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Secondary Neoplasms After Hematopoietic Cell Transplant for Sickle Cell Disease

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PURPOSE To report the incidence and risk factors for secondary neoplasm after transplantation for sickle cell disease.

METHODS Included are 1,096 transplants for sickle cell disease between 1991 and 2016. There were 22 secondary neoplasms. Types included leukemia/myelodysplastic syndrome (MDS; n = 15) and solid tumor (n = 7). Fine-Gray regression models examined for risk factors for leukemia/MDS and any secondary neoplasm.

RESULTS The 10-year incidence of leukemia/MDS was 1.7% (95% CI, 0.90 to 2.9) and of any secondary neoplasm was 2.4% (95% CI, 1.4 to 3.8). After adjusting for other risk factors, risks for leukemia/MDS (hazard ratio, 22.69; 95% CI, 4.34 to 118.66; *P* = .0002) or any secondary neoplasm (hazard ratio, 7.78; 95% CI, 2.20 to 27.53; *P* = .0015) were higher with low-intensity (nonmyeloablative) regimens compared with more intense regimens. All low-intensity regimens included total-body irradiation (TBI 300 or 400 cGy with alemtuzumab, TBI 300 or 400 cGy with cyclophosphamide, TBI 200, 300, or 400 cGy with cyclophosphamide and fludarabine, or TBI 200 cGy with fludarabine). None of the patients receiving myeloablative and only 23% of those receiving reduced-intensity regimens received TBI.

CONCLUSION Low-intensity regimens rely on tolerance induction and establishment of mixed-donor chimerism. Persistence of host cells exposed to low-dose radiation triggering myeloid malignancy is one plausible etiology. Pre-existing myeloid mutations and prior inflammation may also contribute but could not be studied using our data source. Choosing conditioning regimens likely to result in full-donor chimerism may in part mitigate the higher risk for leukemia/MDS.

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INTRODUCTION

Hematopoietic cell transplantation can restore normal hematopoiesis in those with sickle cell disease (SCD), but complications include graft failure, graft-versus-host disease (GVHD), infection, organ dysfunction, secondary neoplasm, and death.¹ Rates and risks for graft failure, GVHD, and mortality are known from phase II clinical trials and data reported to registries.¹⁻⁹ These studies show that survival is highest in children and after human leukocyte antigen (HLA)-matched sibling transplantation.¹⁻³ Alternative donor transplantation extends access but increases risks for transplant-related complications and mortality.^{1,4-9} The predominant transplant conditioning regimen is myeloablative (busulfan with cyclophosphamide or fludarabine) and complicated by growth failure and infertility.^{1,2,10} Less-intense regimens have lower toxicity early after transplantation but require longer follow-up of recipients to ascertain longer-term sequelae.^{4,5,7,8}

Secondary solid neoplasms after transplantation for malignant and nonmalignant hematologic diseases develop at twice the rate expected on the basis of general population rates (observed to expected ratio, 2.1; 95% CI, 1.8 to 2.5) and reached three-fold for patients followed for ≥ 15 years in one report.¹¹ In that report, risks were higher for patients transplanted age ≤ 30 years, received a radiation-containing conditioning regimen, those who had a history of chronic GVHD, and those who were male regardless of age. Secondary solid neoplasm is also higher after transplantation using busulfan/cyclophosphamide regimen for acute and chronic myeloid leukemia with incidence rates 1.4 times higher than the general population.¹² Risk factors include age (≥ 35 years at transplantation), poor performance score pretransplant, and chronic GVHD.¹² Specific to nonmalignant disease, a large study from the Center for International Blood and Marrow Transplantation in patients surviving at least 2 years after transplantation reported incidence rates of 5.6% for Fanconi anemia, 1.7% for other bone marrow

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CONTEXT

Key Objective

What are the risks for secondary neoplasm after hematopoietic cell transplantation for sickle cell disease (SCD)?

Knowledge Generated

Studying an observational cohort with 6,631 person-years, we observed a 10-year incidence of 2.4% for any secondary neoplasm, especially leukemia and myelodysplastic syndrome, compared with other nonmalignant disease with the exception of Fanconi anemia. The risk was highest after low-intensity total-body irradiation-containing conditioning regimens. Persistence of host cells exposed to low-dose radiation may have triggered leukemia and myelodysplastic syndrome through mechanisms similar to that seen in atomic bomb survivors is likely to be one of several factors contributing to neoplasms. A third of secondary neoplasms were solid neoplasms and most occurred in children age 8-11 years at transplantation and after full-dose busulfan regimens. Full-dose busulfan is the accepted conditioning regimen for gene therapy/editing trials and these findings are of clinical relevance.

