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The Emerging Role of Gemcitabine in Conditioning Regimens for Hematopoietic Stem Cell Transplantation

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The specific combination for conditioning regimens in hematopoietic stem cell transplantation continues to be a premier area of focus in research. Although conditioning regimens have significantly evolved over time, obstacles continue to persist, including regimen-related toxicities, graft-versus-host disease, and disease relapse. Gemcitabine (2',2'-difluoro 2'-deoxycytidine, dFdC) is a pyrimidine nucleoside analog that distinguishes itself from other agents in the class by possessing a favorable pharmacokinetic and cytotoxic profile, while maintaining acceptable toxicities. Given the desirable properties, gemcitabine has garnered much attention and been assessed in several conditioning regimens. In this article, we review the pharmacology of gemcitabine with other nucleoside analogs and report the findings of pivotal trials conducted in both autologous and allogeneic transplantation. The positive results suggest a potential future role for gemcitabine and necessitate the need to conduct studies to further define its role.

INTRODUCTION

The use of hematopoietic stem cell transplantation (HSCT) has evolved over the past 50 years and now represents a potentially curative therapy for patients worldwide with various forms of malignant and nonmalignant diseases. Although the risks associated with HSCT have been significantly reduced, obstacles remain with regimen-related toxicities (RRT), graft-versus-host disease (GVHD), and disease relapse. Because of the impact on these various areas, refining HSCT conditioning regimens continues to be a premier area of focus. Research has been dedicated to finding the specific combination of chemotherapy agents, total body irradiation, and targeted therapies to optimize disease control, while also limiting treatment-related toxicities and GVHD. Advances, such as optimizing the systemic exposure of certain drugs through prospective pharmacokinetic-based monitoring, have aided in reducing toxicities. Incorporating novel agents into conditioning regimens has helped improve overall antitumor activity [1-3]. For example, nucleoside analogs have become a focus of interest, given their broad therapeutic activity and mild extramedullary toxicities. Although fludarabine already has an established role in allogeneic HSCT, other agents within the class are also showing promise. Recently, clinical studies have successfully incorporated gemcitabine into HSCT conditioning regimens. In this review, we will discuss the potential role of gemcitabine in HSCT and summarize the available clinical data.

GEMCITABINE PHARMACOLOGY

Gemcitabine (2',2'-difluoro 2'-deoxycytidine, dFdC) is a pyrimidine nucleoside analog [4,5]. Although mechanistically similar, gemcitabine distinguishes itself structurally from cytarabine by a fluorine group substituted at position 2' on the furanose ring (Figure 1). Gemcitabine, like other nucleoside analogs, requires cellular uptake via nucleoside transporters and intracellular phosphorylation for activation. Cellular uptake of the highly lipophilic gemcitabine molecule across the cell membrane involves specific nucleoside transport proteins through both active processes and facilitated diffusion. Specifically, 2 types of human nucleoside transporters have been identified: equilibrative (sodium independent) and concentrative (sodium dependent). Although both transporters are involved in the cellular uptake of gemcitabine, the bidirectional equilibrative carriers, such as human ENT1, have been identified as the primary transport [4,6,7].

Upon cellular uptake, gemcitabine undergoes phosphorylation by deoxycytidine kinase (dCK) to an intermediate metabolite gemcitabine monophosphate. The presence of dCK is rate limiting in gemcitabine activation [8,9]. Although at a much reduced substrate affinity compared with dCK, thymidine kinase 2 is also thought to play a minor role

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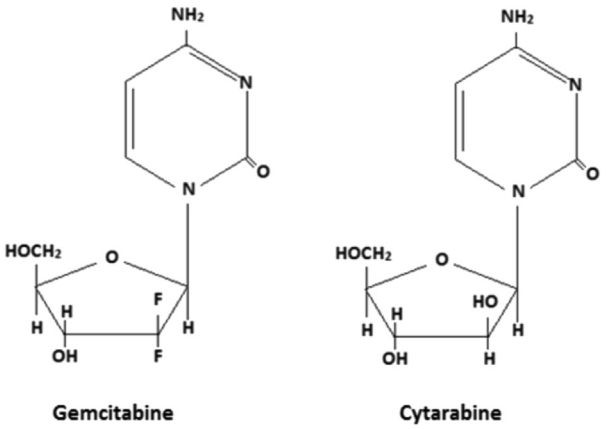


Figure 1. Nucleoside Analog Structures.

in phosphorylating gemcitabine [4]. The monophosphate metabolite is then further converted by other nucleotide kinases to the active metabolites gemcitabine diphosphate (dFdCDP) and, most importantly, triphosphate (dFdCTP). It is dFdCTP that is subsequently incorporated into DNA, resulting in DNA synthesis inhibition (Figure 2) [6-9].

