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Research Article

Noteworthy HSCT Outcomes in Fanconi Anemia based on Protocols with or without Fludarabine: A Systematic Review

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Abstract

Over the past decades, hematopoietic stem cell transplantation (HSCT) has been the sole curative modality in Fanconi anemia (FA) patients; with the inaugural treatment dating back to the early 1970s. Despite the length of time elapsed from the first treatment, no unified standard preparative and prophylactic protocol has been established ever since. Here, we aimed to systematically review the literature from 1977 to 2023 with a focus on types of conditioning regimen used, including Fludarabine (FLU)- and non-FLU-based regimens, and their effects on the primary and secondary outcomes of HSCT.

We electronically and manually searched in PubMed, Scopus, and Web of Science databases alongside Google Scholar. We assessed the primary study domains, selection, and outcome using the official Newcastle-Ottawa Scale quality assessment for cohort studies. We categorized cohorts into treatment groups, and the characteristics of patients' and donors', besides intervention characteristics as well as outcomes, were synthesized.

Among a total of 596 studies, 26 cohorts were included in this systematic review. The studies were heterogeneous in all issued terms. The FLU-based group (n = 10) and non-FLU-based group (n = 6) were similar in GF incidence, while aGvHD incidence was slightly higher in the former. The average rates of OS were reported to be lower in the FLU-based group in comparison to the second group.

In conclusion, our data suggests better post-HSCT outcome in patients who underwent a FLU-based regimen. In patients who were not exposed to total body irradiation, lower risk of developing secondary malignancies in long-term follow-up was evident.

Registration: The paper was registered on PROSPERO, CRD42023421643 ID.

Keywords: Fanconi Anemia; Hematopoietic Stem Cell Transplantation; Transplantation Conditioning; Fludarabine Phosphate

Abbreviation

FA: Fanconi Anemia; BMF: Bone Marrow Failure; FANC: FA Complementation; SMN: Second Malignant Neoplasms; HSPC: Hematopoietic Stem and Progenitor Cells; MDS: Myelodysplastic Syndrome; AML: Acute Myeloid Leukemia; BMF: Bone Marrow; HSCT: Hematopoietic Stem Cell Transplantation; HDCY: High-dose Cyclophosphamide; XRT: Radiotherapy; AA: Aplastic Anemia; LDCY: Low-dose Cyclophosphamide; TAI: Thoraco-abdominal Irradiation; GF: Graft Failure; OS: Overall Survival; GvHD: Graft versus Host Disease; FLU: Fludarabine; BU: Busulfan; ATG: Anti-thymocyte Globulin; SAE: Serious Adverse Effects; TRM: Transplant-related Mortality; RRT: Regimen-related Toxicity; OM: Oral Mucositis; VOD: Veno-occlusive Disease; SOS: Sinusoidal Obstruction Syndrome; HC: Hemorrhagic Cystitis; CIBMTR: Center for International Blood and Marrow Transplant Research; EBMT: European Group for Blood and Marrow Transplantation; SDTBI: Single-dose Total Body Irradiation; cGy: Centi-grays; LFI: Localized Field Irradiation; aGvHD: Acute Graft versus Host Disease; cGvHD: Chronic Graft versus Host Disease; SCC: Squamous Cell Carcinoma.

Introduction

Fanconi anemia (FA), first discovered by Swiss pediatrician, G. Fanconi, is a subcategory of inherited bone marrow failure (BMF) syndromes; based predominantly on chromosomal instability [1,2]. It is described as a devastating multi-systemic disease with genotypic and phenotypic heterogeneity. Twenty-two FA complementation (*FANC*) family genes (*FANCA* to *FANCW*) have imperative pathological roles. FA is associated with various somatic abnormalities, progressive BMF, and second malignant neoplasms (SMN). BMF usually occurs in

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the first decade of life; it occurs due to the attrition of hematopoietic stem and progenitor cells (HSPC) by elevated DNA repair response and apoptosis, which makes FA the most prevalent inherited BMF syndrome. FA patients have a high risk of SMNs including hematological neoplasms such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), besides various solid tumors [3,4].

The diagnosis, clinical management, and treatment is intricate. Supportive care for these patients include the administration of androgen therapy and synthetic growth factors, along with transfusions [5].

A. J. Barrett et al. successfully cured a 15-year-old boy with FA by grafting bone marrow (BM) from his brother, 46 years ago, for the first time [6]. Since then, hematopoietic stem cell transplantation (HSCT) has been the standard treatment modality for FA patients, which can restore normal hematopoietic characteristics. In addition, it is noteworthy to mention that the prime cord blood HSCT was performed by E. Gluckman in FA boy in 1989 [7]. However, HSCT is complicated in FA patients due to the use of alkylating agents and irradiation requirements in the conditioning regimen.Over the past 30 years, HSCT outcomes in FA patients have improved remarkably. Initial HSCT conditioning regimens were accompanied by excessive toxicity and high rates of mortality, as they comprised of high-dose Cyclophosphamide (HDCY; 200 mg/kg) and radiotherapy (XRT); a successful conditioning regimen in aplastic anemia (AA) treatment [8,9]. From the mid-1980s onward, low-dose CY (LDCY; a 5 – 10-fold reduction, 20 – 80 mg/kg) and thoraco-abdominal irradiation (TAI) or no irradiation, which were instituted by E. Gluckman, became the HSCT 'standard' conditioning regimen for that time. Graft failure (GF) was low (< 10%), and the overall survival (OS) rate had improved (> 80%); however, acute and chronic graft versus host disease (a- and c-GvHD) occurred in 25 – 40 and > 40% of FA patients, respectively. In the following years, Fludarabine (FLU), a purine analog that doesn't affect the chromosomal integrity, was utilized by numerous studies and evidence proved it to be a powerful alternative to CY or high-dose XRT. The use of FLU was continually associated with lower GF rate, transplant complication reduction, and improved OS. Busulfan (BU) was introduced in the early 2000s as an alternative to XRT [9-12].

Refinements to the conditioning regimen allowed for HSCT outcomes to reach a new paradigm [4,10,13]. Nowadays, FLU- and BU-based protocols are the backbones of the conditioning regimen for patients with FA, with or without the addition of anti-thymocyte globulin (ATG), XRT or other chemotherapy agents. Figure 1 presents the timeline of conditioning regimen refinements. Despite the multitude of reports conducted heretofore on FA-HSCT, no unanimous consensus has been reached on the optimal combination of treatment; which could possibly be due to the countless genetic variation engendering the disease. Consequently, we aimed to systematically review the literature with a focus on conditioning regimen types, FLU- and non-FLU-based, and the HSCT outcomes, such as serious adverse effects (SAE), SMNs, OS, and transplant-related mortality (TRM).

