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RESEARCH ARTICLE

Busulfan and subsequent malignancy: An evidence-based risk assessment

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Abstract

Background: The incidence of secondary malignancies associated with busulfan exposure is considered low, but has been poorly characterized. Because this alkylating agent is increasingly utilized as conditioning prior to gene therapy in nonmalignant hematologic and related disorders, more precise characterization of busulfan's potential contribution to subsequent malignant risk is warranted.

Procedure: We conducted a literature-based assessment of busulfan and subsequent late effects, with emphasis on secondary malignancies, identifying publications via PubMed searches, and selecting those reporting at least 3 years of follow-up.

Results: We identified eight pediatric and 13 adult publications describing long-term follow-up in 570 pediatric and 2076 adult hematopoietic cell transplant (HCT) recipients. Secondary malignancies were reported in 0.5% of pediatric HCT recipients, with no cases of myelodysplastic syndrome (MDS) or acute myelocytic leukemia (AML). Fatal secondary malignancies were reported in 0.8% of 1887 evaluable adult HCT recipients, and an overall incidence of secondary malignancies of 4.8% was reported in a subset of 389 evaluable adult patients. We also reviewed long-term results from eight publications evaluating lentiviral- and human promotor-based HSC-targeted gene therapy in 215 patients with nonmalignant conditions, in which busulfan/treosulfan monotherapy or busulfan/fludarabine was the only conditioning. Two malignancies were reported in patients with sickle cell disease (SCD), one of which was potentially busulfan-related. No additional malignancies were reported in 173 patients with follow-up of 5–12 years.

Conclusion: The incidence of busulfan-related secondary malignancies is low, and likely to be substantially less than 1% in pediatric transplant recipients, especially those receiving busulfan monotherapy for nonmalignant conditions other than SCD.

1 | INTRODUCTION

The alkylating agent busulfan has been a core component of conditioning regimens, enabling hematopoietic cell transplantation (HCT) prior and subsequent to its approval in 1999–2002. Busulfan has been particularly essential in autologous lentiviral-mediated gene therapy for nonmalignant genetic disorders, both for investigational programs and approved therapies, and at myeloablative (AUC ≥60 mg*h/L), intermediate, and reduced-intensity (RIC: AUC 10−30 mg*h/L) dose levels. Busulfan-related short-term toxicities are well characterized and include mucositis, seizures, and hepatic veno-occlusive disease. Longer term effects include impaired fertility, organ damage including bronchopulmonary dysplasia, and cancer predisposition.[1–4] The incidence of some indolent effects, particularly regarding fertility in pediatric recipients, has not been precisely characterized, and is confounded because of the co-administration of additional cytotoxic therapies in the majority of HCT settings. For these same reasons, the risk of busulfan-associated secondary malignancy has also been incompletely delineated. The recent identification of non-insertional myeloid malignancy in a 45-year-old patient with sickle cell disease (SCD), who had received autologous HSC-directed gene therapy 3 years previously, resulted in additional discussion of busulfan as a potentially carcinogenic and leukemogenic agent.[5] As the number of LV-mediated gene therapy investigational programs and approved therapies continue to expand, often with compelling efficacy, we believed it was imperative to enable a more optimal characterization of malignancy potential in patients receiving this agent, in order to optimally inform patients and clinicians considering gene therapy options for nonmalignant hematologic and related disorders. This is of particular importance in pediatric settings. We conducted a literature-based evaluation regarding the incidence and nature of busulfan-related and other late-effect post-HCT malignancies.

2 | METHODS

A systematic literature review (SLR) was based on the PICOS strategy (patient, intervention, comparator, outcome, and study design) outlined in Table 1.

EMBASE and MEDLINE (via Proquest Dialog) were searched for the terms busulfan (including variants), transplantation, conditioning, and hematopoietic stem cell transplantation (detailed search strategy is described in Supporting Information Methods) encompassing the publications, in English language from 2012 through September 2022.

Literature was extracted to a data extraction template (DET), and duplicates were removed. The entire list was then screened by title/abstract to identify articles for inclusion/exclusion in the SLR. Exclusion criteria consisted of conference abstracts, reviews without patient outcomes, and out-of-scope publications (conditioning regimens without busulfan comprising at least one of the components).

The search results, specific criteria employed, and publications selected for detailed review are depicted in Figure 1, culminating in cohorts of publications describing results in pediatric or young adult transplant studies with at least 5 years of median follow-up, adult transplant studies with at least 5 years of median follow-up, and adult transplant studies with at least 3 years (but less than 5 years) of median follow-up.