Relevance (S. Lentzsch)

The data will provide the foundation to discuss the risk of secondary malignancies after hematopoietic cell transplant for SCD. It will improve the determination of the risk-benefit ratio, especially in the light of the emerging indication of gene therapy for SCD.*

*Relevance section written by JCO Associate Editor Suzanne Lentzsch, MD, PhD.

failure syndromes (1.7%), 1.1% for severe acquired aplastic anemia, and < 1% for all other diseases, including 300 patients transplanted for SCD.¹³ In the nontransplant setting, two population-based studies report an increased lifetime risk for myeloid leukemia for patients with SCD.^{14,15}

There are also case reports of myeloid malignancy in patients with SCD after engraftment failure.¹⁶⁻¹⁸ The increasing numbers of transplants for SCD and use of donors other than HLA-matched siblings, which carry a higher risk for graft failure,^{1,19} led to the current retrospective cohort study to further our understanding of the incidence and risks for secondary neoplasms after transplantation in this population.

METHODS

Data Source

Deidentified records of transplantations for SCD were reviewed from a publicly available data source BioData Catalyst Powered by PIC-SURE.²⁰ Patients or their legal guardians provided consent for research. The study was approved by the Institutional Review Board, National Marrow Donor Program. Patients were transplanted between 1991 and 2016 in the United States. Data were obtained from transplant centers. Patients were followed prospectively from transplantation and relevant data reported using standardized data collection forms until death, loss to follow-up, or last contact through June 2021. Follow-up schedule is as follows: 3, 6, 12, and 24 months after transplantation and thereafter every 2 years. The consent rate at participating sites was 94.5%. As the median time to onset of neoplasm was 40 months (range, 9-196 months), transplantations after 2016 were excluded to limit

incomplete ascertainment of cases and a potential bias from preferential follow-up of patients considered at higher risk for cancer.

Statistical Methods

For each patient, the number of person-years at risk was calculated from the date of transplant until last contact, diagnosis of cancer, or death, whichever occurred first. The incidence of secondary neoplasm was calculated by treating death as a competing risk.²¹ Fine-Gray regression models examined for risk factors associated with leukemia or myelodysplastic syndrome (MDS) and any secondary neoplasm.²² Definition of conditioning regimen intensity used published criteria²³: myeloablative (busulfan with cyclophosphamide or fludarabine); reduced-intensity (fludarabine and melphalan [≤ 140 mg/m²] with or without alemtuzumab, thiotepa, or 200 cGy total-body irradiation [TBI]); and low-intensity (nonmyeloablative; TBI 300 or 400 cGy with alemtuzumab, TBI 300 or 400 cGy with cyclophosphamide with or without alemtuzumab, TBI 200, 300, or 400 cGy with cyclophosphamide and fludarabine with or without alemtuzumab, and TBI 200 cGy with fludarabine).

Alternatively to the whole cohort analysis, a matched-pair analysis was carried out. A marginal Cox model²⁴ examined for risk factors for leukemia or MDS and any secondary neoplasm after matching cases with controls on age at transplantation, donor type, and survival time (controls had to be alive for at least as long as time interval to development of neoplasm to their matched case). The marginal Cox model allowed us to consider graft failure as a time-dependent factor (when graft failure occurred after the diagnosis of neoplasm, the regression model ignored graft

failure as an event). One hundred and ten controls were selected from the pool of 1,074 patients who were transplanted for SCD. The level of significance was set at ≤ 0.05 (two-sided). Analyses were done using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Patient and Transplant Characteristics