Although the major mechanism through which gemcitabine exerts its activity is inhibition of DNA synthesis, it also exhibits other cytotoxic mechanisms. Other mechanisms include direct inhibition of DNA polymerase, resulting in termination of DNA chain elongation; inhibition of ribonucleotide reductase (RNR), leading to reduced competing deoxyribonucleotide pools necessary for DNA synthesis; and incorporation into RNA resulting in direct apoptosis [4,5,8,9]. Of these, the inhibition of DNA polymerase and RNA incorporation are largely attributed to dFdCTP. In contrast, dFdCDP is thought to inhibit RNR and the subsequent reduction in deoxyribonucleotides, particularly deoxycytidine triphosphate [8,9]. Furthermore, it has been suggested that gemcitabine may also contain cytotoxic activity by inducing topoisomerase I-mediated DNA strand breaks [4].

GEMCITABINE COMPARED WITH OTHER NUCLEOSIDE ANALOGS

Two major factors determine the clinical activity of nucleoside analogs: the substrate specificity for activating nucleoside kinases and the expression of these enzymes within the tumor tissues. The content of dCK is several-fold higher in lymphocytes than in other epithelial cells. The affinity of dCK is higher for gemcitabine compared with the other nucleoside analogs, including fludarabine, cytarabine, and cladribine. This may explain the broader range of clinical activity seen with gemcitabine compared with the other nucleoside analogs [8,10-12].

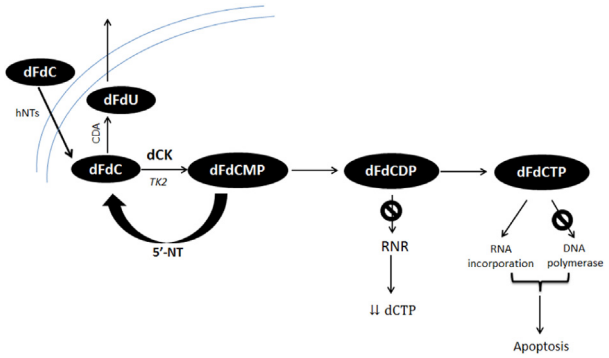


Figure 2. Intracellular Metabolism of Gemcitabine [6-9]. hNTs indicates human nucleoside transporters; TK2, thymidine kinase 2; dFdCMP, gemcitabine monophosphate; CDA, deoxycitidine deaminase; dFdU, 2'2'-difluorodeoxyuridine; dCTP, deoxycytidine triphosphate.

Collectively, the nucleoside analogs share a similar cytotoxic mechanism of inhibiting DNA polymerase at the analog insertion site upon intracellular activation by dCK phosphorylation (Table 1) [4,10,13-19]. Compared with cytarabine and fludarabine, gemcitabine undergoes greater activation to dFdCDP and dFdCTP because of its higher affinity for dCK [9,14]. In addition, gemcitabine possesses an additional cytotoxic mechanism of inhibiting RNR, the major source of deoxynucleotides normally required for DNA synthesis and repair [9]. Although the active metabolites of fludarabine and clofarabine also possess RNR inhibition activity, only dFdCDP results in an irreversible inhibition (Table 2) [9,14,15]. Specifically, in situ assays have identified the active metabolite dFdCDP as inducing the subsequent cellular depletion of deoxynucleotides, resulting in self-potentiation by preferentially incorporating gemcitabine as dFdCTP into DNA [9,10]. Furthermore, gemcitabine exhibits the unique ability of "masked" chain termination. This occurs after dFdCTP DNA incorporation and includes the addition of a single deoxynucleotide by DNA polymerase, predominantly to the 3' end of the extending DNA strand. This specific type of incorporation leads to the masking of the gemcitabine nucleotide from normal DNA polymerase 3' to 5' proof-reading exonuclease activity that normally removes mismatched base pairs [9,10]. This masking phenomenon is unique to gemcitabine, as none of the other nucleoside analog possess a similar mechanism that may help prevent normal DNA polymerase proofreading activity [9,14].