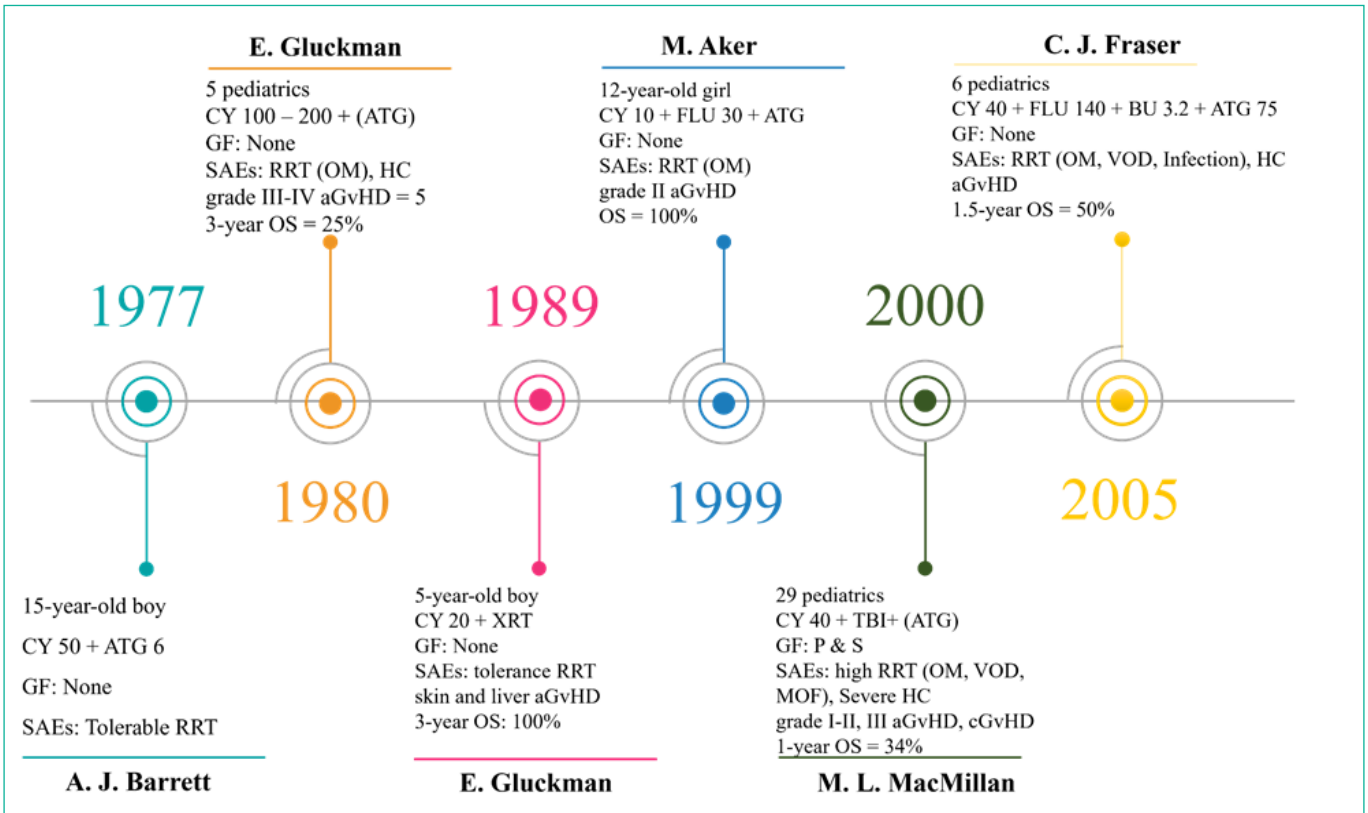

Figure 1: The conditioning regimen refinements' timeline.

Methods

Protocol and Registration

This paper adhered to the latest preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [14]. We registered the paper on PROSPERO with CRD42023421643 ID.

Eligibility Criteria

All articles utilized included cohort studies relating to FA-HSCT between 1977 and 2023. The critical elements of the review, or the PICO framework, are defined as follows:

Participants (P): FA patients without any restrictions,

Intervention (I): HSCT; regardless of regimens and donor characteristics,

Comparison (C): FLU and non-FLU-based regimen protocols,

Outcome (O): Primary outcomes included engraftment, GvHD; and SMNs, OS and TRM were considered secondary outcomes.

Conference abstracts, posters, case reports, case series, and studies that were not available in English were excluded from study.

Information Sources

An advance electronic search was conducted in PubMed, Scopus and Web of Science from 12.14.2023 to 12.18.2023. Google Scholar was also manually searched during those dates.

Search Strategy

We searched all databases using the following three main terms: "Hematopoietic Stem Cell Transplantation", "Fanconi Anemia", and "Transplantation Conditioning". Supplementary Material Table 1 presents the systematic search string.

Selection Process

All articles were exported into EndNote X9 software (Clarivate Analytics, Philadelphia, United States) to reveal and remove duplicates. Two authors independently screened the findings for relevant articles using the title and abstract. Subsequent to the initial screening, the full text of articles that met our eligibility criteria were reviewed. In instances of disagreement, a third reviewer was consulted. Some articles were excluded in instances when full-text articles were not retrievable, even after correspondence with the author.

Data Collection Process

Three authors independently extracted data from the articles according to a predefined extraction outline in Microsoft Excel following the data extraction items summarize in the Supplementary Material Table 2.

Risk of Bias Assessment

Two authors independently assessed the quality and risk of bias of the eligible studies, and in case of any disagreements, a third reviewer was consulted. The primary study domains, selection, and outcome were assessed using the official Newcastle-Ottawa Scale (NOS) quality

Table 1: The baseline, clinical, and HSCT characteristics of twenty-six included cohorts.

Characteristics	# Studies	# Cases	Characteristics	# Studies	# Cases
Collaborations*			**Conditioning Regimen Protocols**		
Single Center	18		FLU-based	10	
Multi-institution	4		non-FLU-based	6	
CIBMTR	2		FLU- vs. non-FLU-based	10	
EBMT	2		**HLA-matching**		
Pt. Counts		2229	MD + (MMD)	20	
HSCT Counts		2287	HID	1	
Median HSCT Age	9		MD + MMD + HID	4	
Pt.'s Sex (F: M)	1044: 1182		**Donor's Sex (F: M)**	11	344: 443
Disease Status			**Donor's Types**		
Mentioned	21		S + (R or U)	11	
AA, MDS, AL		1501, 175, 77	R + (U)	3	
Unknown		164	U	2	
Not Mentioned	5	312	S + R + U	10	
FANC Analysis			**SC Sources**		
Performed	11		BM	2	141
FANCA		243	PB	1	16
FANCC		63	CB	1	93
FANCD2		14	BM, PB	7	
FANCG		28	BM, CB	4	
FANCD1, *FANCE*, *FANCF*, *FANCJ*, *FANCP*		4, 1, 3, 2, 2	All	10	
UnK		241	N. M.	1	151
Not Performed	15	1621			
Mosaicism					
Performed	4				
Not Performed	22				

Abbreviations: CIBMTR: Centre for International Blood and Marrow Transplant Research; EBMT: European Group for Blood and Marrow Transplantation; Pt: Patient; HSCT: Hematopoietic Stem Cell Transplantation; F: Female; M: Male; AA: Aplastic Anemia; MDS: Myelodysplastic Syndrome; AL: Acute Leukemia; FANC: FA Complementation; UnK: Unknown; FLU: Fludarabine; HLA: human leukocyte antigen; MD: Matched Donor; MMD: Mismatched Donor; HID: Haploidentical Donor; S: Sibling; R: Related; U: Unrelated; SC: Stem Cell; BM: Bone Marrow; PB: Peripheral Blood; CB: Cord Blood; N. M.: Not Mentioned.
*Four studies were conducted in North America. Studies from Europe contain Italy (n = 2), the Netherlands, Spain, and the UK apiece reported one study. Three and two studies were from Turkey and Iran, respectively, besides one study apiece from India, Israel, Egypt, and Japan.

assessment for cohort studies [15], which is available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

Synthesis Method

The syntheses were performed to address the study goals. The median of patients' median age at HSCT time with the range reported. Disease data consisted of stage of FA, FANC analysis results alongside mosaicism. The donor's data included HLA- matching, sex and source of SC. The above-mentioned data were reported descriptively.

Data pertaining to conditioning regimen protocols were extracted across all cohorts and categorized into the following treatment groups: FLU- and non-FLU-based with or without radiotherapy utilization. The OS rates were classified into <5-years, 5-years, and >5-years for the purpose of this systematic review, due to the numerous reported survival rates of studies. Outcomes were reported in percentage and average when possible.

Results

Study Selection and Characteristics

A comprehensive search in PubMed, Scopus, and Web of Science identified 105, 186, and 277 records (n = 568), respectively. Thirty-two records were added manually, totaling the number of articles to 600. Twenty-six cohort studies were included in the systematic review. Figure 2 indicates the PRISMA flow diagram. Studies were observational cohorts in design, encompassing 2229 patients whom 58 underwent HSCT twice. Table 1 summarizes the baseline and clinical characteristics of the 26 included cohorts.

Study Risk of Bias

Twenty-six cohorts were qualified based on the standard NOS assessment form. All patients, who underwent HSCT were FU for at least one year, which allowed sufficient data to be collected as a part of outcome. Three studies were excluded due to inadequate or incomplete statistical analysis. Supplementary Material Table 3 describes details on the NOS quality assessment of the included cohorts.

Study Results

The HSCT primary outcomes

Table 4 describes engraftment, GvHD, and SAEs (RRT and HC) on the basis of the conditioning regimen protocol group—FLU-, non-FLU-, and both.

