (i.e., doxorubicin) into toxic metabolites that can damage DNA. A polymorphism in the 5′ promoter region of the gene for CYP3A4 (*CYP3A4*) was found to be associated with fewer cases of therapy-related myeloid neoplasms than wild-type *CYP3A4*. It has been suggested that this polymorphism leads to lower levels of CYP3A4 activity, and thus lower levels of genotoxic metabolites that can enhance DNA damage. Additionally, individuals with certain CYP polymorphisms can be categorized as "poor metabolizers", in that metabolism of certain drugs by CYP enzymes in these individuals occurs at a slower rate. Roddam et al. found that individuals with CYP2D6 and CYP2C19 poor-metabolizer phenotypes were at increased risk of developing therapy-related myeloid neoplasms (134). The *CYP1A1*2A* polymorphism has also been associated with an increased risk of therapy-related myeloid neoplasms (135).

Polymorphisms in detoxification enzymes can affect an individual's ability to detoxify certain substances, including chemotherapeutics. For example, polymorphisms in glutathione *S*-transferase (GST) enzymes, also known as phase II metabolizing enzymes, can affect the cell's ability to detoxify via glutathione conjugation. Several studies have shown that deletions in the gene for GST Mu 1 (*GSTM1*) are associated with an increased susceptibility for AML in adults (136-138). The combination of *GSTM1* deletions and CYP1A1 and CYP2D6 polymorphisms also increases the risk of developing acute leukemia (137). Deletions in *GSTM1* have been linked to treatment outcomes and prognosis, and may also be gender-specific, with a higher percentage found in women (138). Deletions in GST theta-1 (*GSTT1*) have been associated with worse prognosis in patients with de novo AML (139, 140). Deletions in a third GST gene, *GSTP1*, were found to be more relevant to susceptibility to therapy-related myeloid neoplasms. This study examined 89 cases of therapy-related AML and found that individuals with at least one codon 105 Val polymorphism in *GSTP1* were significantly overrepresented among

cases of therapy-related AML after chemotherapy compared to de novo AML (141). Polymorphism constellations have been identified that are more common to therapy-related myeloid neoplasms than normal controls. Polymorphisms of *CYP1A1*2A, NQO1*2* and deletions of *GSTT1* have been shown to modify the risk of therapy-related myeloid neoplasms. The absence of all three polymorphisms decreases the risk of therapy-related myeloid neoplasms 18-fold (OR: 0.054), whereas the presence of all three or just the *NQO1*2* polymorphism increases the risk (OR: 18.42 and 2.09, respectively) (135).

NAD(P)H:quinone oxidoreductase 1 (QR1, *NQO1*) is an enzyme involved in detoxification of quinones and reduction of oxidative stress. *NQO1* polymorphisms such as C609T result in a loss or reduction of enzyme activity (142) and decreased conversion of hydroquinone, a toxic benzene metabolite, to a nontoxic form. This can lead to accumulation of hydroquinone, resulting in oxidative stress, telomere shortening and possibly clonal hematopoiesis (143). The *NQO1* C609T polymorphism was found at a significantly higher rate in cases of therapy-related myeloid neoplasms, patients with chromosome 5 and 7 abnormalities and patients with prior treatment with alkylating agents or topoisomerase II inhibitors compared to the general population. Notably, this polymorphism was more common in therapy-related myeloid neoplasms than in de novo AML or CML (142). A later study indicated that the *NQO1* C609T polymorphism was associated with increased susceptibility to de novo AML as well (144). The *NQO1* C609T polymorphism has also been shown to be related to response to induction therapy for AML. Better response to induction therapy was more commonly associated with the *NQO1* C609T polymorphism. In one study, a higher percentage of *NQO1* C609T-negative patients with de novo AML achieved complete remission as compared to *NQO1* C609T carriers, most likely related to a compromised capacity to detoxify chemotherapeutic agents in *NQO1* C609T-positive patients (138).