Considering all 1,096 eligible patients with 6,631 person-years, we observed 22 neoplasms, which equates to three cases per 1,000 person-years. The completeness index of follow-up (observed ν expected follow-up) after transplantation for the 1,096 patients were 81% and 78% at 8 and 10 years, respectively. Our study population included 22 patients with secondary neoplasm and 1,074 patients without secondary neoplasm after their transplantation for SCD (Table 1). The median age at transplantation for those with secondary neoplasm was 19 years compared with 11 years for those without secondary neoplasm. The predominant conditioning regimen for patients with secondary neoplasm was low-dose TBI (200, 300, or 400 cGy) and the type of donor, haploidentical relative. The predominant regimen for those without secondary neoplasm was busulfan with cyclophosphamide or fludarabine and the type of donor, HLA-matched sibling. Peripheral blood was the predominant graft for transplantations with secondary neoplasm and bone marrow for transplantations without secondary neoplasm. The characteristics of the 22 cases, matched to 110 controls on age, donor type, conditioning regimen intensity, and survival time, are shown in Table 2.

Secondary Neoplasms

The median time to neoplasms was 40 (range, 9-196) months, and 15 of 22 patients are alive. The median age at diagnosis of a neoplasm was 26 (range, 4-57) years. The 10-year incidence of neoplasm was 2.4% (95% CI, 1.4 to 3.8). Types of neoplasm included acute myeloid leukemia ($n = 7$), MDS ($n = 5$), chronic myeloid leukemia ($n = 1$), acute T lymphoblastic leukemia ($n = 1$), T-cell large granulocytic leukemia ($n = 1$), brain tumor ($n = 2$; medulloblastoma and ependymoma grade 2), and other solid tumors ($n = 5$; embryonal rhabdomyosarcoma, Kaposi sarcoma, myxofibrosarcoma, sarcoma not specified, and myofibroblastic tumor of liver; Table 3). Two cases of MDS were reported 18.6 and 40.4 months after second transplantation (Table 3). The 10-year incidence of leukemia and MDS was 1.7% (95% CI, 0.9 to 2.9). The corresponding incidence for solid tumor was 0.7% (95% CI, 0.3 to 1.6).

Risk Factors for Leukemia and Myelodysplastic Syndrome

In a multivariable Fine-Gray regression model, recipients of low-intensity regimens, all of which contained TBI (doses 200, 300, or 400 cGy), were at higher risk for leukemia and MDS (Table 4). A matched-pairs analysis limited to cases and their controls confirmed that recipients of low-intensity regimens were at higher risk for leukemia and MDS (hazard

ratio [HR], 4.33; 95% CI, 1.03 to 18.08; $P = .0445$). In a one-factor Cox regression marginal model, when graft failure was modeled as a time-dependent factor, the risk for leukemia and MDS was higher among patients who experienced graft failure (HR, 3.11; 95% CI, 1.25 to 7.72; $P = .0146$). When conditioning regimen and graft failure were held in the same model, neither met the level of significance, indicating high correlation (low-intensity regimens: HR, 2.96; 95% CI, 0.60 to 14.44; $P = .1802$; graft failure: HR, 2.13; 95% CI, 0.72 to 6.30; $P = .1744$).

Risk Factors for Any Secondary Neoplasm

In a multivariable Fine-Gray regression model, recipients of low-intensity regimens were at higher risk for secondary neoplasms (Table 5). A matched-pair analysis of conditioning regimen intensity confirmed higher risk for any secondary neoplasm with low-intensity regimens (HR, 2.78; 95% CI, 1.22 to 6.33; $P = .0150$). In a one-factor Cox marginal regression model, the risk for any secondary neoplasm was not higher among patients who experienced graft failure (HR, 2.18; 95% CI, 0.96 to 4.92; $P = .0600$). When conditioning regimen and graft failure were held in the same model, a higher risk for any secondary neoplasm was seen with low-intensity regimens (HR, 2.32; 95% CI, 1.03 to 5.24; $P = .0423$) and not graft failure (HR, 1.55; 95% CI, 0.65 to 3.70; $P = .3212$).

DISCUSSION

We observed a higher incidence of leukemia and MDS and any secondary neoplasm compared with a report on transplants for nonmalignant diseases excluding Fanconi anemia.¹³ In the current analysis, the low-intensity conditioning regimens, all of which were TBI-containing (200, 300, or 400 cGy) with or without cyclophosphamide or fludarabine, increased the risk for leukemia or MDS and any secondary neoplasm compared with high-dose busulfan with cyclophosphamide or fludarabine regimens. Onset of leukemia or MDS and graft failure after transplantation are closely correlated. Therefore, it is not surprising that in our examination of the effect of conditioning regimen intensity and graft failure together, neither was identified as a risk for leukemia or MDS, and it is not possible to definitively attribute causation in this setting.