Studies based on the protocols

We categorized cohorts into three groups based on FLU usage. XRT usage was also taken into consideration. First, we individually described each group's study, patients, and protocols. The transplant's outcomes were reported and compared where applicable. Among ten studies in the first group with FLU usage, most studies date around 2010 [19-21,24,27-32], in which the protocols using irradiation were noted as FLU-XRT, and those without irradiation were called FLU-non-XRT. Patients in the second group, without FLU usage (n = 6), were conditioned with CY with or without irradiation, non-FLU-XRT, and non-FLU-non-XRT, respectively [17,22,23,26,33,34]. The third group comprised of ten comparative studies using both—FLU- and non-FLU-based protocols [16,18,25,35-41]. Table 2 defines the patient and clinical characteristics of the cohorts, concerning the conditioning regimen protocols.

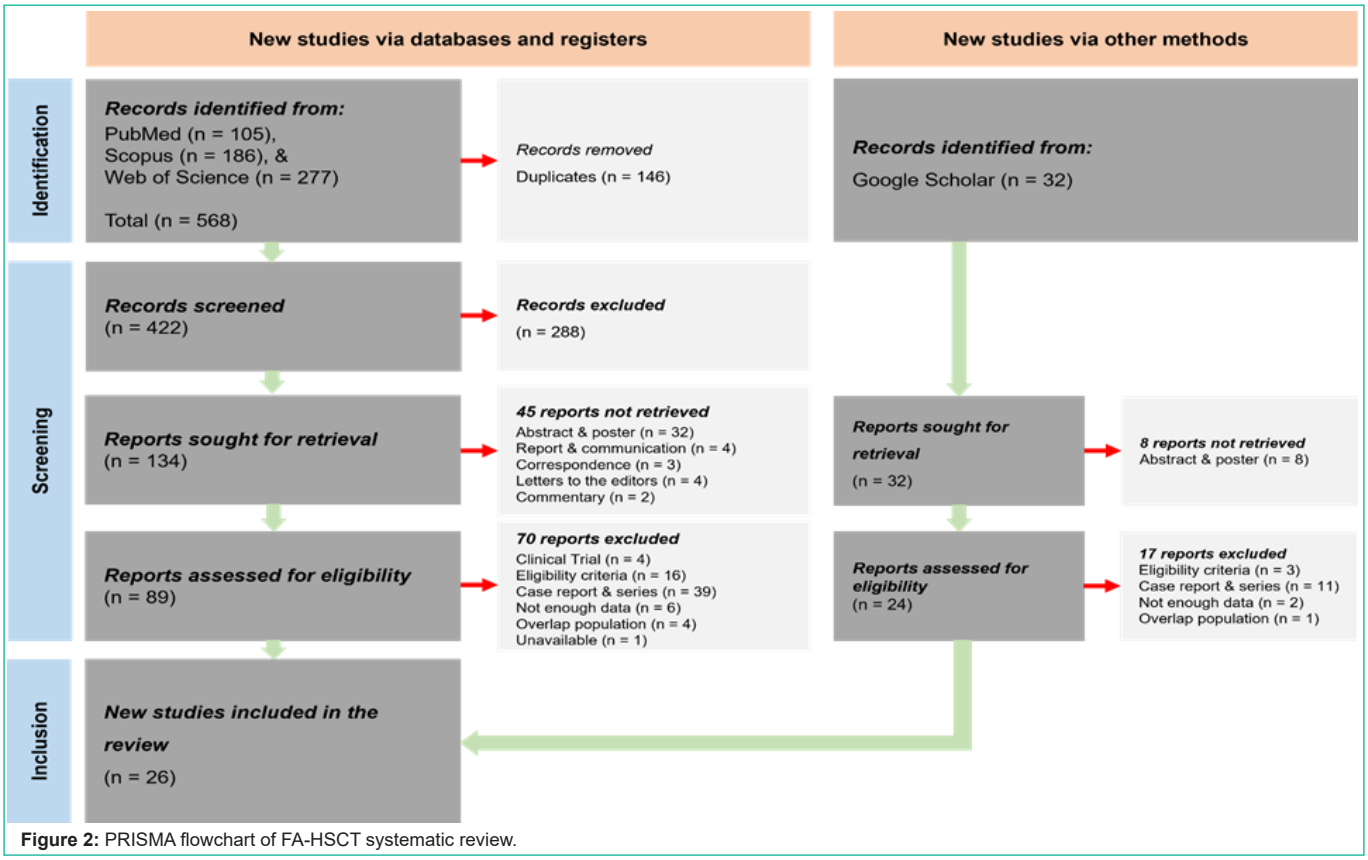

Figure 2: PRISMA flowchart of FA-HSCT systematic review.

Table 2: The patient and clinical characteristics of the cohorts concerning the group.

Group	Study (First Author, Year)	Country	Pt/ HSCT	Median HSCT Age (Range) (yr.)	F: M	Disease Status	*FANC* Group	Mosaicism
FLU-based	S. Chaudhury, 2008	USA	18/21	11.9 (5.5 - 24)	9:9	SAA: 8, MDS-RAEB: 4, AML: 6, CAs: 10	A: 12, C: 1, D1: 1, G: 3, Unk: 1	Yes
	B. Kuskonmaz, 2016	Turkey	26/27	9.6 (5.6 - 17)	7:19	CAs: 7, Ukn: 19	N. M.	N. M.
	P. Anur, 2016	USA	22/23	12.1 (5.4 - 35.6)	14:8	SAA:1, MDS: 6 (der(13), t(13;1;3)), AML: 5	A: 11, C: 2, D2: 1, G: 3, Unk: 5	N. M.
	G. M. Fathy, 2017	Egypt	63	11.2 (2 - 35)	34:29	FA: 63	N. M.	N. M.
	C. L. Ebens, 2018	USA	74	8 (2 - 15)	32:42	SAA: 74	A: 50, C: 10, D2: 3, F: 1, G: 3, J: 2, P: 1, Unk: 4	Yes
	M. Ayas, 2019	Multi-institution	19	9.1 (2.8 - 12.3)	13:6	Pancytopenia + (CAs (+3q: 2, 11q23: 1)): 18, ALL + CAs: 1	N. M.	N. M.
	G. Tuysuz, 2019	Turkey	44	10 (4.3 - 16)	20:24	Severe BMF: 43, CAs: 1 (5q del)	N. M.	N. M.
	R. Uppuluri, 2020	India	19/21	9 (3 - 20)	11:8	CAs (-7): 1, Unk: 18	N. M.	N. M.
	L. Murillo-Sanjuán, 2021	Spain	34	8.4 (4 - 26)	18:16	BMF: 30, MDS: 4	A: 22, D2: 1, E: 1, G: 2, Unk: 8	N. M.
	O. Fink, 2023	Israel	41/46	9.5 (3.2 - 30.1)	21:20	SAA: 32, MDS: 7, AML: 2	A: 26, C: 6, G: 2, Unk: 7	N. M.
non-FLU-based	M. Kohli-Kumar, 1994	Multi-institution	18/19	7.6 (2.7 - 12.6)	6:12	FA: 18	N. M.	N. M.
	E. Gluckman, 1995	CIBMTR	151	10 (1 - 36)	67:84	FA	N. M.	N. M.
	C. Dufour, 2001	Italy	27/29	9 (2.5 - 19.5)	8:19	Hypoplastic: 1, Aplastic: 24, Dysplastic: 2 (CAs: 1)	N. M.	N. M.
	A. Farzin, 2007	USA	35/37	7.6 (2.7 - 22.9)	10:25	Aplasia: 30, Clone or MDS: 4, CMML: 1	A: 20, C: 6, D2: 1, Unk: 8	Yes
	C. M. Bonfim, 2007	Multi-institution	43/47	9 (5 - 29)	20:23	AA: 43, CAs: 12	A: 12, C: 2, F: 1, G: 1, Unk: 27	N. M.
	T. Rostami, 2022	Iran	122	8 (2 - 18)	48:74	Hypoplastic: 113, Clonal Evolution: 9	N. M.	N. M.
Both	F. Locatelli, 2007	Italy	64/66	9 (2 - 20)	25:39	FA	N. M.	N. M.
	J. E. Wagner, 2007	CIBMTR	98	12 (0.8 - 28.6)	46:52	AA: 75, MDS: 14, AML: 7, Other: 2	A: 37, C: 12, D1: 1, D2: 2, G: 4, Unk: 42	Yes
	E. Gluckman, 2007	EBMT	93/96	8.6 (1.4 - 45.4)	54:39	MDS: 8, AL: 4, CAs: 20	A: 7, C: 2, G: 2, Unk: 82	N. M.
	M. Akif Yesilipek, 2009	Turkey	16/17	11 (5 - 17)	6:10	FA	N. M.	N. M.
	P. Stepensky, 2011	Multi-institution	41/42	9.6 (0.5 - 30.8)	25:16	AA: 9, SAA: 26, MDS: 3, AML: 3	N. M.	N. M.
	A. A. Hamidieh, 2011	Iran	53	11.5 (2 - 48)	22:31	Hypocellular: 51, MDS: 2	N. M.	N. M.
	R. Peffault de Latour, 2013	EBMT	795	(0 - 50)	375: 417	AA: 737, MDS/AML: 58	N. M.	N. M.
	S. E. Smetsers, 2016	Netherlands	68/81	8.2 (3.1 - 38.7)	26:42	MDS or Clonal abnormalities: 12, AML: 2	A: 21, C: 18, D2: 4, G: 4, Other: 8, Unk: 13	N. M.
	F. Bernard, 2021	UK	82/92	8.7 (2.2 - 19.8)	45:37	BMF: 69, MDS: 11, AML: 2	A: 25, C: 4, D1: 2, D2: 1, F: 1, G: 4, P: 1, Unk: 44	N. M.
	M. Yabe, 2021	Japan	163/170	8 (0 - 46)	82:81	AA: 118, MDS: 30, AML: 14, ALL:1	N. M.	N. M.