Polymorphisms in genes that regulate DNA damage repair, particularly double-strand breaks, can result in chromosomal translocations or deletions. Defects in the repair machinery can also cause mutations and clonal expansion (23). Polymorphisms in the DNA repair gene *RAD51* have been shown to increase the risk of therapy-related myeloid neoplasms (145-147). When polymorphisms of *RAD51* occur along with polymorphisms of the homeobox gene *HLX1*, which regulates stem cell differentiation, the risk of therapy-related myeloid neoplasms increases synergistically (145). When polymorphisms of *RAD51* (*RAD51* G135C) are present along with variants of another DNA repair enzyme, XRCC3 (*XRCC3* Thr241Met), the risk of therapy-related myeloid neoplasms is higher than the risk of de novo AML (148). Polymorphisms in other DNA repair enzymes such as XRCC1 (*XRCC1* Arg399Gln) have been shown to confer protection against AML (149).

CYTOGENETICS AND THERAPY-RELATED MYELOID NEOPLASMS

In addition to genetic polymorphisms, the link between chromosomal abnormalities and cytogenetic profiles and therapy-related myeloid neoplasms has received considerable attention. This section will present a brief overview based on several comprehensive reviews by Pedersen-Bjergaard and colleagues (18, 150, 151).

Three different cytogenetic subgroups commonly identified in cases of therapy-related myeloid neoplasms have been described. Group 1 includes unbalanced aberrations such as loss of the entire or long arm of chromosome 5 or 7 (–5/5q–, –7/7q–), or gain of chromosome 8 (+8). Patients with group 1 abnormalities often present with therapy-related myeloid neoplasms that are associated with a complex karyotype and point mutations in the genes for AML-1 or p53. Patients tend to be older and have a poor prognosis. This profile is commonly observed in patients who have been exposed to alkylating agents. Group 2 contains balanced translocations or inversions, such as 11q23, 21q22, 17q21, or inv(16)(p13q22). Patients with group 2 abnormalities often present with a single translocation abnormality, tend to be younger and have a favorable prognosis. This cytogenetic profile is commonly associated with the use of topoisomerase II inhibitors. Group 2 balanced translocations often lead to chimeric rearrangements of genes that encode transcription factors involved in the regulation of hematopoiesis. These rearrangements often result in silencing of such transcription factors, leading to decreased differentiation and increased proliferation. Group 3 patients have normal karyotypes. In these patients, the etiology of therapy-related myeloid neoplasms is not known, but may be linked to radiotherapy or immunosuppressive therapy. These patients typically present as therapy-related MDS or therapy-related AML with a favorable or intermediate prognosis, respectively.

The chromosomal aberrations and gene mutations detected in therapy-related myeloid neoplasms and de novo MDS/AML are similar, but the frequencies with which they are observed differ. Most cases of de novo MDS/AML present with a normal karyotype (approximately 50% of cases), with approximately 20% presenting unbalanced abnormalities. In contrast, therapy-related myeloid neoplasm cases more often present with unbalanced abnormalities (approximately 50%) as compared to normal karyotypes (approximately 10%). In both de novo MDS/AML and therapy-related myeloid neoplasms, the types of abnormalities correlate with prior mutagenic treatment.

Pedersen-Bjergaard et al. also classified three different types of mutations that are commonly identified in cases of therapy-related myeloid neoplasms. These differ from the three overarching cytogenetic subgroups discussed above. Class I mutations include activating mutations, such as point mutations or internal tandem duplications (ITD), that result in constitutively active signal transduction pathways and unregulated cell proliferation. Implicated in this class are mutations of the tyrosine kinases FLT3, c-kit, c-Fms, JAK2 and downstream components of the Ras/B-raf pathway. Loss of chromosome 7 or 7q– is also considered a class I mutation based on the resulting leukemogenic properties, which are similar to other class I mutations. Class II mutations are inactivating mutations of hematopoietic transcription factors that result in impaired differentiation. Class II mutations are often caused by translocations, inversions or point mutations leading to chimeric rearrangements of genes that encode hematopoietic transcription factors. Some examples of class II mutations that are associated with therapy-related myeloid neoplasms are mutations in the genes for MLL3 at chromosome 11q23, AML-1 at 21q22, CBF-β at 16q22, nucleoporin Nup98 at 11p15 and nucleophosmin (NPM) (152). The third class of mutations are inactivating mutations of the tumor suppressor p53. These mutations are often associated with deletions of chromosome 5,

