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Treatment of therapy-related myeloid neoplasms with high-dose cytarabine/mitoxantrone followed by hematopoietic stem cell transplant

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Abstract
Few clinical protocols have focused on patients with therapy-related myeloid neoplasms (t-MN). Therefore, we enrolled 32 patients with previously untreated t-MN on a clinical trial testing the effectiveness of a unified induction regimen of high-dose cytarabine and mitoxantrone. The complete remission (CR) rate was 66% (95% CI 47–81%) and the partial remission (PR) rate was 16% (95% CI 5–33%), for an overall response rate of 82%. Day 30 treatment mortality was 9% (3/32), and the most serious induction toxicity was cardiac dysfunction. Among the patients with CR, 13 (62%) received consolidation therapy using an allogeneic hematopoietic cell transplant (HCT), four (21%) received an autologous HCT, and three (16%) received further chemotherapy. We observed long-term disease-free survival in patients who received all three types of consolidation therapy. The remission induction of high-dose cytarabine and mitoxantrone for t-MN is a well-tolerated efficacious combination, which allows aggressive consolidation and long-term disease-free survival.

Keywords: *Therapy-related myeloid neoplasm, high-dose cytarabine, mitoxantrone*

Abbreviations: *Allo=allogeneic; Auto=autologous; ALL=acute lymphoblastic leukemia; AML=acute myeloid leukemia; ANC=absolute neutrophil count; Ca=cancer; Chemo=chemotherapy; CAD=coronary artery disease; CBC=complete blood count; CCyR=complete cytogenetic remission; CHF=congestive heart failure; CI=confidence interval; CLL=chronic lymphocytic lymphoma; COPD=chronic obstructive pulmonary disease; CR=complete remission; DFS=disease-free survival; F=female; HiDAC=high-dose cytarabine; HCT=hematopoietic cell transplant; HLA=human leukocyte antigen; IV=intravenous; M=male; MTX=methotrexate; MUD=matched unrelated donor; N=no; N/A=not assessed; NE=not evaluable; NHL=non-Hodgkin lymphoma; Plts=platelets; PR=partial remission; RD=resistant disease; RT=radiation therapy; t-MN=therapy-related myeloid neoplasm; Tx=transplant; Wbc=white blood cell; WHO=World Health Organization; Y=yes.*

Introduction

Therapy-related myelodysplastic syndrome (t-MDS) and therapy-related acute myeloid leukemia (t-AML) are late complications of cytotoxic therapies used to treat malignant and, increasingly, non-malignant conditions. Based on clinical, morphological, and genetic features, t-MDS and t-AML represent a leukemic spectrum that is recognized by the World Health Organization (WHO) in a singular classification, therapy-related myeloid neoplasms (t-MN) [1]. Morphologically, t-MN most often resembles AML with multilineage dysplasia, also a distinct form of *de novo* AML within the WHO classification.

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The characteristics of t-MN and the timing of its development after a primary diagnosis depend on the exposure to specific agents, the cumulative dose, and dose intensity of the preceding cytotoxic therapy. The classic form of t-MN typically follows 5–7 years after treatment with alkylating agents and/or radiation therapy, and often has complex cytogenetic abnormalities, including loss of part or all of chromosomes 5 and/or 7 [2]. t-MN following chemotherapy with topoisomerase II inhibitors is characterized by translocations involving the *MLL* gene at chromosome band 11q23, or the *RUNX1* (*CBFA/AML1*) gene on chromosome band 21q22, and often occurs within 3 years of the first cytotoxic therapy, rapidly presenting with a high white blood cell count without a myelodysplastic phase [3]. t-MN is being diagnosed increasingly in patients treated for non-malignant primary conditions, either rheumatologic or following solid organ transplant, for which patients had received immunosuppressive therapies not previously thought to cause DNA damage directly [4]. Thus, a growing body of work suggests that the use of any compound that could damage DNA directly, interfere with DNA repair, or suppress the immune system's ability to detect malignant cells increases the risk of t-MN.

There is no standard remission induction therapy for patients with t-MN, nor is there consensus regarding post-remission therapy. Few studies have examined the remission induction rates and outcomes of patients with t-MN exclusively. Clinical trials most often exclude these patients, or include them along with other high-risk patients, such as those who have transformed from a previous myelodysplastic or myeloproliferative disease [5]. Where data are available from prospective clinical trials, the complete remission (CR) rate following standard $7+3$ regimens using cytarabine and an anthracycline appears to be 40–50% among selected patients. Outcomes for patients with t-MN undergoing hematopoietic stem cell transplant (HCT) have been reported in small series, but these patients have often received a variety of induction regimens [6–10].

Because t-MN is a distinct clinical entity, we developed a uniform prospective treatment approach for patients with this disease. We used a single induction regimen, high-dose cytarabine with mitoxantrone, followed whenever possible by HCT. We demonstrate here that it is possible to collect autologous stem cells from selected patients in remission from t-MN, and furthermore, that these stem cells engraft and provide durable and adequate graft function. Our overall approach was efficacious and well tolerated, even in older patients and in those with comorbidities, providing support for treatment strategies with curative intent.