Our observation on the type of neoplasm after transplantation for SCD differed from that reported after transplantation for malignant and nonmalignant diseases.^{11,13,25} Compared with a predominance of solid cancers, including in a report on transplantations for nonmalignant diseases,^{11,13,25} only a third of patients in the current analysis developed solid neoplasms. The predominance of leukemia or MDS may be explained by the fact that the low-intensity regimens rely on tolerance induction through the use of lymphocyte reduction with in vivo T-cell depletion along with mTOR inhibition with sirolimus during recovery.²⁶ This approach leads to establishment of mixed-donor

TABLE 1. Patient and Transplant Characteristics

Characteristic	Cases	Unmatched Controls
No.	22	1,074
Age, years		
Median (range)	19 (1-56)	11 (1-54)
≤ 10, No. (%)	7 (32)	527 (49)
11-19, No. (%)	5 (23)	377 (35)
≥ 20, No. (%)	10 (45)	170 (16)
Sex, No. (%)		
Male	10 (45)	600 (56)
Female	12 (55)	474 (44)
Donor, No. (%)		
HLA-matched sibling	8 (36)	709 (66)
Haploidentical relative	10 (45)	122 (11)
HLA-matched unrelated	2 (9)	111 (10)
HLA-mismatched unrelated	2 (9)	132 (12)
Graft type, No. (%)		
Bone marrow	6 (27)	764 (71)
Peripheral blood	14 (64)	173 (16)
Cord blood	2 (9)	137 (13)
Conditioning regimen, No. (%)		
Myeloablative	6 (28)	601 (56)
Reduced-intensity	2 (9)	263 (24)
Low-intensity	14 (64)	155 (14)
Not reported	—	55 (5)
Myeloablative, No. (%)		
Flu/Bu/in vivo T-cell depletion	—	129 (12)
Flu/Bu	—	9 (< 1)
Bu/cyclophosphamide/in vivo T-cell depletion	6 (28)	415 (39)
Bu/cyclophosphamide	—	48 (4)
Reduced-intensity, No. (%)		
Flu/Mel/thiotepa/in vivo T-cell depletion	—	54 (5)
Flu/Mel/thiotepa	—	2 (< 1)
Flu/Mel/in vivo T-cell depletion	1 (5)	186 (17)
Flu/Mel	1 (5)	15 (1)
TBI 200 cGy/Flu/Cy/thiotepa/in vivo T-cell depletion	—	5 (< 1)
TBI 200 cGy/Mel/in vivo T-cell depletion	—	1 (< 1)
Low-intensity (nonmyeloablative), No. (%)		
TBI (300/400 cGy)/in vivo T-cell depletion	6 (27)	61 (6)
TBI (200-400 cGy)/Flu/Cy/in vivo T-cell depletion	3 (14)	60 (6)
TBI (200-400 cGy)/Flu/Cy	2 (10)	10 (1)
TBI (300/400 cGy)/Cy/in vivo T-cell depletion	2 (10)	7 (< 1)
TBI 400 cGy/Cy	—	1 (< 1)
TBI 200 cGy/Flu	1 (5)	3 (< 1)
TBI (200-400 cGy)/Mel/in vivo T-cell depletion	—	4 (< 1)
TBI (200-400 cGy)/Flu/in vivo T-cell depletion	—	9 (< 1)
Not reported	—	55 (5)

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TABLE 1. Patient and Transplant Characteristics (continued)

Characteristic	Cases	Unmatched Controls
Graft v host disease prophylaxis, No. (%)	1 (5)	
Ex vivo T-cell depletion	1 (5)	14 (1)
CD 34 selection	5 (23)	38 (4)
Post-transplant Cy/sirolimus ± mycophenolate	2 (12)	56 (5)
Post-transplant Cy/CNI/mycophenolate	2 (9)	25 (2)
CNI + mycophenolate	5 (23)	232 (22)
CNI + methotrexate	1 (5)	516 (48)
CNI + sirolimus	1 (5)	5 (< 1)
CNI	1 (5)	93 (9)
Sirolimus	3 (14)	52 (5)
Other	1 (5)	15 (1)
Not reported	—	28 (3)
Chronic graft v host disease, No. (%)		
Yes	4 (18)	257 (24)
None	18 (82)	796 (74)
Not reported	—	21 (2)
Graft failure, No. (%)		
Yes	14 (64) ^{a,b}	205 (19) ^c
No	8 (36)	848 (79)
Not reported	—	21 (2)
Transplant period, No. (%)		
1991-1999	—	68 (6)
2000-2009	7 (32)	287 (27)
2010-2016	15 (68)	719 (67)
Follow-up, median (range), months	97 (48-181)	68 (3-313)