Abbreviations: CIBMTR: Centre for International Blood and Marrow Transplant Research; EBMT: European Group for Blood and Marrow Transplantation; Pt: Patient; HSCT: Hematopoietic Stem Cell Transplantation; F: Female; M: Male; FANC: FA Complementation; FLU: Fludarabine; FA: Fanconi Anemia; CA: Cytogenetic Abnormality; CMML: Chronic Myelomonocytic Leukaemia; SAA: Severe Aplastic Anemia; RAEB: Refractory Anemia with Excess Blasts; AL: Acute Leukemia; ALL: Acute Lymphoblastic Leukemia; BMF: Bone Marrow Failure; UnK: Unknown; N. M.: Not Mentioned.

The studies using FLU-based protocols

Ten studies with FLU-based preparative regimen reported outcomes of 372 HSCTs on 360 patients. The patients' HSCT age ranged from 2 to 35.6 years (median of median: 9.55 years) with a female-to-male ratio of 1:1.

In three cohorts, the protocol consisted of 150 mg/m^2 of FLU, 200 – 450 centi-grays (cGy) of single-dose total body irradiation (SDTBI) with or without LDCY [19,27,29]. In five studies they used FLU (120 – 175 mg/m^2) in tandem with LDCY without or with TBI, at an ionizing dose of 300 cGy [20,24,28,30,31]. High-dose FLU (180 mg/m^2) with LDCY was used in two cohorts [21,32].

The studies using non-FLU-based protocols

A total of 386 patients underwent 396 non-FLU-based HSCTs in six studies. The patients' HSCT age ranged from 1 to 36 years (median of median: 8.5 years) with a female-to-male ratio of 1:1.5.

The conditioning regimen consisted of LDCY in all six cohorts. The patients had TAI or TBI or localized field irradiation (LFI) in four cohorts [13,18,19,22]. Two studies from Brazil and Iran used non-XRT-based protocols—which included LDCY with or without BU and ATG [29,30].

Table 3: The HSCT characteristics of the cohorts concerning the group.

Group	Study (First Author, Year)	Conditioning Regimen	GvHD Prophylaxis	HLA-Matching	F: M	Donor's Type	SC Sources
FLU-based	S. Chaudhury, 2008	CY 40 + FLU 150 + (r-ATG 10, e-ATG, ALEM) + SDTBI 450 cGy	Tac + G-CSF + (TCD)	All	N. M.	all	BM, PB
	B. Kuskonmaz, 2016	CY 20 - 40 + FLU 175 + r-ATG 20 - 40 or Thymoglobulin 5 - 10	CsA + MTX or (Pred) + (TCD)	MD, MMD (1-locus)	11:15	S, R	BM, PB, BM + CB
	P. Anur, 2016	XRT-: CY 40 + FLU 150 + ATG + SDTBI 450 cGy: 18; non-XRT-: CY 40 + FLU 140 + BU 3.2 - 4 + ATG: 4	(CsA or Tac) + TCD	MD, MMD (1-, 2-locus)	N. M.	R, U	BM, PB
	G. M. Fathy, 2017	CY 20 + FLU 120 + ATG 20	CsA + ATG 20	MD	N. M.	S	BM, PB
	C. L. Ebens, 2018	CY 20 - 40 + FLU 140 - 175 + e-ATG 150 + (TBI 300 cGy + S (T))	(CsA or Sir) + (MMF or Pred) + G-CSF + (TCD)	MD, MMD (1-, 2-locus)	N. M.	all	BM, CB, BM + CB
	M. Ayas, 2019	FLU 150 + r-ATG 20 + SFTBI 200 cGy + PTCY 40 - 50	CsA + MMF	All	5:14	S, R	BM, PB
	G. Tuysuz, 2019	CY 40 + FLU 120 - 150 + r-ATG 20 - 30	CsA + (MMF or MP)	MD, MMD (1-, 2-locus)	N. M.	all	all
	R. Uppuluri, 2020	CY 10 + FLU 180 + SDTBI 200 cGy + PTCY 50	Tac + MMF	HID	N. M.	S, R	BM, PB
	L. Murillo-Sanjuán, 2021	CY 20 - 40 + FLU 140 + r-ATG 10 - 15 + (SDTBI 150 - 300 cGy)	CsA + Steroid + (TCD)	MD, HID	N. M.	S, U	all
	O. Fink, 2023	CY 10 - 40 + FLU 180 + (Thymoglobulin 10 or r-ATG 45) or (ALEM 0.6) + ((LDBU 1.6 or TBI 300 cGy) for AD)	CsA + (MMF or MTX)	MD, MMD (1-locus)	17:24	all	all
non-FLU-based	M. Kohli-Kumar, 1994	CY 20 + ATG 120 + TAI 400 cGy + *S (L & K)*	CsA + Pred + ATG	MD	1:17	S	BM, CB
	E. Gluckman, 1995	CY 15 - 25 + LFI 500 (400 - 1500) cGy + (ATG): 82; CY 15 - 20 + TBI 600 (300 - 800) cGy: 20; CY ≥100 + (ATG): 25; Variable: 24	(MTX) + (CsA) or Both, (TCD)	MD	67:84	S	N. M.
	C. Dufour, 2001	CY 20 + (ATG) + TAI 500 (500 - 600) cGy: 12; CY 20 (20 - 80) + (ATG) + TBI 500 (300 - 600) cGy: 10; CY 120 (100 - 200) + (ATG): 5	CsA + (MTX)	MD	9:16	R	BM, CB
	A. Farzin, 2007	CY 20 + h-ATG 120 + TAI 400 cGy: 30; CY 40 + h-ATG 120 + TBI 450 cGy: 1; CY 40 + h-ATG 120 + TBI 400 cGy: 1; CY 20 + h-ATG 120 + TBI 450 cGy: 2	CsA + Corticosteroids + h-ATG 120	MD, MMD (1-locus)	14:21	S	BM, CB
	C. M. Bonfim, 2007	CY 60	CsA + MTX	MD	22:21	S, R	BM
	T. Rostami, 2022	CY 60 + BU 0.8 + r-ATG 7.5 - 10	CsA + MTX	MD	52:70	all	all
Both	F. Locatelli, 2007	FLU-: (CY 1200 + FLU 120): 25; non-FLU-: (CY 1200 + (Thio) + (LFI)): 30, Variable: 9	CsA + ((MTX) + (ATG) + (Corticosteroids) + (G-CSF)), TCD	MD, MMD (1-locus)	N. M.	all	all
	J. E. Wagner, 2007	FLU-: (CY + FLU + (BU) + ATG + (XRT)): 46; non-FLU-: (CY + (BU) + (ATG) + TBI/TAI/LFI): 52	CsA + MTX, Tac, TCD	MD, MMD (1-locus)	N. M.	U	BM
	E. Gluckman, 2007	FLU-: (CY 40) + FLU + (BU <8) + (ATG/ALG) + (TBI 500 cGy): 57; non-FLU-: (CY + (BU) + (ATG/ALG)) + (TBI 500 cGy/TLI 400 cGy)): 35	CsA + ((Pred + ATG) + (MTX)), MMF, Tac	All	N. M.	U	CB
	M. Akif Yesilipek, 2009	FLU-: (CY 40 + FLU 120 - 150 + ATG 60 - 90): 10; non-FLU-: (CY 20 + ATG 30 - 60 + TAI 500 cGy): 6	CsA + (MMF)	MD	N. M.	all	PB
	P. Stepensky, 2011	FLU-: ((CY 20 - 40) + FLU 150 - 180 + (BU 4) + (e-ATG 90 or r-ATG 40) + (ALEM) + (TBI 200 cGy)): 17; non-FLU-: (CY 10 - 60 + ((BU 8) or (r-ATG 40) or (TAI/TLI))): 24	CsA + ((Dac) + (MMF) + (Tac))	MD, MMD (1-, 2-locus)	N. M.	all	all, BM + (CB or PB)
	A. A. Hamidieh, 2011	FLU-based: (CY 20 + FLU 150 + h-ATG 40); non-FLU-based: (CY 60 + BU 0.8 + h-ATG 10)	CsA + (MTX)	MD, MMD (1-locus)	27:26	S, R	BM, PB
	R. Peffault de Latour, 2013	FLU- vs. non-FLU-: 233 vs. 492; XRT- vs. non-XRT-: 307 vs. 270; ATG- vs. non-ATG-: 369 vs. 356	CsA + (TCD- vs. non-TCD-: 94 vs. 513)	MD	119:135	S, U	BM, PB
	S. E. Smetsers, 2016	FLU- vs. non-FLU-: 41 vs. 27; XRT- vs. non-XRT-: 32 vs. 36	TCD	MD, MMD (1-locus)	N. M.	R, U	all
	F. Bernard, 2021	FLU- vs. non-FLU-; TBI/TAI- vs. non-TBI/TAI-; ALEM- vs. non-ALEM-	(CsA) or (Tac) + TCD- vs non-TCD-	All	N. M.	all	all
	M. Yabe, 2021	FLU- vs. non-FLU-; ATG/ALG- vs. non-ATG/ALG-; XRT- vs. non-XRT- (TBI/TAI/TLI)	(Tac) or (CsA) + TCD- vs non-TCD-	MD, MMD (1-locus)	N. M.	all	all