Materials and methods

Study subjects

The trial was reviewed and approved by the Institutional Review Board (IRB) at The University of Chicago, and patients provided written informed consent. To qualify for enrollment, patients had to be $\geq$10 years of age and had to have received prior cytotoxic chemotherapy, radiation, or a drug known to affect the properties of DNA or cell growth for some non-myeloid disorder. All patients underwent a pre-treatment bone marrow biopsy with hematopathology review and cytogenetic analysis. All cases were categorized according to WHO criteria [1]. Exclusion criteria included prior therapy for t-MN, uncontrolled medical disease, positive human immunodeficiency virus (HIV) serology, and women who were pregnant or breast-feeding. The primary endpoint of this clinical trial was the remission induction rate. Secondary endpoints included toxicity, relapse-free survival, overall survival, and the feasibility of autologous stem cell collection.

Treatment and dose modifications

All study subjects were registered with the Protocol and Data Management Office. Induction chemotherapy consisted of preservative-free high-dose cytarabine (HiDAC) at 3000 mg/m^2 in 1000 mL D5W (5% dextrose in water) by intravenous (IV) infusion over 4 h, once on day 1 and once on day 5 (for a total of two doses) followed by mitoxantrone 30 mg/m^2 in 100 mL D5W by IV infusion through a central line over 60 min, starting immediately after the end of each dose of HiDAC, once on day 1 and once on day 5 (for a total of two doses). This regimen was based on studies of leukemia cell dynamics [11]. Dose reductions of HiDAC and mitoxantrone to 2000 mg/m^2 and 20 mg/m^2, respectively, were specified for three patients over the age of 70 years. One younger patient with a left ventricular ejection fraction of 38% also had a reduction in mitoxantrone. Ancillary care included dexamethasone 0.1% eye drops, two drops to each eye every 6 h for 48 h after each HiDAC dose, and allopurinol 300 mg once daily for at least 7 days (for non-allergic patients with normal renal function). Prophylactic antibiotics and antiemetic drugs were administered according to institutional guidelines.

Patients with a suitable human leukocyte antigen (HLA)-matched donor proceeded to an allogeneic HCT after initial cytoreduction whenever possible. Patients without such a donor proceeded to an autologous transplant only if CR was achieved. Patients without suitable allogeneic donors and those who had an inadequate response to induction

chemotherapy were removed from the study treatment and followed. A minimum of 2 weeks was required between peripheral blood count recovery and administration of chemotherapy to mobilize hematopoietic stem cells.

Mobilization chemotherapy consisted of preservative-free HiDAC 2000 mg/m^2 in 250 mL of D5W by IV infusion over 2 h, every 12 h for six doses, together with etoposide 5 mg/kg by continuous IV infusion over 12 h, every 12 h for six doses, beginning on day 1, for a total cumulative dose of 30 mg/kg [12]. Granulocyte colony-stimulating factor (G-CSF) 10 μg/kg was given subcutaneously daily, beginning on day 14 until completion of leukapheresis. Apheresis was begun when the total white blood cell count reached $>10\ 000/\mu$L, and the peripheral blood CD34+ cell count was $>10/\mu$L. The target yield for collection of autologous stem cells was 4×10^6 CD34+cells/kg. Stem cells were cryopreserved using standard techniques according to institutional guidelines.

The preparative regimen for autologous HCT consisted of busulfan given at a dose of 3.2 mg/kg by IV infusion via central venous catheter over 3 h daily for 4 days beginning on day -7 (for a total of 12.8 mg/kg) followed by etoposide 60 mg/kg IV over 4 h on day -3. Busulfan was dosed according to ideal body weight or adjusted ideal body weight (ideal body weight + 25% of the difference between ideal and actual body weights), whichever was lower. Seizure prophylaxis was administered with Dilantin 300 mg p.o. daily, beginning 24 h prior to the first busulfan dose and continuing until 48 h after the last busulfan dose. Autologous stem cell reinfusion occurred on day 0, and G-CSF 5 μg/kg/day was administered subcutaneously beginning on day 0 and continuing until the neutrophil count was $\geq 1500/\mu$L for 2 days or $\geq 5000/\mu$L for 1 day. The preparative regimens for allogeneic HCT were not specified and varied, usually performed on other IRB-approved protocols at The University of Chicago for patients who had appropriate HLA-compatible donors [13–16].

Assessment of response

To assess initial cytoreduction from induction chemotherapy, a bone marrow exam was performed 12–14 days after beginning chemotherapy, and a second bone marrow exam was performed after peripheral blood count recovery, defined by an absolute neutrophil count $>1500/\mu$L, hemoglobin >10 g/dL, and platelet count $>100\ 000/\mu$L, which generally occurred between days 28 and 30. If patients had not achieved peripheral count recovery by days 35–40, a bone marrow exam was performed to assess bone marrow cellularity and disease state.

Response criteria were defined as: CR, $<5\%$ bone marrow blasts with recovery of peripheral blood counts as above; complete cytogenetic remission (CCyR), the disappearance of any pre-existing cytogenetic abnormality; partial remission (PR), $>5\%$ bone marrow blasts, but less than the pre-treatment blast percentage within the bone marrow; resistant disease (RD), no significant cytoreduction in bone marrow leukemic cells from pre-treatment levels; and not evaluable (NE), patients who died during induction chemotherapy or who withdrew from follow-up before assessment could be made.