Abbreviations: Bu, busulfan; CNI, calcineurin inhibitor; Cy, cyclophosphamide; Flu, fludarabine; HLA, human leukocyte antigen; Mel, melphalan; TBI, total body irradiation.

^aSix of 14 graft failures occurred after development of secondary neoplasm.

^bOne patient with primary graft failure.

^cFourteen patients with primary graft failure.

chimerism that is sufficient for production of donor-type red blood cells and reversal of the SCD phenotype but not total eradication of host cells.²⁷ We hypothesize that exposure to low-dose radiation of surviving host cells contributed to development of leukemia and MDS. Studies have suggested two different mechanisms for oncogenesis after exposure to low-dose radiation. For onset of leukemia with a short latency period, low-dose radiation may have led to clonal expansion, and for onset of leukemia with a long latency period, a multistep leukemogenic process.²⁸ The fact that some patients developed leukemia or MDS before graft failure supports our hypothesis that low-dose radiation can trigger malignant transformation in residual host cells in the setting of mixed chimerism. However, it is also possible that prior exposure to inflammation related to SCD and post-transplant proliferative stress also contributed or that the two factors (radiation plus prior stress-induced abnormalities) were additive.^{29,30}

A report on 120 SCD transplantations (TBI 300 or 400 cGy-containing regimens) recorded eight secondary neoplasms.³¹ Of the five cases of acute myeloid leukemia or MDS in that report, four had graft failure and one had low-donor chimerism with imminent graft failure. Three cases of other leukemia or lymphoma had mixed-donor chimerism. They postulate regenerative hematopoiesis from pre-leukemic autologous cells exposed to genotoxic transplant conditioning as a potential driver for hematologic malignancy.³¹ Retrospective examination of biospecimens before and after transplantation for two patients in that cohort³¹ showed *TP53*+ mutation at low variant allele frequency levels before transplant that increased over time and development of *TP53*+ acute myeloid leukemia.¹⁸

Ionizing radiation is associated with direct tissue injury and to alter gene expression levels, which can lead to changes in proinflammatory cytokines and in the redox milieu, setting the stage for altered tissue repair and proliferation.³²⁻³⁴

TABLE 2. Patient and Transplant Characteristics of Cases and Controls Matched on Age at Transplantation, Donor Type, and Survival Time

Characteristic	Cases	Matched Controls
No.	22	110
Hemoglobin SS, No. (%)	20 (91)	104 (95)
Hemoglobin Sβ ⁺ , No. (%)	2 (9)	6 (5)
Age, years, No. (%)		
≤ 10	7 (32)	35 (32)
11-19	5 (23)	25 (23)
≥ 20	10 (45)	50 (45)
Sex, No. (%)		
Male	10 (45)	62 (56)
Female	12 (55)	48 (44)
Donor, No. (%)		
HLA-matched sibling	8 (36)	40 (36)
Haploidentical relative	10 (45)	50 (45)
HLA-matched unrelated	2 (9)	10 (9)
HLA-mismatched unrelated	2 (9)	10 (9)
Graft type, No. (%)		
Bone marrow	6 (27)	66 (60)
Peripheral blood	14 (64)	36 (33)
Cord blood	2 (9)	8 (7)
Conditioning regimen, No. (%)		
Myeloablative		
Flu/Bu/in vivo T-cell depletion	—	8 (7)
Bu/cyclophosphamide/in vivo T-cell depletion	6 (28)	28 (26)
Bu/cyclophosphamide	—	3 (3)
Reduced-intensity		
Flu/Mel/thiotepa/in vivo T-cell depletion	—	7 (7)
Flu/Mel/in vivo T-cell depletion	1 (5)	9 (8)
Flu/Mel	1 (5)	2 (2)
TBI 200 cGy/Flu/Mel/thiotepa/in vivo T-cell depletion	—	5 (5)
TBI 200 cGy/Mel/in vivo T-cell depletion	—	1 (1)
Low-intensity (nonmyeloablative)		
TBI (300/400 cGy)/in vivo T-cell depletion	6 (27)	18 (16)
TBI (200-400 cGy)/Flu/Cy/in vivo T-cell depletion	3 (14)	18 (17)
TBI (200-400 cGy)/Flu/Cy	2 (10)	7 (7)
TBI (300/400 cGy)/Cy/in vivo T-cell depletion	2 (10)	2 (2)
TBI 400 cGy/Cy	—	1 (1)
TBI 200 cGy/Flu	1 (5)	1 (1)
Graft v host disease prophylaxis, No. (%)	22	110
Ex vivo T-cell depletion	1 (5)	1 (1)
CD 34 selection	1 (5)	8 (7)
Post-transplant Cy/sirolimus ± mycophenolate	5 (23)	25 (23)
Post-transplant Cy/CNI/mycophenolate	2 (12)	12 (11)
CNI + mycophenolate	2 (9)	11 (10)