complex karyotypes and poor outcomes (24, 27). There is some evidence that class I and II mutations act in a cooperative manner (18, 150, 151, 153-155). For example, the transcription factor RUNX1 and Ras may cooperate to influence the progression from MDS to therapy-related AML, and RUNX1 and 7q– loss are commonly observed in therapy-related MDS induced by alkylating agents (28, 32, 151, 155). Continued research will shed much-needed light on the functional relationships between the different classes of mutations and therapy-related myeloid neoplasms. In this vein, Pedersen-Bjergaard also described eight different genetic pathways leading to therapy-related myeloid neoplasms based on recurrent abnormalities seen in patients that are dependent on the type of prior mutagenic therapy.

Mutations or disruptions of key regulatory genes may also underlie the development of therapy-related myeloid neoplasms. The gene for the leukemia-related candidate tumor suppressor EGR1 is lost in some cases of MDS/AML with –5 or 5q– deletions. EGR-1 is a member of the Wilms tumor protein family of transcription factors. In animal models, loss of one *EGR1* allele (*EGR1*$^{-/+}$) was associated with leukemogenesis due to haploinsufficiency (156). G-CSF has been shown to preferentially stimulate the proliferation of monosomy 7 patient bone marrow cells, and these cells contained increased levels of activity of signal transducer and activation of transcription STAT1 and STAT5 (157).

Epigenetic mechanisms of silencing of regulatory genes that play integral roles in cell proliferation and differentiation have become a new focus in anticancer research. For example, chromosome 5q deletion leads to suppression of the gene for catenin alpha-1 (*CTNNA1*) during myeloid cell transformation. Demethylation and restoration of *CTNNA1* expression resulted in decreased proliferation and increased apoptosis (158). There is also evidence that methylation of p15 plays a role in the development of therapy-related myeloid neoplasms. p15(INK4B) is a putative tumor suppressor that is a key regulator of cell cycle arrest. Methylation and silencing of the p15 gene promoter were recently documented in 55 of 81 patients with therapy-related myeloid neoplasms. There was a significant association between methylation of the p15 gene promoter and deletion of the long arm of chromosome 7 ($P = 0.0006$), and methylation frequency was significantly associated with an increased percentage of marrow blasts (159).

CURRENT AND NOVEL TREATMENTS FOR THERAPY-RELATED MYELOID NEOPLASMS

Hematopoietic SCT

Treatment options for therapy-related myeloid neoplasms are extremely limited and prognosis is poor, particularly for the elderly. The median survival after diagnosis of therapy-related myeloid neoplasms is 6-12 months (1, 160). According to National Comprehensive Cancer Network guidelines, the management of therapy-related myeloid neoplasms is based on performance status (which is a reflection of age, comorbidities, persistence of primary disease and prior therapy) and types of cytogenetic abnormalities. For patients with poor prognostic indicators, treatment often consists of best supportive care or referral to a clinical trial. Those with good performance indicators and favorable karyotypes may be treated similarly to de novo AML, with standard induction therapy consisting of cytarabine/anthracycline, followed by consolidation therapy and hematopoietic SCT (HSCT). Even with such treatments, however, relapse occurs in the majority of patients and the prognosis remains poor.