3 | RESULTS

From the 455 records identified and extracted, only four papers were excluded at the abstract level and 396 when assessing full text. Because alkylating agent-mediated myeloid malignancies predominantly arise 4–7 years following exposure,[6,7] we placed particular emphasis on studies providing long-term results (median follow-up of at least 3 years), in both pediatric (or mixed pediatric/adult) or adult settings. We also evaluated results, with an emphasis on secondary malignancies, from publications detailing autologous LV-based gene therapy clinical trials utilizing self-inactivating LVs incorporating human promotors in hematologic and related disorders, also with emphasis on studies for which at least 5 years of median follow-up is available. Excluded from this evaluation are long-term results from LV-based gene therapy studies in cerebral adrenoleukodystrophy (CALD), a program using a vector containing a viral promotor associated with in vitro moderate transactivating capability, and the only LV-based program to-date associated with insertional mutagenesis.[8–11]

Forty-nine papers were then comprehensively assessed based on the previous inclusion criteria, and 25 were excluded for (a) non-hematologic pediatric condition; (b) case-controlled risk-analysis; (c) pediatric study less than 3-year follow-up; (d) adult study less than 3-year follow-up; and (e) malignancy incidence not evaluable. A list of 24 records were included for comprehensive review, categorized as follows: pediatric/young adult study greater than or equal to 3-year

TABLE 1 PICOS strategy for the SLR.

Population	Patients undergoing allogeneic or autologous hematopoietic stem cell transplant
Intervention	Busulfan conditioning
Comparator	NA
Outcomes	Secondary malignancies, solid tumors, acute myeloid leukemia, myelodysplastic syndrome
Studies	No restriction (except for "human" only)

Abbreviation: SLR, systematic literature search.
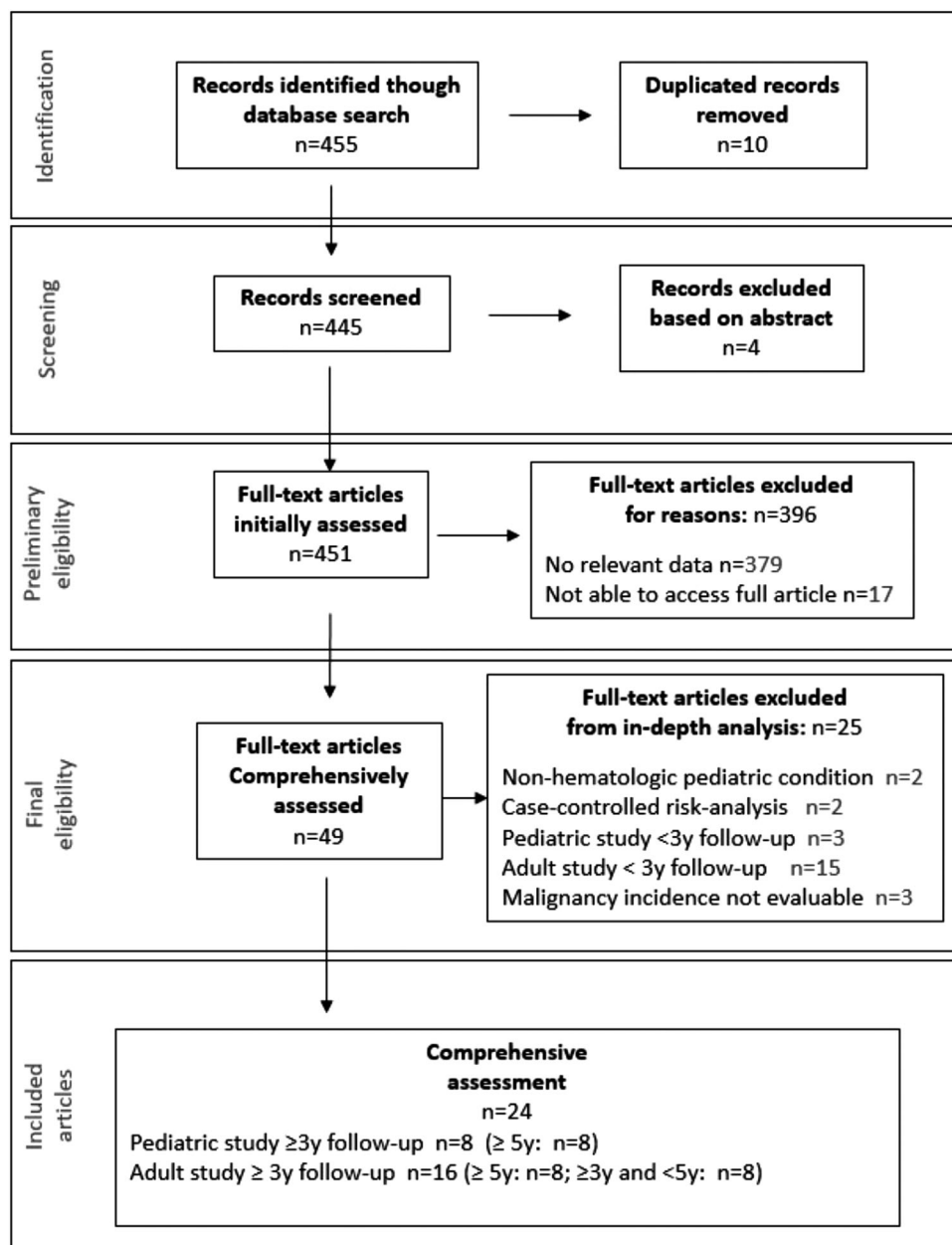
FIGURE 1 PRISMA flow diagram. For excluded publications, "no relevant data" indicates absence of information regarding subsequent or secondary malignancies.