Similarly, after autologous HCT, a bone marrow exam was performed after peripheral blood count recovery, or between days 28 and 30 to determine remission status. The same response criteria were used as after induction chemotherapy. Time to engraftment was achieved for neutrophils on the first day of an absolute neutrophil count $>500/\mu$L for three consecutive days, and $>20\ 000/\mu$L without transfusion support for platelets. Following autologous transplantation, a bone marrow biopsy was performed approximately every 4 months to evaluate for recurrent disease. Patients were followed until death. Relapse was defined as bone marrow blasts $>5\%$ if the patient had achieved a CR, or the recurrence of any clonal cytogenetic abnormality. Median follow-up for surviving patients was 306 days (maximum 1972+). Patient status was current as of July 2009.

Toxicity assessment

While receiving chemotherapy in the hospital, each patient underwent a daily assessment of toxicity with electrolyte and renal studies and complete blood counts (CBCs). Liver function tests, lactate dehydrogenase, and uric acid levels were obtained twice per week. Grade 3 or greater toxicities were captured. Treatment-related mortality was defined as any death not due to relapse and before subsequent consolidation therapy was administered. Comorbidity assessment was made according to the Charlson Comorbidity Index [17]. t-MN itself was not assigned a score, since it was the index condition, but prior malignancies were scored.

Statistical methods

Categorical variables were summarized using frequency and percentage. Mean and standard deviation were reported for normally distributed continuous variables; for variables with a skewed distribution, median and range were reported. Overall survival (OS) was defined as the time between the first day of induction therapy and death. For CR

patients, relapse-free survival (RFS) was defined as the time between the first day of induction therapy and initial failure, either relapse or death from any cause, whichever occurred first. The Kaplan–Meier estimator was used to describe OS and RFS graphically for patients with different transplant types [18]. From these estimates, the median survival and survival rates at 12 months were estimated. Cox proportional hazards (PH) regression analysis was used to test the effect of transplant type on OS and RFS [19]. Since transplant did not take place at the start of the induction therapy, it was treated as a time-dependent covariate in the Cox PH regression analysis.

Results

Remission induction

Thirty-two previously untreated patients with t-MN were enrolled on this clinical trial between February 2003 and February 2009 at The University of Chicago. During this period, we evaluated 116 patients with t-MN at our center, 60 of whom were eligible for participation in this trial. Among the eligible patients, 28 did not enter this trial: 11 enrolled in other clinical trials, seven sought only consultation and received care locally, six refused participation in a clinical trial, and four received therapy off-protocol. Therefore, the patient population enrolled on this clinical trial represented about 50% of eligible t-MN patients seen at our facility. Patients' baseline characteristics are listed in Table I. Median age was 56 years old (range 23–83), and 38% were >60 years old. Fifteen patients (47%) had a Charlson Comorbidity Index [17] of ≥ 3, indicating that they were at high-risk for toxicity from the treatment, due either to older age or underlying conditions. In 28 patients (88%), t-MN developed following cytotoxic therapy for a malignant disease, following rheumatologic disease in two (6%), and with immunosuppressive therapy after solid organ transplants in two patients (6%). The latency interval between patients' primary cytotoxic treatment and development of t-MN was highly variable, from less than 1 year to more than a decade, with a median latency of 3.6 years (range 0.9–23 years). The greatest fraction of patients (28%) experienced a latency of 4–9 years, and in eight patients (25%), the latency was 2 years or less.

Table II summarizes the cytogenetic abnormalities seen in our cohort. An abnormal karyotype was seen in 24 patients (81%), with a complex karyotype observed in 11 (35%). Among patients with a clonal cytogenetic abnormality, only 14 (45%) had abnormalities of chromosomes 5 or 7, leading to loss of 5q or 7q, or both. Nine patients (29%) had recurring or non-recurring balanced rearrangements, including t(9;11) in five patients, and t(8;21), inv(16), t(6;9), or t(6;11)(q21;q23), each observed in one patient. Five t-MN patients (16%) had a normal karyotype.

The response to induction chemotherapy is summarized in Table III, and 28 patients were fully evaluable for anti-leukemic response. Overall, 21 patients (66%, 95% CI 47–81%) attained a CR, including one patient who was treated at a reduced dose. Five patients (16%, 95% CI 5–33%) achieved a PR, two patients (7%) had chemotherapy resistant disease (RD), and four patients were not evaluable, three because they died during induction and a fourth who did not undergo bone marrow evaluation following chemotherapy. Out of the 21 patients who achieved a CR and had detectable cytogenetic abnormalities prior to induction therapy, 10 (48%) achieved a CCyR, including three patients who had a complex karyotype. Fifty-seven percent of patients with cytogenetic abnormalities of chromosomes 5 and/or 7 had a CR, compared to 67% of patients with balanced translocations, and 100% of patients with a normal karyotype, reflecting previous findings from the German AML Cooperative Group (AMLCG) study that cytogenetic risk group is a strong prognostic indicator of survival for patients with t-MN [20].