For patients with therapy-related myeloid neoplasms, the best hope for a cure at present is allogeneic HSCT. Kroger and colleagues recently reported the results of a retrospective analysis of 461 patients with therapy-related myeloid neoplasms who underwent allogeneic SCT and were reported to the European Group for Blood and Marrow Transplantation (EBMT) (161). The investigators found that 3-year relapse-free and overall survival was 33% and 35%, respectively, with higher overall survival rates for younger patients (< 40 years) and those in complete remission at the time of transplantation. For patients under 40 years of age in complete remission and without poor cytogenetic abnormalities, the 2-year survival rate was 62%. In contrast, a patient aged over 40 years with poor cytogenetics and not in complete remission had an estimated 2-year survival rate of 24%. An analysis of 70 patients who underwent allogeneic HSCT in France yielded similar results, in that poor outcomes were associated with age over 37 years and absence of complete remission at the time of transplantation, as well as male sex and use of intensive conditioning therapy (162). The estimated 2-year survival rate in this study was 30%. These results highlight the importance of cytogenetics and age as prognostic indicators for allogeneic HSCT in patients with therapy-related myeloid neoplasms.

Several groups have examined the role of prior inductive therapy (both intensity and type of agent) and myeloablative conditioning prior to HSCT in outcomes for patients with therapy-related myeloid neoplasms. A recent retrospective review of data submitted to the Center for International Bone Marrow Transplant Research (CIBMTR) analyzed outcomes in 868 patients with therapy-related myeloid neoplasms who received allogeneic transplants, 77% of whom received myeloablative conditioning prior to transplant, over a period of 15 years (163). Disease-free survival at 1 and 5 years was 32% and 21%, respectively, and overall survival was 37% and 33%, respectively. Of note, treatment-related mortality was not reduced in patients who received reduced-intensity/non-myeloablative conditioning. Poor-risk genetics, age over 35 years, disease not in complete remission and non-sibling-related donor or mismatched unrelated donor were associated with adverse effects on survival.

A retrospective study conducted by the EBMT analyzed outcomes of 593 patients with therapy-related MDS/AML who received either autologous or allogeneic HSCT with or without prior chemotherapy. For patients that underwent allogeneic HSCT, 3-year survival was 50% in those given chemotherapy prior to transplant and 40% if no chemotherapy was given prior to transplant ($P = 0.01$) (164). A retrospective analysis of chemotherapy plus transplantation (autologous and allogeneic) as compared to chemotherapy alone in high-risk MDS and therapy-related AML failed to find a significant difference in overall survival between the two groups (4-year survival from complete remission = 34.3% and 25.5%, respectively; $P = 0.29$), although disease-free survival was higher in the chemotherapy plus transplantation group (28.9% and 17.3%, respectively; $P = 0.017$) (165). Interestingly, in this study, the investigators found no difference between patients who received allogeneic transplants as compared to autologous transplants.

In 2001, Witherspoon and colleagues analyzed patient and treatment characteristics associated with improved outcomes in patients

who received allogeneic HSCT (related and nonrelated donors) (166). The investigators found that a preparative regimen was a significant factor in disease-free survival, with patients who received targeted bisulfan (BU) therapy having higher 5-year disease-free survival compared to nontargeted BU therapy or TBI plus chemotherapy (30% versus 19% and 8%, respectively). Nonrelapse mortality was also marginally significantly affected by the preparative regimen ($P = 0.9$). The overall disease-free survival for this group was only 19%, which was markedly lower than some of the later studies described above. This may reflect the fact that a majority of the patients analyzed (77 of 110) had as their primary malignancy a hematological neoplasm. Recent evidence suggests that the prognosis of secondary leukemias is quite different for patients with previous hematological neoplasms versus solid tumors (3, 167). Pullarkat et al., for example, analyzed data from patients with therapy-related myeloid neoplasms subsequent to adjuvant chemotherapy for breast cancer who received HSCT (167). Of the 12 patients who underwent SCT (11 allogeneic and 1 autologous), 11 were in remission at a median follow-up of nearly 2 years.