From a pharmacokinetic (PK) perspective, gemcitabine also exhibits favorable attributes that further enhance its mechanisms of action. Similar to clofarabine triphosphate (Cl-F-ara-ATP), dFdCTP has a slow cellular elimination half-life exhibiting both monophasic and biphasic properties (Table 3). At higher cellular concentrations ($\geq$ 100 μM), dFdCTP exhibits more biphasic elimination with a prolonged terminal half-life of 15 to 24 hours as compared with monophasic elimination of 4 to 6 hours in lower cellular concentrations [4,10,13-19]. This unique property of gemcitabine aids in the self-potentiating mechanism by reducing the elimination and further promoting the accumulation of the active metabolite. Specifically, dFdCTP is thought to reduce elimination by blocking deoxycytidylate monophosphate deaminase, its key catabolic enzyme. By blocking deoxycytidylate monophosphate deaminase, the elimination half-life changes from a monophasic to biphasic elimination, resulting in higher cellular dFdCTP concentrations, further enhancing the cytotoxic activity of gemcitabine [14].

Parental gemcitabine at high intracellular levels acts as a substrate inhibitor of dCK, thus explaining that levels of dFdCTP peak with intracellular gemcitabine levels of around 20 μmol/L [8,10,11]. In addition to inherent PK advantages, infusion times of gemcitabine have a direct effect on the activity of gemcitabine. Prolonged infusions at a fixed-dosed rate (FDR) of 10 mg/m^2/minute avoid the saturation of dCK activity by maintaining extracellular gemcitabine concentrations below 15 to 20 μM [20-22]. This FDR strategy has resulted in increased concentration-time curves of dFdCTP in leukemic cells as compared with the standard infusion times [8,23]. Furthermore, DNA synthesis remains suppressed up to 24 hours after the initiation of the gemcitabine infusion [24].

Table 1
Nucleoside Analog Comparison: Mechanism of Action [4,10,13-19]

Comparison	Gemcitabine	Cytarabine (Ara-C)	Fludarabine	Clofarabine
Chemotherapy class	Pyrimidine analogs		Purine analogs	
Mechanisms of action				
Inhibition of DNA polymerase	Intermediate	Strong	Strong	Strong
Inhibition of DNA chain elongation	Intermediate	Strong	Strong	Strong
Inhibition of RNA synthesis	Weak	Weak	Strong	Weak
Other	• Masked DNA chain termination	-	• Prevents DNA ligation	• Induces apoptosis
Major active metabolites	• Diphosphate (dFdCDP) • Triphosphate (dFdCTP)	• Triphosphate (ara-CTP)	• Triphosphate (F-ara-ATP)	• Triphosphate (Cl-F-ara-ATP) • Monophosphate (Cl-F-ara-A)

Ara-CTP indicates cytarabine triphosphate; F-ara-ATP, fludarbine triphosphate; Cl-F-ara-ATP, clofarabine triphosphate; Cl-F-ara-A, clofarabine monophosphate.

Although more active in certain malignancies, FDR administration also results in increased myelosuppression and limited nonhematologic toxicities, such as elevations of transaminases [25-28]. The increased toxicities with gemcitabine FDR infusions have prevented its widespread use in oncology practice, instead favoring the shorter 30-minute infusion, despite the PK and pharmacodynamic advantages of FDR.

GEMCITABINE ACTIVITY

In addition to its broad use for solid malignancies, gemcitabine has also demonstrated in vitro and clinical activity in hematologic malignancies, such as leukemia, non-Hodgkin's lymphoma (NHL), and Hodgkin's lymphoma (HL) [11,12,23,29]. Specifically, fludarabine and gemcitabine combinations have demonstrated synergy in phase 1 studies for relapsed or refractory acute myelogenous leukemia, with minimally active concentrations of both agents resulting in a 3- to 4-fold increase in activity compared with either agent alone [29]. Additionally, when combined with traditional alkylating agents, such as melphalan and busulfan, nucleoside analogs are able to inhibit DNA repair of alkylator-mediated DNA damage [30-32].