All patients were evaluable for toxicity of induction chemotherapy (Table IV). Grade 3–4 cytopenias and infection were common. Mucositis was uncommon. Non-infectious diarrhea occurred in 16% of patients. An estimate of ejection fraction was made for each patient prior to initiation of induction chemotherapy, and was re-determined based on patient symptomatology. Of the four patients (13%) who developed left ventricular systolic dysfunction after induction therapy, one had a reduced ejection fraction of 43% at baseline and received a reduced dose of mitoxantrone along with desrazoxane. The other three had normal cardiac function at baseline, although one patient had documented coronary artery disease, and the other two had received doxorubicin for their preceding malignancies (patient 18, 500 mg; and patient 26, 550 mg). All three of these patients received full doses of mitoxantrone. The decrease in ejection fraction was highly variable: one patient had an absolute decrease of $<10\%$, one declined by 20%, and two had severely depressed function to $<20\%$.

Five patients died during or after induction chemotherapy: one patient (patient 29) without a history of coronary artery disease experienced cardiogenic shock while neutropenic and died on day 15; one (patient 27) suffered an intracranial hemorrhage after falling while pancytopenic on day 23; and

Table I. Patient data.

Patient study ID	Age/sex at diagnosis	Primary disease	Primary therapy	Latency (years)	Significant comorbidities	Charlson Comorbidity Index: total combined score	Cytogenetic abnormalities	Response to induction	Post-remission therapy	Relapsed? Y/N	Disease-free survival (days)	Survival at last follow-up (days)
1	43F	Breast Ca	Chemo/RT	5.7		0	t(6;9)	CR	Allo-matched sibling	Y	377	827
2	56F	Breast/ovarian Ca	Chemo	8.6	Diabetes mellitus	2	Complex, including −2,der(7),−16, der(19)t(16;19)	RD	No transplant	N	N/A	103
3	49M	Nasopharyngeal Ca	RT	2	Chronic sinusitis	2	None	CR*	No transplant	N	1938+	1972+
4	48F	Breast Ca	Chemo	3	Psoriasis	2	t(9;11)	PR	No transplant	N	67	101
5	63F	Lupus	MTX	6.1		2	Complex, including del(5),−7,t(12;17), add(19),add(13),−3	CCyR	No transplant	Y	32	94
6	37M	Hodgkin lymphoma	Chemo/RT	5.4		0	−6,−7	CCyR	Allo-MUD	N	1755+	1798+
7	45F	Breast Ca	Chemo	2.2		2	t(9;11)	CCyR	Allo-MUD	N	1591+	1625+
8	56M	Renal transplant	Mycophenolate mofetil	4.4	Renal disease, hypertension	3	None	CR*	Allo-matched sibling	Y	380	528
9	23M	ALL	Chemo	6		0	−7	PR	Allo-matched sibling	N	530	568
10	68M	CLL	Chemo	7.6		2	Complex, including −5,inv(7),r(7), t(17;22),t(9;19)	CCyR	Autotransplant	Y	95	353
11	65M	Heart transplant	Cyclosporine, azathioprine, prednisone	15.4	Diabetes mellitus, heart transplant	6	−7,del(11)	CCyR	Autotransplant	N	349	400
12	63M	Esophageal Ca	Chemo/RT	6.6	Hypertension, diabetes mellitus, CAD	3	Complex, including del(5),del(11), idic(21),add(22)	NE	No transplant	N	N/A	28
13	41F	Breast Ca	Chemo/RT	0.9		2	t(9;11)	PR	Allo-haplocord	N	77	127
14	50F	Crohn's disease	Azathioprine, azulfidine, infliximab	2.6	Crohn's disease	1	None	CR*	Allo-mismatched related	N	197	236
15	62F	NHL	Chemo/ ibritumomab	9.9	Mild COPD	3	Complex, including t(5;11),+8, add(10),−11	CR	Allo-MUD	N	280	332
16	46F	Breast Ca	Chemo/RT	3.2		2	t(8;21)	CCyR	Allo-matched sibling	N	1148+	1183+
17	67F	Breast Ca	Chemo/RT	1	Hypertension	4	None	CR*	No transplant	N	26	68
18	67F	Breast Ca	Chemo/RT	6.5	Hypertension	2	Complex, including −5,del(7), del(1),del(3),−12	CCyR	Allo-MUD	Y	76	807
19	33F	Breast Ca	Chemo/RT	1.2	Spina bifida	2	t(9;11)	CR	Allo-double cord	Y	103	673
20	52M	Melanoma, Prostate Ca, sarcoma	RT	1.8	Hypertension, coronary artery disease	3	inv(16)	CCyR	Autotransplant	N	883+	917+

(*continued*)

Table I. (*Continued*).