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TABLE 2. Patient and Transplant Characteristics of Cases and Controls Matched on Age at Transplantation, Donor Type, and Survival Time (continued)

Characteristic	Cases	Matched Controls
CNI + methotrexate	5 (23)	28 (25)
CNI + sirolimus	1 (5)	1 (1)
CNI	1 (5)	9 (8)
Sirolimus	3 (14)	11 (10)
Other	1 (5)	2 (2)
Not reported	—	2 (2)
Chronic graft v host disease, No. (%)		
Yes	4 (18)	24 (22)
No	18 (82)	86 (78)
Transplant period, No. (%)		
1991-1999	—	8 (7)
2000-2009	7 (32)	18 (16)
2010-2016	15 (68)	84 (76)
Follow-up, median (range), months	97 (48-181)	73 (12-313)

Abbreviations: Bu, busulfan; CNI, calcineurin inhibitor; Cy, cyclophosphamide; Flu, fludarabine; HLA, human leukocyte antigen; Mel, melphalan; TBI, total body irradiation.

TBI-containing and limited-field radiation regimens are known risks for secondary neoplasms after transplantation for malignant and nonmalignant hematologic diseases, but the TBI doses were substantially higher than that used for SCD transplantation, and most secondary neoplasms were solid tumors.^{11,25} In a report that studied the effect of TBI dose on the risk for subsequent neoplasm, the highest risks were seen after single-fraction TBI 600-1,000 cGy and the second highest risk was after fractionated TBI 1,440-1,750 cGy compared with chemotherapy-alone regimens.²⁵ That report²⁵ did not show a difference in the risk for secondary neoplasms after receiving TBI doses 200-450 cGy compared with chemotherapy-alone regimens, which differ from our observations for SCD. In our study, all low-intensity regimens included TBI (200-400 cGy), while none of the patients receiving myeloablative regimens and only 23% of the patients receiving reduced-intensity regimens received TBI. In another report, transplantation of peripheral blood increased the risk for acute myeloid leukemia and MDS.³⁵ We examined for an effect of graft type and found none (results not shown).

Two reports on clonal hematopoiesis in SCD offer differing results, with one reporting higher odds and the other no differences in the odds for clonal hematopoiesis for those with SCD compared with African American controls.^{36,37} These reports^{36,37} used tests with varying sensitivity, which may explain the different conclusions. No doubt, in some patients, low variant allele frequency levels before transplant would have progressed over time, but we lack the evidence to conclude all leukemia and MDS arose from clones, before transplant. Furthermore, without an agreement on the sensitivity or even a predictive nature of the finding of clonal hematopoiesis in a setting such as SCD, it is challenging to implement in clinical practice.