follow-up, $n = 8$ (≥ 5 years: $n = 8$); and adult study greater than or equal to 3-year follow-up, $n = 16$ (≥ 5 years: $n = 8$; ≥ 3 years and <5 years: $n = 8$).

Eight allogeneic HCT publications were identified describing long-term outcomes in 642 predominantly pediatric patients, including median follow-up of at least 5 years in 602 patients, as detailed in Table 2.[12–19] No secondary malignancies were reported in six of these studies (involving $n = 504$ patients). In one study, three of 66 (4.5%) pediatric leukemia patients receiving busulfan-based multiagent conditioning regimens developed secondary malignancies, specifically thyroid ($n = 1$), basal cell skin cancer ($n = 1$), and meningioma ($n = 1$), in contrast to 20/174 (11.5%) of patients receiving TBI-based regimens without busulfan.[18] In one additional study, a single patient developed

a secondary melanoma, but it was not specified whether this patient had received busulfan-based ($n = 72$ patients) or TBI-based ($n = 77$) conditioning.[19] Thus, for 570 evaluable pediatric patients receiving busulfan-based conditioning, secondary malignancies were reported in three patients, representing a 0.5% incidence. There were no reported cases of acute myelocytic leukemia/myelodysplastic syndrome (AML/MDS) in the pediatric population. These findings are in contrast to secondary malignancies occurring in 131/2721 (4.8%) of pediatric patients receiving TBI-based regimens in the absence of busulfan.

Of note, in one study evaluating long-term effects in very young pediatric patients (age <2 years; 82% with nonmalignant conditions) receiving multiagent busulfan-based conditioning between 1993 and 2008 (predominantly busulfan, cyclophosphamide, and ATG), late

TABLE 2 Secondary malignancies and additional observations in pediatric (or young adult) allogeneic transplant evaluations with at least 5 years of follow-up.

Publication	Objective	Study population, chronology, follow-up	Conditioning	Secondary malignancies	Miscellaneous
Saglio BMT 2020	HCT long-term effects *Ped acute leukemia* Multicenter (AIEMOP: Italy)	Age: 3-18 y (n = 670) HCTs: 2000-2012 f/u: 3-16 y	Bu/CTX: n = 197 TBI/CTX: n = 473	Bu/CTX: 0 TBI/CTX: 18% (n = 85) p = .019	Cataracts 24% (TBI) vs. 4% (Bu) p = .0001
Allewelt BBMT 2016	HCT (UCB) late effects *Very young ped* Nonmalignant (82%) Single center (Duke: US)	Age: <2 y (n = 102) HCTs: 1993-2008 f/u: 13 y med (7-22 y)	Bu: n = 102 Bu/Cy/ATG (73.5%)	None	Late effects included: dental complications (92%), short stature (56%), cognitive defects (54%), abnormal puberty (28%)
Rahal Haem 2018	HCT late effects *Ped β-thalassemia* Multicenter (France)	Age: 3-11 y (n = 99; Bu: n = 96) HCTs: 1985-2012 f/u: 12 y med (2-30 y)	Bu/Cy: n = 86 Bu/Flu/ThioT: n = 10	None	Fertility and puberty effects detailed in text
Mitsuhashi BBMT 2016	HCT outcomes *Older ped/adult ALL* Multicenter (Japan)	Age: >15 y; (n = 2130) 43%-61% <40 y Bu(po/iv): 37, 41 y med (15-70 y) TBI: 31 y med (16-68 y) HCTs: 2000-2012 f/u: Bu(po/iv): 8, 2 y med (<1-12.5 y) TBI:	Bu(po)/Cy: n = 60 Bu(iv)/Cy: n = 42 TBI/Cy: n = 2028	Bu/Cy: 0 TBI/Cy: 1% (n = 22)	Malignancies in TBI cohort not specified (resulted in mortality in n = 13)
Anur BMT 2016	HCT late effects *Ped/adult FA (BMF/MDS/AML)* Single center (MSKCC: US)	Age: 12 y med (5-36 y) (n = 22) HCTs: 1999-2012 f/u: Bu 7 y med (2-15 y)	Bu/Cy/Flu: n = 4 TBI/Cy/Flu: n = 18	Bu/Cy/Flu: 0 TBI/Cy/Flu: 11% (n = 2)	Malignancies in TBI cohort were SCC (cervical, oral) at 4-7 y post HCT
Kato BJH 2015	HCT (UCB) outcomes *Infant ALL KMT2A (MLL)* Multicenter (Japan)	Age: <1 y (n = 132) HCTs: 1996-2011 f/u: 5 y med (1-17 y)	Bu/CTX: n = 90 TBI/CTX: n = 28	Bu/CTX: 0 TBI/CTX: 7% (n = 2)	Malignancies in TBI cohort were thyroid carcinoma at 9-12 y post HCT
Bernard BMT 2014	HCT late health effects *Ped leukemia* Multicenter (LEA: France)	Age: 9 y med(<1-20 y) (n = 240) HCTs: 1982-2008 f/u: 10 y med (<1-26 y)	Bu/CTX: n = 66 TBI/CTX: n = 174	Bu/CTX: 4.5% (n = 3) TBI/CTX: 11.5% (n = 20)	Malignancies in Bu cohort included: thyroid (1), basal cell or meningioma (1)
Bresters BMT 2010	HCT late effects *Ped; heme-malignancy 54%* Single center (Leiden, Neth)	Age: 6 y med (<1-18 y) (n = 162) HCTs: through 2007 f/u: 7 y med	Bu/Cy: n = 72 TBI: n = 77	One melanoma in pt with cGvHD (conditioning regimen not specified)	