Patient study ID	Age/sex at diagnosis	Primary disease	Primary therapy	Latency (years)	Significant comorbidities	Charlson Comorbidity Index: total combined score	Cytogenetic abnormalities	Response to induction	Post-remission therapy	Relapsed? Y/N	Disease-free survival (days)	Survival at last follow-up (days)
21	83M	NHL, Prostate Ca	Chemo	1.1	Hypertension, chronic renal insufficiency, CAD	8	+13	NE	No transplant	N	N/A	233
22	36M	Hodgkin lymphoma	Chemo	1.7		2	None	CR*	Allo-matched sibling	N	692+	732+
23	66M	Esophageal/ Prostate Ca	Chemo/RT	2.7		5	Complex, including inv(9), der(5)t(5;6), −6,der(7), t(7;15),del(11)	PR	No transplant	Y	56	164
24	44M	NHL	Chemo	8.4		0	del(7)	PR	Allo-matched sibling	N	43	74
25	65F	Breast Ca	Chemo/RT	1.6		4	Not analyzed	CR*	Autotransplant	Y	385	519
26	56F	NHL	Chemo/RT	14.6	COPD	3	Complex, including add(5),r(7),−16, add(19),−21, −22	RD	No transplant	N	N/A	63
27	77M	Prostate Ca	RT	2		5	Complex, including del(5),−17, add(7),−18, add(15)	NE	No transplant	N	N/A	23
28	78F	Hodgkin lymphoma	Chemo	3.2	Hypertension	5	−7	CCyR	No transplant	Y	252	401
29	53F	ALL	Chemo/RT	2.7	Diabetes mellitus	5	t(6;11)(q21;q23)	NE	No transplant	N	N/A	15
30	55F	Multiple myeloma	Chemo	4		3	Complex, including −3,−4,−5, add(6),−7,−8, −9,−13,−15,−16, −17,−20	CCyR	Allo-haplocord	N	212+	280+
31	59M	Lymphoplasmacytic lymphoma	Chemo	5.1	CHF with chronic diastolic dysfunction	3	t(9;11),del(11)(q13;q23)	CCyR	Allo-matched sibling	N	126+	180+
32	49F	Hodgkin lymphoma	Chemo	23	Hypothyroidism	2	Complex, including del(5),dic(7;12),add(11)	CR	Allo-haplocord	N	100+	140+

*Cytogenetic remission could not be determined, since either no detectable cytogenetic abnormalities were present at diagnosis or baseline cytogenetic data were not available (patient 25).

ALL, acute lymphoblastic leukemia; Allo, allogeneic; Ca, cancer; CAD, coronary artery disease; Chemo, chemotherapy; CHF, congestive heart failure; CLL, chronic lymphocytic lymphoma; CCyR, complete cytogenetic remission; COPD, chronic obstructive pulmonary disease; CR, complete remission; F, female; M, male; MTX, methotrexate; MUD, matched unrelated donor; N, no; NE, not evaluable; N/A, not assessed; NHL, non-Hodgkin lymphoma; PR; partial remission; RD, resistant disease; Y, yes.

one (patient 12) had a history of coronary artery disease and suffered a myocardial infarction at day 28. Two deaths (patients 4 and 5) occurred after they were discharged following induction chemotherapy, both due to sepsis while neutropenic. Patient 4 died with *Enterococcus faecium* sepsis on day 101, and patient 5 had presumed *Aspergillus* sepsis and died on day 94.

Post-remission therapy

Allogeneic HCT had the highest priority for t-MN patients on this study, and several sources of allogeneic stem cells, including matched related, matched unrelated, umbilical cord, and HLA-haploidentical cells combined with umbilical cord blood cells, were used [13–16]. The median time from remission induction to allogeneic HCT was 88 days (range 61–503 days). Thirteen of 21 patients (62%) who achieved CR underwent allogeneic HCT (see 'Appendix,' Table SI). In addition, two of five patients (60%) who had achieved PR proceeded directly to allogeneic HCT without additional chemotherapy, and one of the five (patient 24) received high-dose cytarabine and etoposide prior to allogeneic HCT (Table SI).

For patients who were not candidates for allogeneic HCT, due to older age, lack of an available donor, or patient choice, we tested whether adequate autologous stem cells could be collected following an additional course of chemotherapy. Only patients in CR were candidates for autologous stem cell collection. In several additional cases, autologous stem cells were collected as a back-up stem cell source prior to undergoing an allogeneic HCT, or when it was uncertain whether an HLA-compatible donor would be available.

Of the 10 patients in CR who underwent mobilization chemotherapy, we were able to collect adequate numbers of autologous stem cells in seven (70%), as shown in Table V. The total number of stem cells collected ranged from 2.9×10^6 CD34+ cells/kg to 30.3×10^6 CD34+ cells/kg. Cytogenetic analysis was not performed routinely on cells collected during apheresis. Mobilization chemotherapy was well tolerated and most toxicities were related to cytopenias (Table IV). Grade 3–4 thrombocytopenia was seen in three patients and may have contributed to gastrointestinal hemorrhage in one patient. One patient died suddenly at home while pancytopenic after

Table II. Cytogenetic abnormalities in 31 patients* with t-MN.

Karyotype	Number (%)
Normal karyotype	5 (16)
Abnormal karyotype	25 (81)
Complex karyotype	11 (35)
Clonal abnormalities†	26 (84)
Clonal abnormalities of chromosome 5, 7, or both (± other abnormalities)	14 (45)
Abnormal chromosome 5	3 (10)
Abnormal chromosome 7	5 (17)
Abnormal chromosomes 5 and 7	6 (19)
Recurring balanced rearrangements	
t(6;9)(p23;q34)	1 (3)
t(8;21)(q22;q22)	1 (3)
t(9;11)(p22;q23)	5 (16)
inv(16)(p13.1q22)	1 (3)
Non-recurring balanced rearrangements	
t(6;11)(q21;q23)	1 (3)
Trisomies	
+8	1 (3)
+13	1 (3)

*One patient was not studied prior to treatment.
†≥3 unrelated clones.
t-MN, therapy-related myeloid neoplasms.