Another plausible explanation for myeloid malignancies may be continued hemolysis and proinflammatory factors.^{30,38} Mixed-donor chimerism and hemolysis when donor cells were < 50% have been reported after full-dose busulfan and cyclophosphamide regimen but none of the patients in a report from France reported secondary neoplasm.¹⁰ The median age at transplantation was 8 years in that study, and all patients had \geq 5 years of follow-up.¹⁰ By contrast, we observed eight secondary neoplasms in children age \leq 11 years and all except one neoplasm occurred within 5 years after transplantation. Consistent with published reports,^{11,12} we also observed a predominance of solid neoplasms after busulfan and cyclophosphamide regimen, but these were recorded in young children and with a relatively short latency period. The sole case of leukemia in a 4-year-old without graft failure may be explained by mixed-donor chimerism and chronic hemolysis and perhaps impending graft failure.

Two cases of acute myeloid leukemia have been observed in SCD after autologous transplantation for gene therapy.^{39,40} In one case, the absence of vector sequences in the leukemic cells exculpated vector-driven insertional oncogenesis,³⁹ and in the other, the presence of vector (not near any potential oncogenes) exculpated busulfan mutagenesis as the transduced cells were cryopreserved during conditioning.⁴⁰ A unifying hypothesis is that pre-existing clonal hematopoiesis mutations in patient's cells were selected by the bottleneck of ex vivo culture for transduction and oligoclonal repopulation that occurred in the first cohort of patients who received relatively low cell dose.

Using data that spanned nearly 3 decades from multiple centers in the United States allowed us to study the

TABLE 3. Secondary Neoplasms

Age, Years	Conditioning Regimen	Neoplasm	Time to Onset, Months	Time to Graft Failure, Months	Status
1	TBI/Flu/Cy	MDS ^a	54	2	Alive
4	Busulfan/Cy ^b	AML	9	NA	Dead
6	Flu/melphalan ^b	T-cell LGL	9	NA	Alive
8	Busulfan/Cy ^b	Medulloblastoma	36	NA	Alive
8	Busulfan/Cy ^b	Embryonal rhabdomyosarcoma	124	NA	Alive
10	Busulfan/Cy ^b	Ependymoma	65	NA	Alive
10	Busulfan/Cy ^b	Sarcoma not specified	60	NA	Alive
11	TBI/Flu/Cy	Kaposi sarcoma	35	NA	Alive
18	Flu/melphalan	MDS ^a	196	Primary graft failure	Dead
18	TBI/Flu/Cy ^b	AML ^c	44	48	Alive
18	Busulfan/Cy ^b	AML	101	14	Dead
19	TBI ^b	CML	45	4	Alive
20	TBI ^b	AML ^c	67	35	Alive
27	TBI/Flu/Cy ^b	MDS ^c	82	85	Alive
32	TBI/Flu/Cy ^b	AML	12	NA	Dead
37	TBI ^b	Myofibroblastic tumor (liver)	31	NA	Alive
37	TBI/Cy ^b	AML	26	35	Dead
37	TBI ^b	MDS ^c	32	35	Dead
39	TBI/Cy ^b	T-cell ALL	36	NA	Alive
40	TBI/Flu	AML	77	4	Dead
42	TBI ^b	Myxofibrosarcoma	36	25	Alive
56	TBI ^b	Monosomy 7	14	4	Alive

NOTE. Melphalan dose: 140 mg/m²; busulfan dose > 8 mg/kg IV or > 12 mg/kg oral.

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; Cy, cyclophosphamide; Flu, fludarabine; LGL, large granulocytic leukemia; MDS, myelodysplastic syndrome; TBI, total-body irradiation.

^aTwo patients recorded MDS after second transplantation for graft failure. Conditioning regimen for second transplant for one patient was TBI 300 cGy/alemtuzumab and the other, TBI 300 cGy/cyclophosphamide/fludarabine. Both recorded graft failure after second transplantation.

^bIn vivo T-cell depletion.

^cFour patients received a second transplant to treat leukemia/MDS. Three of these patients received busulfan (13, 13, and 15 mg/kg)/fludarabine and the remaining patient received TBI 900 cGy/cyclophosphamide/fludarabine. There were two graft failures, one after busulfan/fludarabine regimen and the other after TBI 900 cGy/cyclophosphamide/fludarabine.