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia; ATG, anti-thymocyte globulin; BMF, bone marrow failure; Bu, busulfan; cGvHD, chronic graft-versus-host disease; CTX, chemotherapy; Cy, cyclophosphamide; FA, Fanconi anemia; Flu, fludarabine; f/u, follow-up; HCT, hematopoietic cell transplant; MDS, myelodysplastic syndrome; Neth, Netherlands; ped, pediatric; pt, patient; SCC, squamous cell carcinoma; TBI, total body irradiation; ThioT, thiotepa; UCB, umbilical cord blood; y, years.

effects included dental complications (92%), short stature (56%), cognitive deficits (54%), and pubertal abnormalities (28%).[13] In one study evaluating HCTs in 99 pediatric β-thalassemia patients receiving transplant between 1985 and 2012, detailed fertility effects were reported as follows: 12 of 33 (33%) pre-pubertal females experienced normal puberty with younger age at HCT (median 2.5 years) relative to those who had delayed puberty (median age 8.7 years). Pre-pubertal males experienced subsequent normal puberty in 18/22 (82%) of instances. Eleven of 27 (40%) of evaluable females had at least one successful pregnancy; and four of 21 (19%) of evaluable males fathered at least one child. Information regarding percentages within groups actively seeking pregnancy/fatherhood was not provided. A majority of these patients received either oral busulfan or intravenous therapy prior to the implementation of pharmacokinetically guided therapy.[14]

Thirteen publications in adult hematologic malignancies were identified describing long-term results for busulfan/chemotherapy combination pretransplant conditioning regimens, encompassing 2076 patients, predominantly in allogeneic HCT settings.[20–32] Three additional publications described long-term results in adults (also with hematologic malignancies) when busulfan was combined with TBI and additional chemotherapy agents, involving 744 patients.[33–35] Five of the busulfan/chemotherapy publications and each of the three busulfan/TBI/chemotherapy publications involved median follow-up exceeding 5 years, summarized in Table 3; the remaining eight selected studies involved median follow-up of between 3 and 5 years, summarized in Table 4.

For 11 of the 13 studies evaluating busulfan/chemotherapy conditioning regimens, long-term outcomes were reported as fatal secondary malignancies, involving 1887 patients. (It is presumed that these malignancies, resulting in patient deaths, are identified in contrast to localized or less aggressive secondary neoplasms such as noninvasive skin cancers, which were reported in >5% of patients in some adult series.) Incidences of fatal secondary malignancies ranged from 0% to 5.9% across studies (median incidence 0.7%). Overall there were 16 fatal secondary malignancies reported subsequent to HCT in 1887 patients, representing a 0.8% incidence. Of note, of the four studies with the highest incidence of fatal secondary malignancies, three of these four employed reduced intensity (RIC) conditioning, as did two of the four studies with the lowest incidences.