GEMCITABINE USE IN AUTOLOGOUS HSCT

Gemcitabine/Docetaxel/Melphalan/Carboplatin

Several gemcitabine-containing conditioning regimens have been assessed in the autologous HSCT setting. In 1 of the earlier reports of gemcitabine use by Nieto et al., gemcitabine at FDR of 10 mg/m^2/minute was combined with a previously described regimen consisting of high-dose docetaxel, melphalan, and carboplatin [33]. The length of gemcitabine infusion was escalated from 9 to 20 hours (total dose of 12,000 mg/m^2), which was established as its maximum tolerated dose (MTD) (Figure 3).

A total of 52 heavily pretreated patients with refractory solid tumors and lymphomas were enrolled. The major nonhematologic RRT included stomatitis and gastrointestinal toxicity (Table 4). Thirty-one of 34 patients (91%) with measurable disease before HSCT responded to gemcitabine/docetaxel/melphalan/carboplatin (Gem-DMC), with 50% experiencing a complete response (CR). Median duration of response was 19 months (range, 4 to 30+ months). At median follow-up time of 24 months (range, 13 to 33 months), 79% of patients were alive, with 54% of them remaining disease free. Median event-free survival (EFS) was 26 months, although overall survival (OS) had not yet been reached (Table 5) [33].

PK and pharmacodynamic analyses were conducted by measuring plasma gemcitabine and 2',2'-deoxydifluorouridine levels, as well as intracellular dFdCTP levels in mononuclear cells. Gemcitabine exhibited a linear PK behavior at high doses, with a linear increase of its area under the curve with the duration of infusion from 9 to 20 hours ($r^2 = .71$, $P < 10^{-5}$), with no major differences observed in clearance ($r^2 = .005$, $P = .60$), maximum concentration ($P = .14$), volume of distribution ($P = .95$), half-life ($P = .85$), or steady-state concentration ($P = .37$). Serial measurements of intracellular dFdCTP levels throughout treatment showed an exponential increase after the last of the 4 daily treatments, consistent with the self-potentiating mechanisms of gemcitabine activation [33].

This regimen is also currently being evaluated in other oncologic venues. Specifically, Gem-DMC with combination bevacizumab is being assessed in an ongoing phase 2 trial for patients with refractory germ-cell tumors (Clinical Trials: NCT00936936) [34]. Treatment in this phase 2 trial consists of patients receiving bevacizumab on day -14, followed by 2 different high-dose chemotherapy (HDC) regimens initiated on day -5 with stem-cell support on day 0. The 2 HDC regimens consist of Gem-DMC as conditioning for the first HSCT, followed by ifosfamide/carboplatin/etoposide for the second HSCT. In the preliminary analysis of the first 21 patients, tumor markers normalized in 14 of 17 evaluable patients after HDC cycle 1. Mucositis was the most common toxicity. At a median follow-up time of 23 months (range, 3 to 43 months), 14 of 21 patients were alive and in CR [34,35].

Table 2
Nucleoside Analog Comparison: Pharmacology [4,10,13-19]

Comparison	Gemcitabine	Cytarabine (Ara-C)	Fludarabine	Clofarabine
Affinity for enzymes				
dCK	++++	+++	+	+++++
RNR activity				
Metabolite with activity	dFdCDP	None	F-ara-ATP	Cl-F-ara-ATP
RNR inhibition	Irreversible	None	Reversible	Inhibits
RNR strength of inhibition	Potent	None	Weak	Potent
Affinity for deactivating enzymes				
dCMP deaminase	Substrate	Substrate	N/A	N/A

dCMP indicates deoxycytidylate monophosphate; dCK, deoxycytidine kinase; RNR, ribonucleotide reductase; dFdCDP, gemcitabine diphosphate; F-ara-ATP, fludarabine triphosphate; and Cl-F-ara-ATP, clofarabine triphosphate.

Table 3
Nucleoside Analog Comparison: Pharmacokinetics [4,10,13-19]

Comparison	Gemcitabine	Cytarabine (Ara-C)	Fludarabine	Clofarabine
Triphosphate cellular elimination	Slow	Rapid	N/A	Slow
Elimination half-life	Monophasic: 4-6 h Biphasic*: 15-24 h	Biphasic: α half-life: 7-20 min β half-life: 2-3 h	Monophasic: 15- <24 h	Triphasic: β half-life: 8-24 h γ half-life: >24 h
Self-potentiation	Yes	No	Yes	Yes

* At higher cellular concentrations ≥ 100 μM

Gemcitabine/Busulfan/Melphalan

Although the BEAM (carmustine [BCNU], etoposide, cytarabine, and melphalan) regimen has long been considered 1 of the standard HDC regimens utilized before autologous HSCT for patients with chemosensitive relapsed HL and diffuse large B cell lymphoma, relapse remains a major concern [10,36]. Patients with primary refractory tumors or high-risk relapse, such as those whose tumors relapse within 1 year, are at a particularly higher risk for relapse after BEAM.