incidence and risks leading to leukemia or MDS and any secondary neoplasm after transplantation for SCD. Yet, there are limitations that merit discussion. First, although our follow-up is rather complete for 8 years after transplantation, there is the possibility of ascertainment bias as we cannot be sure all secondary neoplasms are reported in the cohort we studied as well as after transplantations when subjects declined consent to participate in research. Follow-up reporting beyond 2 years after transplantation is at 2-year intervals, and 5.5% of patients declined to participate in the research. Second, our cohort without a secondary neoplasm has a modest median follow-up of 6 years. Our data suggest the median time to neoplasm is 3.3 years, but with longer follow-up, we may observe additional cases of neoplasms, especially solid neoplasm, that would allow for examination of risk factors for this type of neoplasm.¹¹ Third, the data source used for the current

analysis limited our ability to examine other potential risk factors that may increase the risk for secondary neoplasm. These include systematic examinations of pretransplant biospecimens for myeloid mutations, markers for hemolysis, proinflammatory markers, and serial measurements of donor chimerism. Our definition of graft failure relied on < 5% donor cells, an accepted definition for data obtained from transplant registries.⁴¹ Some patients may have had recurrence of SCD symptoms despite donor chimerism > 5%.⁴² Fourth, non-irradiation-containing low-intensity regimens were not studied as these are not typically used for SCD transplantation and there were none in our population.

After a controlled analysis, risks for leukemia and MDS or any secondary neoplasm were higher after low-intensity TBI-containing regimens in contrast to the higher-intensity regimens that typically do not include TBI. Persistence of host cells exposed to low-dose radiation is likely one of

TABLE 4. Multivariable Fine-Gray Model for Leukemia and Myelodysplastic Syndrome

Variable	Hazard Ratio (95% CI)	P
Age, years		
≤ 10	1.00	.8357
11-19	1.25 (0.22 to 7.02)	.7988
≥ 20	1.57 (0.34 to 7.28)	.5634
Donor type		
Matched sibling	1.00	.6967
Mismatched relative	1.07 (0.33 to 3.52)	.9111
Matched unrelated	2.78 (0.51 to 15.08)	.2365
Mismatched unrelated	1.30 (0.14 to 12.47)	.8191
Conditioning regimen intensity		
Myeloablative	1.00	.0003
Reduced-intensity	2.61 (0.27 to 24.92)	.4034
Low-intensity	22.69 (4.34 to 118.66)	.0002

several factors that predispose patients with SCD to leukemia and MDS. The occurrence of solid neoplasms in young children are of specific relevance to the field, considering full-dose busulfan is preferred for gene therapy and gene editing trials and for HLA-matched sibling transplantation in children. Whether we will see a pattern in that most solid neoplasms occur with higher-intensity regimens we of course cannot know.

Cancer therapy with radiation, topoisomerase II inhibitors, and platinum preferentially selects for mutations in DNA damage response genes (*TP53*, *PPM1D*, and *CHEK2*), and sequential sampling has shown DNA damage response clones out-compete other clones leading to therapy-related myeloid malignancy.⁴³ Avoiding these agents in regimens intended to

TABLE 5. Multivariable Fine-Gray Model for Any Secondary Neoplasm

Variable	Hazard Ratio (95% CI)	P
Age, years		
≤ 10	1.00	.8371
11-19	0.68 (0.19 to 2.40)	.5519
≥ 20	0.80 (0.23 to 2.74)	.7222
Donor type		
Matched sibling	1.00	.3525
Mismatched relative	3.15 (0.80 to 12.40)	.1012
Matched unrelated	2.41 (0.52 to 11.23)	.2635
Mismatched unrelated	1.85 (0.36 to 9.39)	.4585
Conditioning regimen intensity		
Myeloablative	1.00	.0025
Reduced-intensity	0.91 (0.16 to 5.29)	.9141
Low-intensity	7.78 (2.20 to 27.53)	.0015

establish mixed-donor chimerism with persistence of host cells may in part mitigate the higher risk for myeloid malignancy after SCD transplantation. It may be prudent to preferentially choose regimens, regardless of the specific agents used, likely to result in full-donor chimerism without persistent host cells. Consideration for examining pretreatment bio-samples for the presence of pathogenic mutations in a Clinical Laboratory Improvement Amendments–certified laboratory may identify a subset of patients with SCD at higher risk for secondary neoplasm, warranting close surveillance, including, perhaps, periodic screening for mutations after treatment. We believe there is an urgent need for guidelines for cancer surveillance in patients with SCD undergoing curative treatments.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**Secondary Neoplasms After Hematopoietic Cell Transplant for Sickle Cell Disease**

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