The overall incidences of secondary malignancies were reported in four of the adult studies, involving 389 patients. Nineteen of these 389 (4.8%) patients encountered secondary malignancies, with the incidence ranging between 0% and 10.7%. Importantly, 16 of the 19 secondary malignancies were reported from a single study in high-risk hematologic malignancies in an older adult population (median age 61 years, 33% above 65 years at the time of HCT).[32] Malignancies in this study included one of the previously mentioned fatal cancers, and 15 additional secondary malignancies (seven of which were dermatologic); 14 of these 15 secondary malignancies occurred in patients who had experienced either acute (n = 6) or chronic (n = 8) graft-versus-host disease (GVHD).

The highest incidences of secondary malignancies occurred in the three series in which busulfan and additional chemotherapy agents were administered in conjunction with TBI, as conditioning for hematologic malignancy-directed HCTs in adults. Secondary malignancy incidences in these three studies, involving 744 patients, were 6%, 7.5%, and 8%, respectively.[33–35] Specific malignancies were described in two of these publications and were noninvasive skin cancers, occurring in a total of three patients.

The potential contribution of busulfan to secondary malignancies is potentially best delineated by the experience in autologous hematopoietic stem cell-directed gene therapy studies, several of which utilize busulfan monotherapy or combination exclusively with fludarabine. In these studies, busulfan dosage ranged from reduced intensity (RIC; as low as AUC 10−19 mg*h/L) to fully myeloablative (70–85 mg*h/L). Long-term follow-up results are available for several of these trials, with follow-up durations of 5−12 years available across eight trials encompassing 173 patients, detailed in Table 5.[36–45] Also described in Table 5 are results from nine patients receiving treosulfan (as the carcinogenic risk of this agent is anticipated to be similar to that of busulfan) and 42 patients enrolled in SCD studies for whom follow-up of at least 3 years is available.[46] Notably, the only malignancies occurring in these were in the SCD studies, in two patients receiving therapy as part of the initial Group A process, involving bone marrow harvest, lower CD34^{+} cell doses, and more limited hemoglobin response relative to the more recently treated 35 patients. Both myeloid malignancies harbored mutations in oncogenes RUNX1 and PTPN11, and monosomy 7 cytogenetic abnormalities. In one case, the malignant cell population comprised of gene-corrected cells, with an integration in proximity to VAMP4, a loci not consistent with mutagenic potential.[46,47] In the second case, no gene markings were present in the malignant population, and the AML was considered potentially secondary to busulfan in the context of underlying SCD.[5] Importantly, for the entirety of LV-based gene therapy studies utilizing vectors with human promotors, no malignancies have been reported secondary to insertional mutagenesis. A recent update indicated no clonal hematopoiesis or associated detectable mutations in 10 ADA-SCID gene therapy recipients who received RIC busulfan with 7−10 years of follow-up.[37,48]

4 | DISCUSSION

Alkylating agents, which covalently bind DNA and result in interstrand cross-linkages, are carcinogenic, although the associated malignant risk has been difficult to define because these agents are often used in combination with other cytotoxic therapies, and because of the diverse clinical disorders and follow-up durations in relevant investigations.

We observed that the incidence of busulfan-associated secondary malignancies is low, particularly in pediatric settings, with a majority of long-term evaluations indicating an incidence less than 1%, and an overall aggregate incidence across more than 500 patients of 0.5%. Secondary MDS/AML in the setting of pediatric busulfan exposure was not reported and is likely to be rare. The incidence of post-busulfan secondary malignancies was higher in adult transplant studies, but also relatively limited. The incidence of fatal secondary malignancies

TABLE 3 Secondary malignancies and additional observations in adult transplant evaluations with at least 5 years of follow-up.