Table III. Responses to induction chemotherapy with high-dose cytarabine and mitoxantrone.

	CR (%)	PR (%)	RD (%)	NE (%)	Total
All patients	21 (66)	5 (16)	2 (6)	4† (13)	32
Dose of induction chemotherapy					
Full dose	20 (63)	5 (16)	2 (6)	1 (3)	28
Reduced dose	1 (25)	0 (0)	0 (0)	3 (75)	4
Cytogenetic abnormalities*					
Normal	5 (100)	0 (0)	0 (0)	0 (0)	5
Complex	6 (50)	1 (9)	2 (17)	2 (17)	11
Abnormalities of chromosomes 5/7	8 (57)	3 (23)	1 (8)	2 (15)	14
Balanced translocations	6 (67)	2 (22)	0 (0)	1 (11)	9
Other	1 (50)	0 (0)	0 (0)	1 (50)	2

*Among the patients with complete responses, karyotype data were only available for 18 out of the 19 patients.
†There were three induction deaths due to intracranial bleeding, myocardial infarction, and cardiogenic shock. One patient was unable to undergo induction assessment.
CR, complete remission; PR; partial remission; RD, resistant disease; NE, not evaluable.

mobilization chemotherapy. No autopsy was performed, making it difficult to assess causality.

Among the four patients (13%) who underwent autologous HCT, three had achieved a complete cytogenetic remission and one had an indeterminate cytogenetic response to induction chemotherapy. We observed rapid neutrophil engraftment in all four patients. One patient failed to achieve an adequate platelet count, but the other three patients achieved platelet recovery by 12, 20, and 39 days post-autologous transplant. Thus, it is feasible to collect and engraft autologous stem cells in selected t-MN patients. Two patients subsequently relapsed. One patient with t-MN and inv(16) has remained in CR. The fourth patient died in remission 133 days after successful autologous HCT due to infectious complications.

Survival

The median overall survival was 399 days [Figure 1(A)], and overall survival at 1 year was 51%. Overall patient survival was significantly improved for those patients who achieved a CR (median survival, 673 days) compared to those who had a PR (median survival, 126 days) to induction chemotherapy [Figure 1(B)]. Overall survival at 1 year was 74% for patients who had achieved a CR, but was only 20% for patients who had achieved a PR to induction. Median relapse-free survival was 415 days, with 59% of CR patients remaining relapse-free at 1 year [Figure 1(C)].

Table IV. Grade 3–4 toxicities observed in >10% of patients after each treatment course.

	Grade 3–4 toxicity	Number of patients (%)
Induction ($n=32$)	Neutropenic fever	20 (63)
	Infection/sepsis	19 (59)
	Thrombocytopenia	8 (25)
	Left ventricular dysfunction	4 (13)
		Patient 12: 50%→25%
		Patient 18: 43%→36%
		Patient 26: 58%→10–15%
		Patient 29: 54%→16%
	Non-infectious diarrhea	5 (16)
	Anemia	3 (10)
Stem cell mobilization ($n=10$)	Neutropenic fever	4 (40)
	Thrombocytopenia	3 (30)
	Infection/sepsis	3 (30)
Autologous transplant ($n=4$)	Infection/sepsis	3 (75)

The median survival for patients who received an allogeneic HCT was 673 days, compared to 399 days for patients who received an autologous HCT and 93 days for patients who received no transplant [Figure 1(D)]. Overall survival at 1 year was 72% for patients who had undergone an allogeneic HCT, 75% for patients who had an autologous transplant, and 17% for patients who had not received a transplant. However, since patients who underwent HCT did not receive the HCT at the start of induction therapy, these data may not truly indicate that patients who received a HCT survived much longer than those who did not. Therefore, we further analyzed the effect of HCT on survival by treating it as a time-dependent covariate and found that the effect was not statistically significant ($p=0.83$). Similar results were obtained for relapse-free survival. The rate of relapse-free survival at 1 year was 67% for patients who underwent either allogeneic or autologous HCT, compared to 25% for those who did not have a transplant. When patients were not considered candidates for allogeneic HCT, due to either donor availability or patient preference, there may have been selection bias as to which patients received an autologous transplant versus those who went on to chemotherapy-only consolidation. However, the numbers are very low. With a median follow-up time of 29 months for surviving patients, nine patients (28%) remain alive and disease-free: seven (22%) after allogeneic HCT, one (3%) after autologous HCT, and one (3%) after consolidation with only chemotherapy. The median survival for these nine patients is 917 days (range 140–1972+) (Table I).

Discussion

Because of the paucity of prospective treatment data for patients with t-MN and because these patients are often excluded from frontline clinical trials, we sought to determine the remission induction rate and tolerability of a unified chemotherapy regimen for these patients. Our high-dose cytarabine and mitoxantrone combination resulted in a CR rate of 66% and a PR rate of 16% (95% CI 47–81% and 5–33%, respectively), which compare favorably with the remission rates that have been achieved for patients with *de novo* AML who received similar therapy [21–25]. Among these studies, remission rates of 58–64% have been achieved, with remission rates of 70–84% occurring in patients less than 60 years old. Cumulative cytarabine and mitoxantrone doses in these studies ranged from 8000 to 36 000 mg/m^2 and from 60 to 80 mg/m^2, respectively. We performed our trial as a single-center study over a 6-year period, reflecting the relative rarity of this diagnosis. Further

Table V. Stem cell collection and autotransplant.