In patients for whom a suitable donor is not available, there is evidence that autologous HSCT can be an effective treatment for therapy-related myeloid neoplasms. In 2006, Kroger and colleagues carried out a retrospective analysis of 65 patients with therapy-related myeloid neoplasms who underwent autologous HSCT and were reported to the EMBT (168). Overall survival and disease-free survival following autologous HSCT (35% and 32%, respectively) were comparable to results obtained for allogeneic HSCT (35% disease-free survival) (161). Similar to allogeneic transplantation, significant indicators of outcomes were age and complete remission at time of transplantation. There is some evidence that autologous versus allogeneic transplantation in patients with therapy-related myeloid neoplasms may be associated with slightly improved outcomes in certain carefully selected patients (163, 168). The results of an alternative approach, the use of cells harvested prior to the development of the secondary malignancy, were recently reported for six patients with therapy-related myeloid neoplasms. The results indicated that early stored SCT may provide prolonged survival in some patients with therapy-related myeloid neoplasms (169). The safety/efficacy of an inductive chemotherapeutic regimen consisting of cytarabine/mitoxantrone prior to autologous transplantation for patients with therapy-related myeloid neoplasms is currently being evaluated in a nonrandomized, open-label phase II clinical trial. Enrollment will be capped at 35 subjects, and the study is expected to be completed in 2010. Primary outcomes include remission rate, toxicity, disease-free survival and overall survival (170).

Novel chemotherapeutic strategies

Standard AML treatment (i.e., induction followed by consolidation therapy) for therapy-related myeloid neoplasms is complicated by several factors, including poor hematopoietic reserves due to prior chemotherapy or myeloablative regimes. Patients tend to be more resistant to standard agents, and many cannot tolerate the toxicity associated with treatments used to overcome resistance. In addition, many of the agents that are used for second-line therapy are the same as those used for first induction therapy, with lower response rates. That being said, a recent retrospective review of outcomes of patients who undergo aggressive induction therapy for therapy-related myeloid neoplasms suggests that some patients, particularly those with a low-risk profile (i.e., fewer comorbidities, younger age), can not only tolerate such a regimen, but may have a higher rate of complete remission (171). Unfortunately, durability (i.e., long-term survival) was not affected by aggressive induction therapy, indicating that novel approaches to improve the durability of responses are needed. A prospective study of the effect of a priming regimen consisting of low-dose cytarabine, homoharringtonine and G-CSF (CHG) in patients with advanced MDS or therapy-related AML found that CHG as an induction therapy was well tolerated and effective (46.9% complete remission), but that more effective subsequent chemotherapy is needed to improve overall survival (172).

A number of agents that target various components of mitogenic/proliferative signaling pathways are currently in clinical trials for MDS and AML, including therapy-related myeloid neoplasms. One group of agents within this loosely defined category are the farnesyltransferase inhibitors (FTIs; i.e., lonafarnib and tipifarnib), which target protein substrates of protein farnesyltransferases, including, potentially, Ras proteins, which are frequently dysregulated in MDS/AML. Dose–response and safety/efficacy trials of tipifarnib in patients with acute or refractory/relapsed AML show that the drug is well tolerated and safe (173). In a recent phase II trial in 136 poor-risk (elderly, adverse cytogenetics) patients who were ineligible for conventional induction chemotherapy and would otherwise have received palliative or supportive care, oral tipifarnib resulted in a complete response rate of 15% and an overall response rate (complete + partial) of 34%. The overall response rate in patients > 75 years of age was 30%. A phase II trial of tipifarnib in patients with high-risk MDS also showed promise, with an overall response rate of 34% (174). Results of a prospective phase II trial of lonafarnib in 16 patients with MDS or therapy-related AML were somewhat less encouraging. Lonafarnib showed limited activity in this study, and gastrointestinal toxicities necessitated a decrease in dose from the initial design. Interestingly, of the two patients with partial responses, one had secondary AML (175). Clearly, additional trials are needed to evaluate the effects of these drugs specifically in patients with therapy-related myeloid neoplasms.