Abbreviations: CY: Cyclophosphamide; ATG: Anti-thymocyte Globulin; XRT: Radiation Therapy; FLU: Fludarabine; BU: Busulfan; CsA: Cyclosporine A; MP: Methylprednisolone; MTX: Methotrexate; TCD: T Cell Depletion; CS: Corticosteroid; MMF: Mycophenolate mofetil; Tac: Tacrolimus; G-CSF: Granulocyte Colony-stimulating Factor; HLA: human leukocyte antigen; MD: Matched Donor; MMD: Mismatched Donor; HID: Haploidentical Donor; N. M.: Not Mentioned; F: Female; M: Male; SC: Stem Cell; S: Sibling; U: Unrelated; R: Related; BM: Bone Marrow; CB: Cord Blood; PB: Peripheral Blood.

The comparative studies using both protocols (FLU- and non-FLU-based)

The patients in the third group (n = 1473) were conditioned with different protocols with or without the addition of FLU. The patients' HSCT age ranged from <1 to 50 years (median of median: 9 years) with a female-to-male ratio of 1:1.

Six cohorts encompassed 388 patients who underwent HSCT in using FLU [16,18,25,35,36,38], whereas 648 adhered to a non-FLU protocol. The remaining cohorts (n = 4) did not disclose the number of patients in the two different settings [37,39-41]. Table 3 defines the HSCT characteristics of three groups.

Engraftment and GF

The incidence of GF was similar in FLU- and non-FLU-based studies (average: 5.05 vs. 5.56%), each ranging from 0 – 22% vs. 0.8 – 12%, respectively. FA-HSCT patients experienced primary GF in the FLU-based cohorts, while secondary GF was reported more in the

Table 4: The primary HSCT outcomes of the cohorts concerning the conditioning regimen protocol group—FLU-, non-FLU-, and both.

Group	Study (First Author, Year)	Engraftment Status	aGvHD (I-II, III-IV)	cGvHD (L, E)	SAEs (RRT and HC)
FLU-based	S. Chaudhury, 2008	None	4/18 (22%); 3, 1	1/18 (5%)	OM, MOF, Polycythaemia, IDDM
	B. Kuskonmaz, 2016	1/26 (3.8%); P	2/26 (7.7%) (I-II)	1/26 (3.8%); L	OM (grade ≥III), VOD, Toxicity (Liver & Kidney); HC
	P. Anur, 2016	None	N. M.	None	Hypothyroidism, DM, NC, Cataracts, Hearing loss, IDDM (XRT-)
	G. M. Fathy, 2017	2/53 (3.7%); P: 1, S: 1	8/63 (12.7%) (N. M.)	4/63 (6.3%); L	PRES
	C. L. Ebens, 2018	3/74 (4%)	7%, 4%	5%	N. M.
	M. Ayas, 2019	None	8/19 (42%); 5, 3	1/19 (5.3%); E	OM, SOS; HC
	G. Tuysuz, 2019	2/44 (4.5%); P	12/44 (27%) (III-IV)	2/44 (4%)	VOD; HC
	R. Uppuluri, 2020	2/19 (10.5%); P	11/16 (68%); 9, 2	4/16 (25%)	OM, Toxicity (Liver)
	L. Murillo-Sanjuán, 2021	1/34 (2%); S	9/34 (26%) (II-IV)	3/34 (9%)	H Pulmonary
	O. Fink, 2023	9/41 (22%); P	13/41 (31.7%); 10, 3	9/41 (22%); L: 4, E: 5	MOF
non-FLU-based	M. Kohli-Kumar, 1994	1/18 (5%); S	None	3/18 (16%); L: 2, E: 1	OM (grade I-II), Seizure, HT; HC
	E. Gluckman, 1995	**4%**, 0% vs. 4% (**XRT-**)	38%, 54% vs. 45%	39%, 54% vs. 45%	N. M.
	C. Dufour, 2001	2/25 (8%); S	9/25 (36%); 7, 2; **100%** vs. 25% (**CY ≥100**)	3/24 (12.5%); L: 1, E: 2	M (grade I-III) (3/5 vs. 8/22), Toxicity (Liver & Kidney) (1/5 vs. 3/22); HC (2/5 vs. none)
	A. Farzin, 2007	2/39 (5%); P: 1, S: 1	8/35 (23%); 3, 2	4/34 (12%); L: 1, E: 3	OM, Toxicity (Liver, Kidney, GI, Skeletal, Endocrine, & Pulmonary)
	C. M. Bonfim, 2007	5/42 (12%); P: 1, S:4	8/42 (19%); 7, 1	12/41 (29%); L: 2, E: 10	OM (grade I-IV) 43/43, Toxicity (Liver & Kidney) (1/43), HT (5/43 (11.6%%)); HC (1/43 (2.3%))
	T. Rostami, 2022	1/122 (0.8%); S	18/122 (14.7%) (III-IV)	6/111 (5.4%)	OM, VOD, PRES, HC
Both	F. Locatelli, 2007	4/64 (6.25%); P: 2, S: 2	23/63 (36%) (II-IV)	13/51 (25.4%); L: 8, E: 5	N. M.
	J. E. Wagner, 2007	5 vs. 13	30/96 (31.25%); 13, 17; 16% vs. 42.5%	16/55 (29%)	N. M.
	E. Gluckman, 2007	N. M.	17 (19%), 19 (20%)	16%	VOD, ILD (Pneumonitis), ARDS; HC
	M. Akif Yesilipek, 2009	P: 1/10 vs. 0/6	1 vs. 3	L: 1 vs. 2	HC, VOD, vs. M, BOS, ICH, Pulmonary Aspergillosis, Pneumonia
	P. Stepensky, 2011	2/41 (4.8%); P: 2/17 vs. 0/24	10 (41%), **1 (4%)** vs. 6 (35%), **8 (47%)** (**FLU-**)	L: 6/20 vs. 4/6, E: 2/20 vs. 2/6	3.9 vs. **10.6** (**non-FLU-**)
	A. A. Hamidieh, 2011	4/53 (7.5%); P: 2 vs. 0, S: 2 vs. 0	4/38, 1/38 vs. 17/38, 17/38 (**non-FLU-**)	L: 3/43 vs. 5/43, E: 1/43 vs. 2/43	N. M.
	R. Peffault de Latour, 2013	93/795 (11%); P: 8%, S: 3%; **9** vs. 75 (**FLU-**); **23** vs. 43 (**TCD-**)	32% (II-IV)	14%	N. M.
	S. E. Smetsers, 2016	P: 5, S: 7	5, 7	L: 2, E: 4	N. M.
	F. Bernard, 2021	P: 8, S: 2	5 (6.1%) (II-IV)	L: 1, E: 1	N. M.
	M. Yabe, 2021	P: 1, S: 1	17, 9	L: 22, E: 15	N. M.