	Patient study ID	No. of apheresis collections	No. of stem cells collected ($\times 10^6$ CD34$^+$ cells/kg)	No. of stem cells used for autologous HCT ($\times 10^6$ CD34$^+$ cells/kg)	Time to neutrophil engraftment (days to ANC > 500)	Time to platelet engraftment (days to Plts > 20 000/μL)	DFS (days)	Survival post-Auto Tx (days)	Last known status
Patients successfully collected	10	3	2.92	2.92	13	39	95	194	Relapse
	11	5	3.53	3.53	13	N/A (no engraftment)	349	133	Died in remission
	14	1	15.43	0*	N/A	N/A	197	N/A	Died in remission
	15	3	8.93	0*	N/A	N/A	280	N/A	Died in remission
	18	1	11.61	0*	N/A	N/A	76	N/A	Relapse
	20	1	30.31	10.1	11	20	883+	817+	CR
	25	1	5.46	5.46	11	12	385	393	Relapse
		Reason for collection failure							
Patients who failed to collect	3	Wbc count too low							
	17	Sudden death while pancytopenic after mobilization chemotherapy							
	28	Wbc count too low							

*Eventually received an allogeneic HCT.
HCT, hematopoietic cell transplant; ANC, absolute neutrophil count; Plts, platelets; DFS, disease-free survival; N/A, not assessed; Wbc, white blood cell.

studies to evaluate this regimen as a direct comparison to more standard doses of cytarabine and anthracyclines would require multi-institutional cooperation to be feasible. We look forward to other combinations under investigation now (e.g. cytarabine/amonafide) for patients with t-MN [26].

Although the overall percentage of patients with a cytogenetic abnormality was similar to what we have previously reported for our cohort at The University of Chicago [2], the distribution of the cytogenetic abnormalities differed, tending toward patients with a more favorable prognosis. For example, in our overall series, 70% of patients had a clonal abnormality of chromosomes 5 and/or 7, but in this clinical trial, only 45% of patients had these chromosomal abnormalities. Patients were not selected for this clinical trial based upon their karyotype, since we generally were not aware of a patient's cytogenetic abnormalities when the induction regimen was chosen.

Acute left ventricular dysfunction developed in a total of four (13%) patients after induction therapy. Of those, only one had baseline cardiac dysfunction, and desrazoxane was given in addition to a reduced dose of mitoxantrone. Because most of our patients were not treated at our center for their primary disease, we do not have complete records as to how much prior anthracycline each patient had received. Therefore, it is difficult to predict which patients will develop this serious complication of induction, but we would advise caution in using our combined regimen for patients with pre-existing left ventricular dysfunction.

Our findings are similar to published experience of treating patients with AML with mitoxantrone or high-dose daunorubicin, in which cardiac toxicity is seen at the 5–10% range [22,27]. Seiter *et al.* found significant cardiac toxicity in 165 patients with AML treated with multiple rounds of mitoxantrone-based induction chemotherapy for AML [22]. In their study, 25% of patients experienced a decrease in ejection fraction after three cycles of chemotherapy, increasing to 33% of patients by cycle 5. Although our patients received only one cycle of induction therapy, they had all received prior chemotherapy, given the nature of t-MN. A feature common to our experience and published reports is cardiac decompensation with sepsis [22,28], and therefore aggressive management of neutropenic fever is advised. Because successful transplant requires adequate cardiac function, careful attention must be given to the cardiac status of patients treated with our induction regimen.

Our study demonstrates the feasibility of stem cell mobilization and collection in patients with t-MDS/t-AML, since in seven out of 10 patients in whom autologous stem cell collection was attempted, we were able to cryopreserve $\geq 2.0 \times 10^6$ CD34+ cells/kg (Table V). Failure to collect sufficient autologous stem cells from three patients is higher than in other studies that have used high-dose mitoxantrone for stem cell mobilization [29], which may reflect a bias of our small sample size or the nature of