In preclinical studies, exposure of chemotherapy-refractory T and B cell lines individually to gemcitabine, busulfan, and melphalan resulted in no significant effect on cell proliferation. Variations of 2-drug combinations (busulfan/melphalan, gemcitabine/busulfan, and gemcitabine/melphalan) led to mean inhibition of proliferation rates of 16%, 18%, and 22%, respectively. In contrast, exposure to triple therapy with gemcitabine, busulfan, and melphalan (Gem/Bu/Mel) led to an average inhibition of proliferation rate of 48%, significantly greater than any of the 2-drug combinations ($P < .001$) [10].

These preclinical observations led to a dose- and schedule-finding study of Gem/Bu/Mel, where 3 different schedules of gemcitabine were assessed in combination with busulfan and melphalan in 133 patients with refractory lymphoid malignancies, including HL, NHL, and myeloma. Gemcitabine was infused daily for 6 days on days -8 to -5 and -3 to -2; as 3 doses on days -8, -6, and -3; or as 2 doses on days -8 and -3 (Figure 4). Each dose of gemcitabine was administered with a loading dose of 75 mg/m^2 i.v. bolus, targeting a steady-state concentration of 15 μmol/L, followed by FDR of gemcitabine, followed by the corresponding doses of busulfan and/or melphalan [10].

The cutaneous and mucosal toxicity profile of Gem/Bu/Mel was more pronounced with the daily 6-dose and 3-dose gemcitabine schedules, whereas the 2-dose gemcitabine schedule resulted in a much ameliorated profile of mucositis, rash, and self-limited asymptomatic transaminase elevation (Table 6). Following the 2-dose gemcitabine schedule, doses were escalated from level 1 to 9, with level 9 established as the MTD (daily dose of 2775 mg/m^2 and total dose of 5550 mg/m^2).

Response rates among HL patients were 88%, with 62% of patients in CR. At a median follow-up of 24 months (range, 3 to 63 months), the EFS and OS rates were 54% (44 of 80 patients) and 72% (58 of 80 patients), respectively, with a median EFS of 43 months and median OS not reached (Table 5) [10]. The high antitumor activity of Gem/Bu/Mel in HL raises the question of how it compares with current standard HDC regimens.

In a contemporary cohort study of all patients with refractory or high-risk relapsed HL who underwent transplantation at MD Anderson between 2005 and 2010, Gem/Bu/Mel, BEAM, and busulfan/melphalan (Bu/Mel) were compared [36]. A total of 180 patients were analyzed, 84 of whom received Gem/Bu/Mel, 39 received Bu Mel, and 57 received BEAM. Of all patients treated, 86 patients and 94 patients were primary refractory and poor-risk relapsed patients, respectively. Of note, the Gem/Bu/Mel arm included significantly more patients with primary refractory disease and other poor prognostic features, such as positron emission tomography (PET)—positive tumors and tumor growth at time of HDC, as well as extranodal disease and bulky tumors (> 5 centimeters) at relapse or progressive disease.

The median follow-up times were 32 months (range, 12 to 68 months), 36 months (range, 3 to 72 months), and 49 months (range, 8 to 66 months) for the Gem/Bu/Mel, Bu/Mel, and BEAM cohorts, respectively. The Bu/Mel and BEAM groups had similar EFS rates (33% versus 39%, $P = .60$). Despite having worse prognostic features, the Gem/Bu/Mel cohort had significantly improved EFS rates when compared with the combined EFS rates of Bu/Mel and BEAM groups