Publication	Objective	Study population, chronology, follow-up	Conditioning	Secondary malignancies	Miscellaneous
Kebraei BBMT 2018	HCT outcomes; Bu in ALL *Adult ALL* Multicenter (CIBMTR)	Age: 18–60 y (n = 1118) HCTs: 2005–2014 f/u: Bu: 43 m med (3–98 m) TBI: 63 m med (3–125 m)	Bu/CTX: n = 299 TBI/CTX: n = 819	Fatal secondary malignancy incidence 0.7% in each cohort	Mortality in Bu cohort (n = 135) predominantly ALL relapse (57% of deaths), also GVHD (12%), organ failure (9%)
Yoon BMT 2018	HCT outcomes (autologous) *NHL: high-risk or relapsed* Single center (Catholic U, Korea)	Age: 17–65 y (n = 121) HCTs: 2007–2014 f/u: 5.5 y med (21–130 months)	Bu/melphalan/thiotepa (RIC): n = 121	Fatal secondary MDS 56 months post HCT (n = 1, 0.8%); no additional secondary malignancy reported	
Kennedy BBMT 2016	HCT outcomes *B-lymphoid malignancies* Single center (Vanderbilt U)	Age: 53–55 y med (33–70 y) HCTs: 2005–2013 f/u: 5.5 y med (Bu)	Bu/Flu RIC: n = 61 Flu/Cy/Ritux RIC: n = 33 all: ATG if unrelated	Fatal secondary malignancy in n = 2 (5.9%) in Bu RIC cohort	Mortality in Bu cohort (n = 61) predominantly relapse (38% of deaths), also GVHD (29%), infection (18%)
Ghosh JAMA Onc 2020	HCT outcomes (RIC) *NHL* Multicenter (CIBMTR)	Age: 55 y mean (19–76 y) HCTs: 2008–2016 f/u: ≥90% 4 y	Bu/Flu: n = 458 Flu/CTX: n = 1276 Flu/Cy/TBI: n = 89	Fatal 2nd malignancy: n = 4 (2%) Bu Fatal 2nd malignancy: n = 16 (3%) CTX Fatal 2nd malignancy: n = 1 (3%) TBI	Mortality predominantly due to NHL relapse (37%–53%), GVHD (9%–20%), infection (10%–17%), organ failure (6%–13%)
Sakellari Ann Hem 2018	HCT long-term outcomes *Adult/Ped ALL* Single center (Papanicolaou: Greece)	Age: 29 y med ± 12 (n = 151) HCTs: 1993–2016 f/u: Bu 14 y med (<1–26 y) TBI 7.5 y med (<1–14 y)	Bu/Cy: n = 67 TBI/Cy: n = 84	Bu/Cy: 2.9% (n = 2) TBI/Cy: 4.4% (n = 4)	Malignancies not specified
Salhotra Leuk Lymph 2022	HCT outcomes; intensified conditioning regimen *Adult high-risk leukemia* Single center (City of Hope)	Age: 18–50 y (n = 32) HCTs: 2000–2014 f/u: 17 y med (14–19 y)	TBI + Bu (AUC 700–900 μM/min) + etoposide	Noninvasive skin cancers (SCC, BCC) in n = 2 (6%) at 7 and 11 y post HCT	All patients received chemotherapy and TBI
Ousia Clin Transplant 2020	HCT outcomes; *Heme malignancy* Single center (U Calgary)	Age: 18–72 y (n = 700) HCTs: 2003–2017 f/u: 5 y med (<1–15 y)	Bu+Flu+TBI+ATG (MAC); post-HCT MTX/cyclosporine	7.5% 10 y incidence	All patients received chemotherapy and TBI
Michelis BBMT 2019	HCT outcomes (plerixafor) *AML* Single center (U Toronto)	Age: 49 y med (38–58 y) HCTs: 2010–2013 f/u: 67 months med	Bu+Flu+TBI+plerixafor (n = 12)	n = 1 (8%) 2nd malignancy (skin cancer) 45 and 53 months (recur) post-HCT, surgical rx	All patients received chemotherapy and TBI

Note: HCTs were allogeneic unless otherwise specified.

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia; ATG, anti-thymocyte globulin; BCC, basal cell carcinoma; Bu, busulfan; CTX, chemotherapy; Cy, cyclophosphamide; Flu, fludarabine; f/u, follow-up; GvHD, graft-versus-host disease; HCT, hematopoietic cell transplant; MDS, myelodysplastic syndrome; MTX, methotrexate; NHL, non-Hodgkin's lymphoma; ped, pediatric; pt, patient; RIC, reduced intensity conditioning; SCC, squamous cell carcinoma; TBI, total body irradiation.

TABLE 4 Secondary malignancies and additional observations in adult transplant evaluations with predominantly 3–5 years of follow-up.