Other agents that target signal transduction pathways currently in clinical trials for MDS and AML, including patients with therapy-related myeloid neoplasms, include sorafenib, a small-molecule inhibitor of Raf kinase, and AZD-6244 (selumetinib), an inhibitor of MEK kinase. In a phase I clinical study in 42 patients with MDS or AML (including therapy-related AML), sorafenib was well tolerated at a dose of 300 mg b.i.d. (176). A phase II safety/efficacy trial of sorafenib in patients with MDS, including those with therapy-related MDS, is currently enrolling. Several other phase I/II clinical trials of sorafenib in combination with other chemotherapeutic agents (i.e., cytarabine) or the histone deacetylase inhibitor vorinostat in patients with AML or high-risk MDS are under way.

An interesting new class of agents that is currently being explored in MDS/AML, including therapy-related myeloid neoplasms, are the proteasome inhibitors. Proteasomal degradation is essential to cellular homeostasis. Thus, proteasome inhibitors have been explored as potential cell cytotoxic agents. Bortezomib is a proteasome inhibitor that has been shown to be effective in multiple myeloma patients. The safety and tolerability of bortezomib in combination

with tipifarnib in patients with acute AML, including patients with therapy-related AML, who are unfit for conventional therapy are currently being evaluated in a phase II clinical trial.

Gemtuzumab ozogamicin is a conjugate of an anti-CD33 antibody and the toxin calicheamicin that is currently approved for the treatment of elderly (> 60 years) patients with AML. While gemtuzumab ozogamicin as monotherapy and in combination with other agents continues to be investigated in different populations (MDS, de novo AML, children with relapsed/refractory AML), differential analyses of patients with therapy-related myeloid neoplasms have not been reported (177-179). Several current clinical trials of gemtuzumab ozogamicin in combination with standard chemotherapy (i.e., cytarabine and daunorubicin hydrochloride) are under way and enrolling patients with therapy-related AML. The observation that gemtuzumab ozogamicin is well tolerated and shows therapeutic promise in patients with poor-risk profiles is encouraging in terms of its potential efficacy in the treatment of therapy-related myeloid neoplasms.

Epigenetic changes associated with MDS/AML, such as dysregulated methylation status, have prompted an active area of investigation into inhibitors of DNA methyltransferases (hypomethylating agents) and histone deacetylases, either alone or in combination, in the treatment of de novo and therapy-related MDS/AML, including relapsed/refractory disease. The hypomethylating agent decitabine is currently indicated for the treatment of MDS, including therapy-related MDS. The results of a phase II study of decitabine as a single agent in older patients (≥ 60 years) with previously untreated AML, including those with therapy-related AML, were recently reported (180). Of 33 patients enrolled, 15 had therapy-related AML. The response rate was similar for de novo and therapy-related AML. Most clinical trials focus on patients with high-risk MDS/AML, refractory/relapsed MDS/AML or elderly patients with previously untreated MDS/AML who are considered ineligible for standard therapy. Some studies include patients with therapy-related MDS/AML as eligible for enrollment, and it is likely that, while not explicitly stated, many others do so as well. Unfortunately, while decitabine, as well as another hypomethylating agent, azacitidine, are both effective in high-risk MDS, 50% of high-risk MDS patients still fail to achieve a meaningful response, and these agents offer only a modest survival benefit.

CONCLUSIONS

While prognosis remains rather grim for patients with therapy-related myeloid neoplasms, clinical studies of novel agents, particularly the hypomethylating agents, and combinations of agents continue to offer small but meaningful improvements in response rates and survival. Improvements in pre- and post-transplantation management also offer some hope for patients. Cytogenetics has been shown to be the most important prognostic indicator for several treatment regimens, and the ability to eventually predict which treatments will be most effective based on a patient's karyotype is one of the ultimate goals of therapy. There is increasing recognition that different treatment approaches will likely be more appropriate for young versus elderly patients, the latter being typically eligible for only palliative and supportive care. Novel agents, such as the signal transduction inhibitors and hypomethylating agents, offer the promise of improved outcomes, but are also aiding in our understanding of the mechanism of disease in therapy-related myeloid neoplasms. Elucidation of the underlying mechanisms of the development of therapy-related myeloid neoplasms remains a high priority in managing and preventing one of the most devastating complications of cancer recovery.

DISCLOSURES

The authors state no conflicts of interest.

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