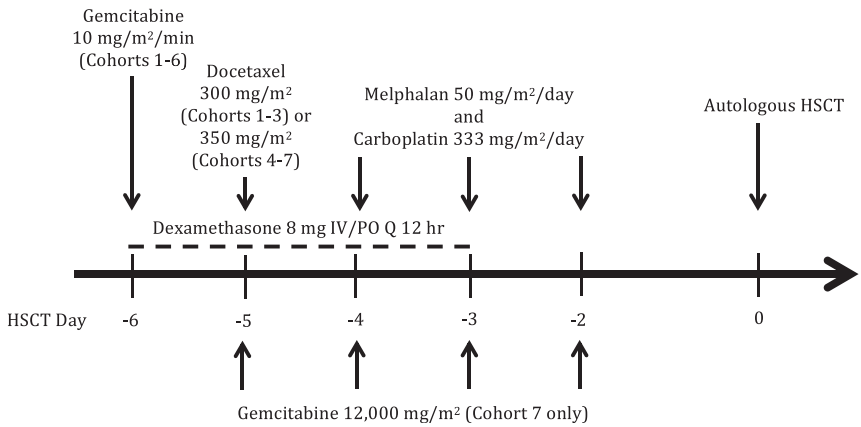


Figure 3. Treatment Regimen [33].

Table 4
Gem-DMC Toxicities [33]*

Toxicities	Patients, n (%)	Median Onset (HSCT day)	Median Duration, d
Asthenia			
Grade I-II	22 (44.2)	-	-
Grade III-IV	-	-	-
Diarrhea			
Grade I-II	9 (17.3)	Day +14	-
Grade III-IV	4 (7.7)		-
Enterocolitis			
Grade I-II	2 (3.8)	-	5
Grade III-IV	4 (7.7)	-	
Erythematous Rashes			
Grade I-II	5 (9.6)	-	-
Grade III-IV	2 (3.9)	-	-
Mucositis			
Grade I-II	24 (46.2)	Day +2	4
Grade III-IV	14 (26.9)		
Myoarthralgia			
Grade I-II	29 (55.8)	Day −3	2
Grade III-IV	-	-	-
Onycholysis			
Grade I-II	12 (23.1)	-	-
Grade III-IV	-	-	-
Peripheral neuropathy			
Grade I-II	13 (25)	-	-
Grade III-IV	-	-	-
Gemcitabine MTD	9 (17.3)	-	-
Transaminitis			
Grade I-II	16 (30.8)	Day −3	7
Grade III-IV	9 (17.3)		

* As reported in ≥ 5% of the study population.

(57% versus 35%, $P = .01$). The median EFS times were not reached for Gem/Bu/Mel and were 13 months and 12 months for Bu/Mel and BEAM, respectively. As for OS, the rates for the Gem/Bu/Mel, Bu/Mel, and BEAM groups were 82%, 52%, and 59%, respectively, with median OS times not reached for Gem/Bu/Mel, and 63 months and 53 months for Bu/Mel and BEAM, respectively (Table 5). Gem/Bu/Mel resulted in improved OS compared with the combined Bu/Mel and BEAM groups (82% versus 54%, $P = .04$) [36].

Furthermore, in the specific analyses of the Gem/Bu/Mel subgroup, negative PET status at the time of HDC, history of only receiving 1 prior salvage therapy, gemcitabine naïve patients, and disease status all significantly correlated with better EFS. Specifically, the difference in EFS between the CR2 and CR3 patients approached significance ($P = .06$), whereas the difference between PR2 and CR3 patients did not ($P = .90$). This cohort study suggests improved clinical outcomes with Gem/Bu/Mel in primary refractory or poor-prognosis relapsed HL patients as compared with the 2 other treatment cohorts [36].

Table 5
Gemcitabine Response Rates [10,33,36,37,40-42]

Regimen	Cohort	Patients, n	RR (%)	ORR (%)	CR (%)	EFS (%)	OS (%)
Gem-DMC [33]	All	52	-	-	-	54	79
Gem/Bu/Mel [10]	B-DLCL	17	88	-	60	-	-
	HL	41	88	-	62	54	72
Gem/Bu/Mel [36]	HL	84	91	-	74	57	82
Bu/Mel [36]	HL	39	67	-	58	33	52
BEAM [36]	HL	57	88	-	56	39	59
Gem/Bu/Mel [37]	B-DLCL	30	-	100	69	80	83
	Burkitt's	3	-	100	100	-*	-*
	FL	2	-	100	100	-†	-†
	T-NHL	11	-	66	66	73	73
GN-CyBV [40]	HL	92	-	-	-	67	83
Gem-BM [41]	HL/T-NHL	22	-	-	-	70	85
	NHL	33	-	-	-	30	35
G-FM [42]	HL	15	-	-	-	49	87

RR indicates response rate; ORR, overall response rate; CR, complete response; EFS, event free survival; OS, overall survival; A, cytarabine; B = BCNU, carmustine; BU, busulfan; C, carboplatin; Cy, cyclophosphamide; D, docetaxel; E = V, etoposide; F, fludarabine; G or Gem, gemcitabine; M or Mel, melphalan; N, vinorelbine.