Publication	Objective	Study population, chronology, follow-up	Conditioning	Secondary malignancies	Miscellaneous
Epperla BBMT 2020	HCT outcomes (RIC) NHL: DLBCL Multicenter (CIBMTR)	Age: 53–59 y med (22–73 y) HCTs: 2008–2016 f/u: 48 months med (Bu)	Bu/Flu: n = 151 Flu/Mel: n = 296 BEAM: n = 115	Fatal 2nd malign: n = 0 Bu Fatal 2nd malign: n = 1 (1%) Flu/mel Fatal 2nd malign: n = 1 (1%) BEAM	
Sakellari Leuk Lymph 2015	HCT outcomes (autologous) NHL and HL (76% rel/refr) Single center (Papanicolaou)	Age: 34 y med HCTs: 2000–2011 f/u: 42 months (Bu) - 64 months (BEAM) med	Bu/etop/mel: n = 50 BEAM: n = 87	None	
Cornillon Acta Haem 2016	HCT outcomes (RIC, ATG dose) Heme malignancy; 2 centers (France)	Age: 58–61 med (25–74 y) HCTs: 2000–2011 f/u: 36 months med	Bu+Flu (RIC) + ATG (n = 168)	Fatal 2nd malign n = 1 (0.6%)	
Vaezi IJHOSCR 2016	HCT outcomes AML/ALL single center (Shariati, Iran)	Age: ≥18 y HCTs: 2013–2014 f/u: ≥2 y	Bu/Cy (n = 122)	n = 1 (0.8%) secondary astrocytoma	Sicca syndrome (34%); cataract (13%); 9%–15% ovarian failure (F); >50% primary gonadal dysfunction (M)
Oudin Haematol 2014	HCT outcomes (RIC) AML/MDS 2 centers (France)	Age: 57 y med HCTs: 2005–2011 f/u: 39 months med (20–76 months)	Bu/Flu/ATG RIC (n = 165)	Fatal 2nd malign: n = 1 (0.6%)	
Chen BBMT 2013	HCT outcomes (RIC; Bu-dose); AML/MDS 2 centers (DFCI/MGH)	Age: 61–64 med (55–68 y) HCTs: 2004–2009 f/u: 3.2–4.4 y med	Bu/Flu RIC (n = 217)	Fatal 2nd malign: n = 4 (1.8%): EBV-PTLPD; recurrent oral SCC; NSCLC; AML (donor-derived)	All 2nd malignancies occurred in lower dose (3.2 mg/kg) Bu cohort (vs. 6.4 mg/kg)
Damlaj BBMT 2016	HCT outcomes (RIC) AML/MDS Single center (Mayo)	Age: 60–61 med (18–72 y) HCTs: 2008–2014 f/u: 40 months med	Bu/Flu RIC (n = 47) Flu/Mel RIC (n = 134)	Fatal 2nd malign: n = 1 (2.1%)	
Mehta Transplant Cell Ther 2021	HCT outcomes High-risk myeloid and other malignancy; older adults Single center (MD Anderson)	Age: 61 med (24–75) HCTs: 2012–2015 f/u: 43 months med	Bu/Flu (fractionated dose) (n = 150)	Fatal 2nd malign n = 1 (0.7%) Addtl 2nd malign n = 15 (8.7% 3 y inc.); incl. 7 skin cancers	14 of 15 2nd malignancies occurred in pts with either cGHVD (n = 8) or aGVHD (n = 6)

Note: HCTs were allogeneic unless otherwise specified.

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia; ATG, anti-thymocyte globulin; BEAM, carmustine, etoposide, cytarabine, melphalan; Bu, busulfan; CTX, chemotherapy; Cy, cyclophosphamide; DLBCL, diffuse large B-cell lymphoma; EBV-PTLPD, Epstein–Barr post-transplant lymphoproliferative disorder; etop, etoposide; Flu, fludarabine; f/u, follow-up; GvHD, graft-versus-host disease (a/c: acute/chronic); HCT, hematopoietic cell transplant; HL, Hodgkin's lymphoma; MDS, myelodysplastic syndrome; Mel, melphalan; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; ped, pediatric; pt, patient; RIC, reduced intensity conditioning; SCC, squamous cell carcinoma; TBI, total body irradiation.

TABLE 5 Secondary malignancies and efficacy overview in self-inactivating lentiviral gene therapy studies utilizing busulfan- or treosulfan-based conditioning and at least 5 years of follow-up.

Disorder	Patients	Treatment chronology	Conditioning	Efficacy/survival	Secondary malignancy
ADA-SCID Kohn 2021	n = 40 (ped) n = 13 (ped)	2012–2016 2017	Bu RIC (non-cryo) Bu RIC (cryo)	98% efficacy 92% efficacy	None
X-SCID Mamcarz 2019 DeRavin 2016	n = 8 (infant) n = 5 (7–23 y)	2016–2018 pre-2016	Bu RIC (AUC 22 mg*h/L) Bu RIC (AUC 10–19 mg*h/L)	88% efficacy varied efficacy	None
Wiskott-Aldrich Aiuti 2013 Ferrua 2020	n = 34 (1–35 y) predominantly pediatric	2010–2018	Bu (AUC ~60 mg*h/L) + Flu n = 7 (UK) Bu (AUC ~82 mg*h/L) + Flu n = 5 (FR) Bu (AUC 70–85 mg*h/L) + Flu n = 5 (US) Bu (AUC ~48 mg*h/L) + Flu n = 17 (IT)	31/34 alive in 2020 —mortality not rx-related —skin: 100% resolution in surviving patients —platelet: incomplete recovery	None
β-Thalassemia Marktel 2019	n = 6 (3–13 y) n = 3 (adult)	2015–2017 2015–2017	Treosulfan (myeloablative) Treosulfan (myeloablative)	3/4 peds: transfusion-independent 2/3 adults: decreased transfusion requirements	None
β-Thalassemia Thompson 2018	n = 22 (12–35 y)	2013–2016	Bu (myoablative)	15/22 transfusion-independent, incl. 12/13 non-β⁰/β⁰	None
X-CGD Kohn 2020	n = 9 (2–27 y) predominantly adult	2016–2018	Bu (AUC 65–75 mg*h/L)	7/9 alive in 2020 —mortality not rx-related —6/7 normalized PMN function	None
MLD Biffi 2013 Fumagalli 2022	n = 33 (<1–12 y) predominantly young pediatric	2010–2020	Bu (AUC ~67 mg*h/L)	26/29 alive in 2020 —mortality not rx-related —22/26 normalized cognitive development	None
Sickle Cell Kanter 2022 Goyal 2022 Hsieh 2020	n = 42 (12–38 y)	2016–2020 earlier for initial n = 9	Bu (AUC ~82 mg*h/L)	Median Hb: increased from 8.5 (pre-rx) to ≥11 g/dL No severe vaso-occlusive events in n = 25 subset (3.5 y annual rate pre-rx)	n = 2 AML: 3 and 5.5 y post-rx n = 1 potentially Bu-related n = 1 in gene-corrected cells; no evidence of insertional mutagenesis; VAMP4 integ.