Abbreviations: P: Primary; S: Secondary; a-GvHD: Acute Graft versus Host Disease; c-GvHD: Chronic Graft versus Host Disease; L: Limited; E: Extensive; SAE: Severe Adverse Event; RRT: Regimen-related Toxicity; HC: Hemorrhagic Cystitis; OM: Oral Mucositis; HT: Hypertension; MOF: Multiple Organ Failure; IDDM: Insulin Dependent Diabetes Mellitus; VOD: Veno Occulsion Disease; NC: Neurologic Complication; SOS: Sinusoidal Obstruction Syndrome; N. M.: Not Mentioned.

non-FLU cohort. Fifty-nine patients were efficaciously engrafted in three cohorts who were conditioned with FLU-XRT-based protocol [19,27,29]. The highest primary GF incidence was 22% (9 out of 41), and 10.5% (2 out of 19), subsequently [21,32].

An Iranian cohort reported the least secondary GF incidence at 0.8% (1 out of 122) [34]; whereas, the Brazilin cohort had the highest secondary GF rate at 9.5% (4 out of 42) in the non-FLU-non-XRT setting [33], followed by 8% (2 out of 25) of Italian patients with the non-FLU-XRT regimen [26]. The CIBMTR report in 1995 compared the usage of XRT and lack thereof in non-FLU-based protocols. In LDCY setting 4% GF in LFI and 0% GF in TBI was observed, whereas in the non-XRT HDCY regimens, the rate of GF was at 4% (*P-value = 0.009*) [23].

The CIBMTR report in 2007 stated 11% GF (5 out of 46) and 25% (13 out of 52) failed apt engraftment, in FLU and non-FLU-based regimens, respectively [18]. Three cohorts reported a 10%, 11.7%, and 18.1% primary GF rate in the FLU-based setting, whereas none was reported non-FLU-based [36-38]. Similarly, 9 out of 233 vs. 75 out of 492 patients experienced GF in the EBMT report (*P-value= 0.013*) [16]. FLU-based conditioning was associated with statistically decreased GF (*P-value = 0.01*) [39].

The GvHD incidence (acute and chronic)

AGvHD incidence was slightly higher in FLU-based regimens 27% vs. 23% in non-FLU. On the contrary, cGvHD was more common in the non-FLU setting (8% vs. 20.1%, FLU- vs. non-FLU-based).

The lowest aGvHD incidence in the FLU setting with the same protocol was reported in two studies; 7% grade I-II, 4% grade III-IV: 4% and grade I-II: 7.7% [20,28]. The highest frequency of grade I-II (56.2%) and III-IV (12.5%) was reported in the Indian cohort [21]. A multi-institution study described lower incidence; grade I-II: 26.3% and III-IV 15.7% [29]. Eighteen patients did not develop aGvHD in the non-FLU-XRT setting [22]. A study on 151 HSCT revealed that aGvHD incidence was at 45% in the FLU-non-XRT vs. 38% and 54% (LFI and TBI) FLU-XRT settings, respectively.

The conditioning regimen using ATG had lower aGvHD occurrence rates [23]. Conversely, the Italian study described significantly higher aGvHD incidence; 100% vs. 25% in XRT- vs. non-XRT setting, respectively (*P-value = 0.0001*) [26]. Two non-FLU-non-XRT studies with LDCY protocol with and without BU reported 14.7% and 19% aGvHD incidence, respectively [33,34].

The development of aGvHD was statistically significantly lower in recipients of the FLU-containing regimens in the CIBMTR report (16% vs. 42.5%; *P-value < 0.001*) [18]. Three studies by M. A. Yesilipek (10% vs. 50%; respectively), P. Stepensky (41.6% vs. 82.3%; *P-value = 0.002*), and A. A. Hamidieh (45.5% vs. 78.6%; *P-value= 0.03*) were consistence with J. E. Wagner's study [36-38].

In a study of 22 American patients using FLU-based protocol, with and without XRT, no cGvHD was reported [27]. In contrast, the highest cGvHD rate, 25% and 22%, was reported in two cohorts with FLU administered at 180 mg/m^2 with and without XRT, respectively [21,32]. The incidence was similar in non-FLU-based cohorts whether they received irradiation or not; 21.5% vs. 17.2%, respectively [17,22,23,26,33,34]. The studies by M. A. Yesilipek (10% vs. 33.3%), P. Stepensky (33.3% vs. 23.5%), and A. A. Hamidieh (9.3% vs. 16.2%) did not find any disparity between cGvHD development and types of conditioning regimens [36-38].

The SAEs

The most frequent SAEs in patients receiving FLU-based conditioning regimens were OM [19,21,28,29], HC, pulmonary hemorrhage [28-31], and VOD/SOS [28-30]. HC (median incidence: 29.3%) and pulmonary hemorrhage (incidence: 5.8%) were reported in four cohorts of the FLU-based group [28-31]. FA-HSCT patients suffered from VOD/SOS [28-30]. In two American studies, the development of insulin-dependent diabetes mellitus was reported in cohorts using FLU-XRT-based regimen [19,27].

OM was reported as gastrointestinal toxicity in all six non-FLU-based studies. All Brazilian patients who received LDCY, presented grade I-IV OM [33]. A study described grade I-III OM in 36.6% (8 out of 22) vs. 60% (3 out of 5) in irradiated and non-irradiated patients, respectively [26]. Among the non-FLU-based Italian cohort, HC development was reported in 40% of patients vs. none in those who were conditioned with HDCY vs. LDCY + XRT [22,26]. Three studies reported liver and kidney toxicity [17,26,33]. Additionally, PRES was reported in the Iranian cohort that utilized a non-FLU-non-XRT-based protocol [34].

A study on sixteen Turkish patients reported VOD in FLU-based groups; however, the sample size was not adequate enough to speculate the correlation, similar to other FLU-based cohorts [36]. Furthermore, OM was described in all (6 out of 6) patients who were conditioned with non-FLU-based protocol, but none (0 out of 10) in patients who received FLU-based conditioning. The EBMT report in 2007 did not find any statistical difference in toxicities between FLU- and non-FLU-based groups [25]; however, P. Stepensky indicated statistically lower cumulative toxicity score of many organs in patients who underwent the FLU protocol (10.6 vs. 3.9) [38].

The HSCT secondary outcomes

Table 5 describes the post-HSCT malignancies, in addition to OS and TRM of the cohorts based on the conditioning regimen protocol groups.

The SMNs

Four cohorts in the FLU group who were irradiated with SDTBI 150 – 450 cGy developed squamous cell carcinoma (SCC) [19,27,31,32]. MDS and Burkitt's lymphoma were reported in two of these studies [27,32]. In an Egyptian study secondary AML was reported in cases without irradiation [24].

SCC was reported in three studies that were non-FLU-based with or without the use of XRT [17,23,33]. Similarly, the Italian cohort, based on a CIBMTR report in 1995, had developed SCC without the utilization of XRT; Secondary AML was also reported in the same study [23]. Two patients who underwent HSCT using non-FLU-XRT protocol also developed SCC [17]. The Brazilian study reported SCC development within 5-years post-HSCT with extensive cGvHD occurrence [33].

SCC was reported in five studies [66, 35, 39-41], while secondary AML occurred in two [36,41]. Lymphoma [16,41] and gliomatosis

Table 5: The secondary HSCT outcomes of the cohorts concerning the conditioning regimen protocol group—FLU-, non-FLU-, and both.