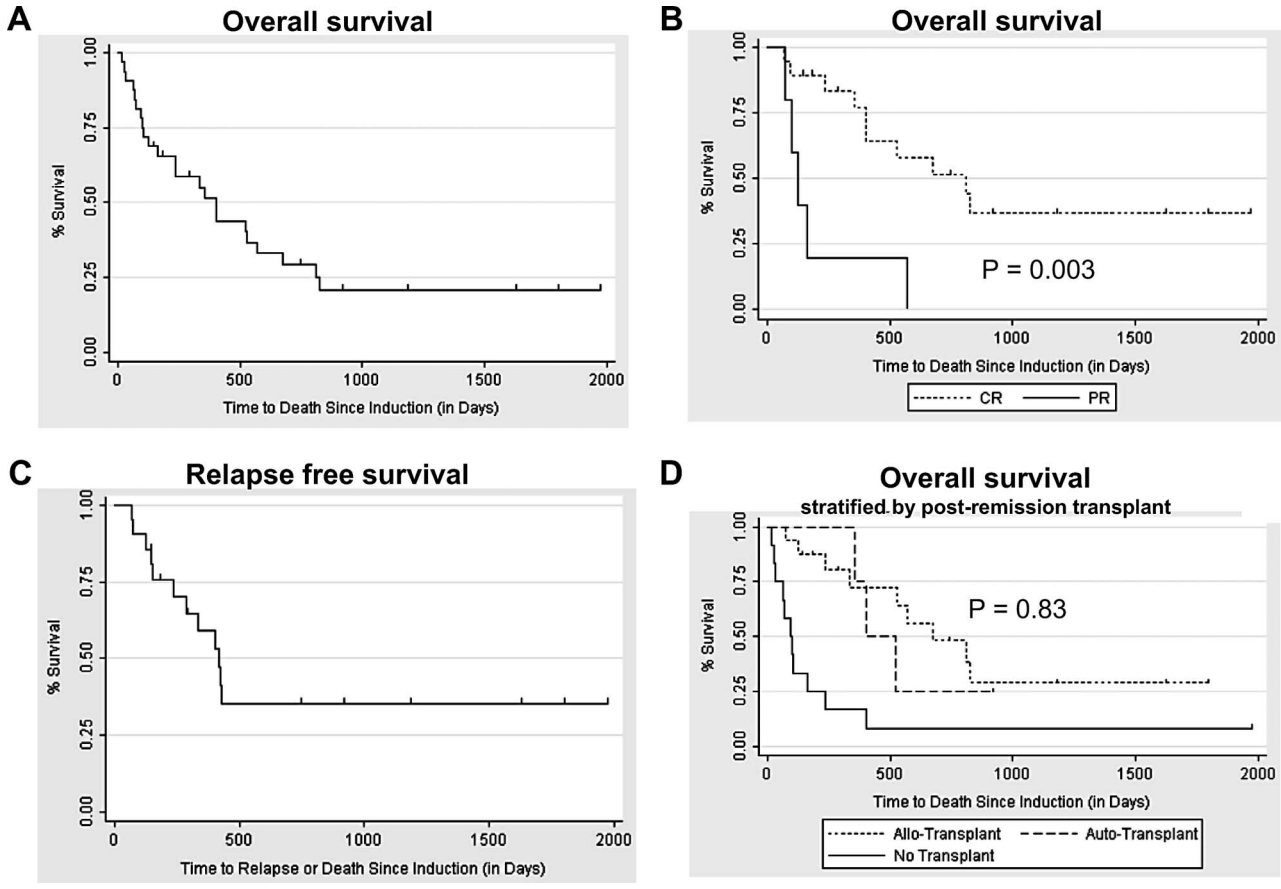

Figure 1. Patient survival. (A) Overall patient survival ($n = 32$). (B) Overall survival based on response to induction chemotherapy. Dashed line, 21 patients who achieved a CR to induction therapy. Solid line, five patients who achieved a PR to induction therapy. (C) Relapse-free survival ($n = 21$). The log-rank test was used to compare the curves, and the p-value is indicated. (D) Survival curves based on post-induction therapy, treating HCT as a time-dependent covariate. Short-dashed line, 16 patients who underwent allogeneic HCT; long-dashed line, four patients who underwent autologous HCT; and solid line, 12 patients who had no transplant. The log-rank test was used to compare the curves, and the p-value is indicated.

pre-treatment with prior therapy in patients with t-MN. Among the four patients who underwent autologous HCT, all four experienced rapid neutrophil engraftment by day +13. However, one patient failed to engraft platelets and died of infectious complications without evidence of t-MN, and two of the other patients took $\geq$20 days to achieve a platelet count above 20 000/μL. Notably, the only patient who has survived long-term after autologous HCT had a good-prognosis AML with inv(16) [30].

Of the 16 patients who underwent allogeneic HCT, nine (56%) are long-term survivors, all of whom had achieved a CR to induction chemotherapy. Among the three patients who had an allogeneic HCT despite only a PR to induction chemotherapy, one patient has had a prolonged survival of more than 500 days. Of the four patients who had an autologous HCT, only one patient survived long-term, and among the three patients who received chemotherapy-only consolidation, we also observed only one long-term survivor. Therefore, although our patient numbers are small, it would appear that the clinical parameter most associated with overall and disease-free survival was the ability to achieve a CR to induction chemotherapy. Once CR was achieved, allogeneic HCT for t-MN provided 29% survival at 3 years, a reasonable outcome given these patients' significant comorbid conditions and extensive prior chemotherapy exposure.

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Appendix

Table SI. Post-induction therapies.

Response to induction	Second induction course	Consolidation chemotherapy	Autologous HCT	Allogeneic HCT
CR ($n=21$)	0	3	4	13 (donors: 5 matched sibling, 4 matched unrelated, 1 mismatched related, 1 double cord, and 2 haplocord)
PR ($n=5$)	0	0	0	3 (donors: 2 matched sibling and 1 haplocord)
RD ($n=2$)	1	0	0	0
Total ($n=28$)*	1	3	4	16

*Four patients were not evaluable, and four patients received no therapy beyond initial induction.
CR, complete remission; PR, partial remission; RD, resistant disease; HCT, hematopoietic cell transplant.