Abbreviations: ADA-SCID, adenosine deaminase severe combined immunodeficiency; Aut Bu, busulfan; MLD, metachromatic leukodystrophy; ped, pediatric; RIC, reduced intensity conditioning; X-CGD, x-linked chronic granulomatous disease; X-SCID, x-linked severe combined immunodeficiency.

is likely lower than 1%, and the overall incidence of secondary malignancies is likely lower than 5%, including a substantial proportion of noninvasive dermatologic malignancies. A substantial proportion of the secondary malignancies was identified in studies with extensive participation of older adults.[29,30,32] It is likely that older age confers a higher susceptibility to conditioning-related malignancies, consistent with this association in more general populations.[38,49] Results in adult populations are unlikely to precisely inform potential malignant risks for pediatric patients.

Notably, in the adult transplant series, secondary malignancies were reported both in cohorts receiving myeloablative and RIC regimens. It is likely that although RIC regimens involve an overall reduced cytotoxic burden, this effect is potentially countered by survival of hematopoietic progenitors, which have been exposed to alkylator and other therapies with potential for mutagenic DNA damage. RIC regimens may also be more likely utilized in older adult cohorts, who are at higher baseline cancer risk.

A limitation of this study is that the overwhelming proportion of both allogeneic and autologous HCTs was performed in settings of hematologic malignancies, in which multiagent conditioning regimens were likely to have been preceded by induction and additional chemotherapies, such that patients' overall exposure to cytotoxic therapy was extensive. This limits the extent to which one may ascribe the specific contribution of busulfan to secondary malignancies or other long-term complications. These results also pre-date the implementation of therapeutic drug monitoring (TDM) to enable more precise busulfan dosing; because the pharmacokinetics of oral and intravenous busulfan are highly variable, there was higher potential for overexposure in these earlier studies than in current programs. An additional limitation is that very few publications provided detailed results regarding other long-term effects of interest—including fertility and neurocognitive and overall development in pediatric transplant recipients. These remain substantial risks for children receiving high-dose combination chemotherapy; as with secondary malignancies, the precise risks conferred by busulfan cannot be determined because of the multiagent nature of the regimens evaluated. There is limited understanding regarding dose relationship for these toxicities, although it is likely that higher exposure is associated with increased risk. Long-term results from an EBMT dataset are likely forthcoming and will hopefully provide more precise information regarding fertility impairment in adult survivors of pediatric transplant.

The most busulfan-specific results are likely to emanate from autologous HSC-directed gene therapy studies, including several in which a single cycle of busulfan preconditioning was utilized exclusively as a conditioning agent, at exposures ranging AUC 10−85 mg*h/L (with reduced-intensity therapy utilized in SCID and myeloablative administration in other conditions). As indicated, long-term results from the earliest LV-mediated studies are now available, with 5−10 years of follow-up for more than 150 patients. In these settings, administration of busulfan conditioning therapy is less confounded by concomitant conditioning agents or prior anticancer therapies. The only potential busulfan-related malignancy (AML) developed in a patient with SCD, a condition increasingly recognized as predisposing to myeloid malignancy, albeit with uncertainty regarding the specific contributing factors or overall increase in risk.[50] Longer term follow-up through 15 years is anticipated and will facilitate optimal risk assessment. Presently, follow-up of more than 5 years in patients receiving busulfan-based conditioning for genetic disorders other than SCD indicates that the risk of secondary malignancy is likely to be very rare.

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