Group	Study (First Author, Year)	Median HSCT FU (Range) (yr.)	OS (Time)	Transplant-Related Morbidity	Secondary Malignancy
FLU-based	S. Chaudhury, 2008	3.2 (1 - 6.5)	72.2 (5-yr.)	GvHD, Infection, ARDS, MOF, Relapsed AML	SCC (V)
	B. Kuskonmaz, 2016	4.5 (0.83 - 10.9)	96 (5-yr.)	GF, Infection	None
	P. Anur, 2016	7.45 (2.2 - 15.3)	100, 84 (5-, 10-yr.)	Secondary Malignancy	SCC, MDS (XRT-)
	G. M. Fathy, 2017	N. M.	68.3 (6-yr)	GF, Infection, Intracranial H, aGVHD, VOD, Relapsed AML	AML
	C. L. Ebens, 2018	7 (3.9 - 9.6)	90 (5-yr)	GvHD, GF, Infection, MOF	N. M.
	M. Ayas, 2019	3.1 (2.7 - 3.6)	89.5 (5-yr.)	GvHD, Recurrence Leukemia	None
	G. Tuysuz, 2019	3 (0.08 - 13.25)	70.5 (3-yr.)	Infection, GvHD, HC	None
	R. Uppuluri, 2020	2.5	68.4 (2.5-yr.)	GvHD, Infection, GF, Ruptured Peliosis Hepatis	N. M.
	L. Murillo-Sanjuán, 2021	6.5 (0.27 - 11.1)	73 (5-yr.)	Infection, GF, PTLD, Relapse, Secondary Malignancy	SCC
	O. Fink, 2023	2.1 (0 - 20.1)	82.9 (2-yr.)	Infection, ARDS, Secondary Malignancy	SCC (O), Burkitt Lymphoma
non-FLU-based	M. Kohli-Kumar, 1994	2.25 (0.5 - 6.25)	100 (2-yr.)	None	N. M.
	E. Gluckman, 1995	2.75 (0.3 - 13.5)	86 (2.75-yr.); **82, 70** vs. 55 (**XRT-**); **91** vs. 70 (**ATG-**) (2-yr.)	N. M.	SCC (T, L), AML (XRT- (LFI))
	C. Dufour, 2001	3 (0.1 - 16.3)	80.8 (3-yr.)	cGvHD, ARSD, MOF	None
	A. Farzin, 2007	10.2 (2.5 - 15.9)	89 (10-yr.)	MOF (ARDS, Cerebral Infarcts, Fulminant Hepatic Failure, Autoimmune Anemia, Autoimmune Thrombocytopenia, Interstitial Pneumonia), Relapsed AML	SCC (T, A, HN)
	C. M. Bonfim, 2007	3.7 (0.6 - 7.9)	93 (3.7-yr.)	GF, MOF, Infection, NC, RRT	SCC (T)
	T. Rostami, 2022	2 (0.2 - 8.5)	84.14, 82.16 (1-, 5-yr.)	Infection, GvHD, GF, Toxicity (CNS)	N. M.
Both	F. Locatelli, 2007	6 (0.25 - 16)	**86** vs. 59 (**FLU-**) (8-yr.)	Secondary Malignancy (non-FLU)	SCC (T)
	J. E. Wagner, 2007	14.6 (6.1 - 19.3)	52 vs. 13 (3-yr.)	GF: 2 vs. 6, GvHD: 5 vs. 3, Interstitial Pneumonitis: 0 vs. 1, OF: 2 vs. 0, Recurrent Leukemia: 1 vs. 1 (**non-FLU-**)	None
	E. Gluckman, 2007	1.8 (0.25 - 10)	50 vs. 25 (**FLU-**) (3-yr.)	Infection, GF, HC, aGvHD, MOF, VOD, ARDS	N. M.
	M. Akif Yesilipek, 2009	2.6 (0.25 - 7.5)	90 vs. 50 (3-yr.)	VOD vs. Infection (FLU- vs. non-FLU-)	AML (non-FLU-)
	P. Stepensky, 2011	2.6 (0 - 12.4)	**83** vs. 35 (**FLU-**) (10.9-yr.)	GvHD: 1 vs. 9, Infection: 2 vs. 1, Secondary Malignancy: 0 vs. 1	Yes (non-FLU-)
	A. A. Hamidieh, 2011	1.1 (0.25 - 13.5)	36.4 vs. 70 (3-yr.)	GF, GvHD (n = 1), PTLD vs. Infection, GvHD (n = 5), H, TTP, ARDS (FLU- vs. non-FLU)	N. M.
	R. Peffault de Latour, 2013 (13)	6 (0 - 28)	65, 52, 36 (**FLU-**) (5-, 15-, 20-yr.)	GvHD, Infection, GF, Toxicity, Relapse, Secondary Malignancy	SCC (Mo, T, O, L, V-Vg), ST, Lymphoma, AL, MDS
	S. E. Smetsers, 2016	5.5 (0 - 23.5)	76.4; 87.8 vs. 59.3 (5-yr.)	Relapsed Leukemia, aGVHD, Infection, MOF, Secondary Malignancy	SCC (HN, O, Vg)
	F. Bernard, 2021	6.2 (5 - 7.3)	79.9; 88.3 vs. 27.3 (5-yr.)	Relapse, Gliomatosis cerebri, cGVHD, BOS	SCC (Gingival, T, O), Gliomatosis cerebri
	M. Yabe, 2021	8.7 (0.1 - 28.6)	81; **87.1** vs. 66.7 (**FLU-**); **87.5** vs. 59.4 (**ATG/ALG-**); 77, 72 (**5-**, 10-, 15-yr.)	MOF, Relapse, GvHD, Infection, PTLD, Secondary Malignancy	SCC (T, M, O), donor-type AML, non-Hodgkin Lymphoma

Abbreviations: FU: Follow Up; Yr.: Year; R: Range; OS: Overall Survival; CY: Cyclophosphamide; ATG: Anti-thymocyte Globulin; TAI: Thoracoabdominal Irradiation; cGy: Centi Grays; S: Shielding; LFI: Limited Field irradiation; TBI: Total Body Irradiation; Ara-c: Cytarabine; FLU: Fludarabine; ALEM: Alemtuzumab; SDTBI: Single Dose Total Body Irradiation; RT: Radiation Therapy; BU: Busulfan; SFTBI: Single Fraction Total Body Irradiation; PTCY: Post Transplant Cyclophosphamide; N. M.: Not Mentioned; GF: Graft Failure; aGvHD: Acute Graft versus Host Disease; VOD: Veno Occulsion Disease; MOF: Multiple Organ Failure; ARDS: Acute respiratory distress syndrome; AML: Acute Myeloblastic Leukemia; HC: Hemorrhagic Cystitis; SCC: Squamous Cell Carcinoma; T: Tongue; L: Larynx; A: Anus; HN: Head and Neck; V: Vaginal; AL: Acute Leukemia; M: Myeloid; ML: Mixed Lineage; MDS: Myelodysplastic Syndrome; N. M.: Not Mentioned.

cerebri, nine years post-HSCT [40], were reported in two and one cohorts respectively. Two Japanese boys developed donor-type AML and non-hodgkin lymphoma six and one year after being conditioned with FLU-based TBI and TAI protocols in M. Yabe's study [41]. A study by P. Stepensky defined SMNs in patients without FLU [38].

The OS and cause of death

Among ten cohorts who received FLU-based conditioning regimen, the average OS rates were reported as following: < 5-year OS at 84.1% (n = 5), 5-year OS at 74% (n = 3), and > 5-year OS was noted at 76.1% (n = 2).

Two studies reported ≥ 90% 5-year OS with a median FU of 4.5 – 7 years in the FLU-based setting (FLU 175 mg/m^2) [20,28]; however, three studies with lower dose FLU (140 mg/m^2) reported 72.2% – 89.5% OS rate [19,29,31]. Three cohorts transplanted with FLU at 120 – 180 mg/m^2 stated < 90% of 3-, 2.5-, and 2-year OS rates, respectively [21,30,32]. Higher OS rate of 84% in the American cohort conditioned within a FLU-based regimen (140 – 150 mg/m^2) was reported [27]. Subsequently, the lowest OS within 6 years was 68.3% in the Egyptian cohort [24].

Subsequently, in the non-FLU-based conditioning regimen, the OS rates were at 90% (< 5-year), 82% (5-year), and 89% (> 5-year) in one, four, and one cohort, respectively.

A 5-year OS rate of 82.16% was reported in the Iranian cohort with the non-FLU-non-XRT protocol [34]. Among three studies conditioned with non-FLU-XRT, the longer the time from HSCT, the lower the OS rate. It means that 2-year OS: 100%, 2.75-year OS: 86%, and 3-year OS: 80.8% [22,23,26]. In parallel, a multi-institution study utilizing LDCY calculated the 3.7-year OS rate of 93% [33] Similarly, the 10-year OS of 89% was reported in the American cohort, which is consistence with non-FLU-XRT settings [17].

Nine studies confirmed the profound difference in OS based on FLU-based regimens compared to non-FLU ones with the median FU time of 3 – 10.9 years [16,18,35-41]. All cohorts except one by A.A. Hamidieh reported higher OS rates in patients who received FLU. They did not find a statistically significant difference in 3-year OS (36.4% vs. 70%; *P-value = 0.112*) [37]. In contrast, The EBMT 2007 report stated a 3-year OS rate of 50% vs. 25% in the FLU- vs. non-FLU-based conditioning regimen, respectively (*P-value = 0.01*). Howbeit, irradiation usage was not described as a statistically determining factor, 32% vs. 47% (XRT- vs. non-XRT; *P-value = 0.21*) [25].

Infection and GvHD development were reported as the main TRM in eight [19-21,24,28,30-32] and six [19-21,24,29,30] cohorts with FLU-based protocol. In tandem, five cohorts reported that the FA-HSCT patients died of GF [20,21,24,28,31]. Secondary AML [19,24,29] and SMNs [27,31,32] occurred in three cohorts each.

Two studies defined MOF and ARDS as causes of death [17,26]. The neurological complication and CNS toxicity were reported in patients who were conditioned with CY-and CY-BU-based protocols, respectively [33,34].

Two studies by F. Locatelli and J. E. Wagner (47% vs. 81%; *P-value < 0.001*) described higher TRM rates in non-FLU- cohorts [18,35]. P. Stepensky and A. A. Hamidieh reported GvHD as TRM in non-FLU groups [37,38].

Discussion

This systematic review is the first conducted on 26 cohorts who underwent HSCT using preparative regimens to investigate post-HSCT complications regarding FLU usage with and without irradiation. FA patients need multidimensional management due to the sophisticated nature of the disease. The disease status, *FANC* genotypes, and mosaicism are effective factors that physicians have to consider to ensure successful HSCT outcomes. The clonal cytogenetic abnormalities evolution can be challenging. Patients fare better with low-intensity conditioning regimens due to the pathophysiology of FA disease; however, recipients with cytogenetic abnormalities require more intensive regimens [42].

Approximately 31% of American transplant recipients with FA had evidence of cytogenetic clones- MDS, or AML; however, no significant difference was found [17], in contrast to S. E. Smetsers's study [39]. Conversely, The EBMT report in 2013 defined BM status as an imperative factor associated with better OS (AA vs. MDS or AML; hazard ratio: 2.1, (95% confidence interval (1.41 – 3.11)) [16]. On the other hand, a retrospective analysis showed a significantly higher 5-year OS in the AA group rather than in the MDS or AML [40,41]. Since BM cellularity has a vital effect on the outcome of HSCT, we suggest mentioning BM status in every cohort.

The diagnosis and management of FA is contingent on the molecular work-up. *FANCA*, *FANCG*, *FANCC*, *FANCD2*, and *FANCB* is distributed in 85%, 9%, 3%, 2%, and 1% of the patients, respectively. The genotype-phenotype association in this disease is vague, which contributes to challenges in prognostic predictions, treatment decisions, and the FU programs [43].

In some studies, the subtype of FA and HSCT association were analyzed indecorously (n= 4). The remaining studies did not mention the subtypes at all (n = 15). In the American cohort it was noted that the complementation group (*FANCA* vs. non-*FANCA*) did not influence OS [17]. In parallel, the CIBMTR report in 2007 did not signify a correlation either [18]. Three out of 12 FA-A, one out of three FA-G, and one out of one FA-D1 patients died due to infection and disease relapse in the American cohort; however, it was stated as statistically not significant [19] unlike former studies. The unified multi-centric Spanish study was similar to the American study [31].

Spontaneous HSPC modifications of *FANC-A*, *B*, *C*, *D2*, *N*, and *-T* germline mutations lead to a somatic genetic rescue (SGR) event. It's worth noting that specific genome sequences, molecular defects, and mitochondrial oxidative metabolism contribute to development pathways and the occurrence rate of the aforementioned event in FA individuals. Ultimately an attenuated cellular and clinical phenotype leading to the late onset and diagnosis of FA individuals are associated with HSPC's SGR event. A recently published retrospective review of literature-reported FA mosaicism cases has indicated that mosaicism may be associated with a lower incidence of BMF or hematologic malignancy, lower requirement for HSCT, and relatively lower mortality during the initial two to four decades of life compared to non-mosaic patients [44].

Twenty-two cohorts had not determined mosaicism, despite its importance. The CIBMTR report in 2007 reported on the influence of mosaicism on HSCT outcomes. An observation suggests the higher GF incidence in recipients of non-FLU-XRT may be due to incomplete ablation of DEB-resistant lymphocytes, which the addition of FLU overcame [18]. Furthermore, an American study demonstrated mixed chimerism (91% donor cells) within two years in one patient with a 76% mosaicism history [17]. Two studies mentioned this matter but had not analyzed the relationship [19,20]. Carrying out a FANC group's analysis and FA/BRCA pathway to determine mosaicism pre-HSCT, contributes to the appropriate conditioning regimen and irradiation usage, leading to better HSCT outcomes. There are contradictory outcomes regarding FLU- and non-FLU-based with or without XRT in the cohorts. The most efficacious engraftments were

observed in patients receiving HSCT utilizing FLU-XRT-based (150 mg/m^2) preparative regimens [19,27,29]. The use of FLU at 180 mg/m^2 associated with 10.5% and 22% of GF in the two studies, respectively, with [21] and without [32] radiotherapy. The EMBT report in 2013 proved the FLU-based regimen has a vital role in efficient engraftment [16]. Unexpectedly, the highest rate of secondary GF (9.5%) was reported in the non-FLU-non-XRT-based protocol in a multi-institution study [33].

Preparative regimens have similar effects on a-and c-GvHD development. All four studies comparing FLU and non-FLU-based regimens showed lower GvHD development in recipients using FLU [18,36-38]. Two cohorts support 'the higher the CY dose, the higher the GvHD incidence' in the non-FLU-non-XRT settings [26,33].

Likewise, post-HSCT toxicities regarding regimens are consistent with the engraftment and GvHD development outcomes; the higher the CY dose, the higher the OM and HC incidences. Overall, a higher OS rate was seen in patients who underwent HSCT in the FLU-non-XRT-based settings. Secondary AML and GvHD are described as the most common TRM in the XRT setting. Howbeit, the patients who were not irradiated expired due to infection and GF. Irradiation can be a risk factor in SCC development [23,27,35,40,41]; however, cohorts with non-XRT-based preparative regimens reported it as well [31-33]. The EBMT report in 2013 could not find a statistically significant relation between XRT-based conditioning regimen with SMNs; although independent risk factors including HSCT age and clonal evolution were an indication.

This is the first systematic review to investigate the effect of preparative regimens on HSCT success, as stated. The evidence was described transparently by ascertaining the scientific goals in advance and employing a systematic approach with precise methods, minimizing the risk of study selection and reporting bias. However, we dealt with several limitations.

The patient's age, disease status and subtypes, the conditioning regimen protocols details, types of irradiations, and incidence of HSCT outcomes were not mentioned in the review of some studies, which restricted conclusions. We did not narrow the inclusion criteria, even though some limitations could be solved and the comparability between cohorts could be enhanced.

The cohorts regarding regimens and their effect on HSCT outcomes are countless, but the evidence is limited due to heterogeneity as well as methodological shortcomings. However, current evidence shows better post-HSCT outcomes in FLU-regimen without irradiation. In addition, evidence suggests a lower risk of developing SMNs in a long-term FU of patients who weren't exposed to irradiation. Due to the heterogeneity of data and the lack of conducive parameters, we suggest future studies prioritize the investigation of the relationship between the patient's genotype and the appropriate conditioning regimen as well as the dosage.

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Author Contributions Statement

P.N. conducted electronic search and selection processes, assessed risk of bias, and wrote the manuscript. M. B. supervised the manuscript. H. K. assessed risk of bias and synthesized method. L. J., Z. K., R. M. and M. M. conducted data collection process. Y. K. checked and edited native English. A. A. H. hypothesized the main idea of the manuscript.

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