* All 3 patients relapsed at 3 months and passed.

† One patient in CR and 1 patient alive with relapsed disease.

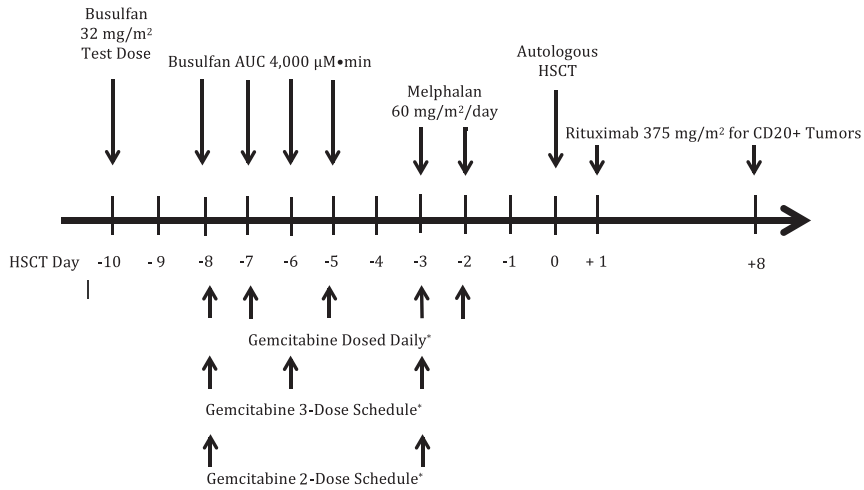
The NHL subgroup included 46 patients, of which 22 patients and 24 patients had primary refractory and poor-risk relapsed disease, respectively. Thirty patients had diffuse large-cell lymphoma (DLCL), 3 patients had Burkitt's lymphoma, 2 patients had follicular lymphoma, and 11 patients had T cell NHL (T-NHL) [37]. They had experienced multiple prior relapses (≤ 1 in 17 patients, > 1 in 29 patients), had a secondary International Prognostic Index score at relapse/progressive disease of 0 to 1 in 7 patients and > 1 in 33 patients, high lactate dehydrogenase at relapse/progressive disease (13 patients), and 50% had a positive PET at the time of HDC. At a median follow-up time of 16 months (range, 2 to 57 months), the EFS rates were 80% and 73% for B cell DLCL (B-DLCL) and T-NHL, respectively (Table 5). The OS rates were 83% and 73% for B-DLCL and T-NHL, respectively. This subgroup analysis suggests that Gem/Bu/Mel has high clinical activity in refractory or poor-risk relapsed NHL [37].

The above study demonstrated that high doses of FDR gemcitabine can be safely combined with Bu/Mel for autologous HSCT with encouraging signs of high activity in HL and NHL. A phase 2 trial is currently underway at MD Anderson to confirm these results with Gem/Bu/Mel in refractory HL patients [38]. A separate phase 2 trial in refractory myeloma patients has recently completed accrual [39].

Gemcitabine in Other Autologous HSCT Regimens

Arai et al. from Stanford University reported on the combination of gemcitabine and vinorelbine to the cyclophosphamide, BCNU, and etoposide regimen in 92 relapsed/recurrent HL patients. Their goal was to allow for a reduction in BCNU dose and, therefore, reduce BCNU-associated toxicities [40]. A gemcitabine dose of 1250 mg/m^2 was defined as the MTD. Of note, 85 patients (92%) underwent transplantation with chemosensitive disease and 58% of patients (53 patients) were in CR at the time of HDC. The authors observed a 15% incidence rate (14 patients) of pneumonitis, seemingly lower than the historical control rate of 35% using higher doses of BCNU. At a median follow-up time of 29 months (range, 8 to 86 months), the 2-year EFS was 67% (95% confidence interval [CI] 57% to 77%) and 2-year OS was 83% (95% CI, 75% to 91%) (Table 5) [40].

Furthermore, Rapoport et al. from the University of Maryland also reported on a modified BEAM regimen, by substituting cytarabine and etoposide with gemcitabine in 55 patients with either relapsed/refractory or high-risk NHL or relapsed/refractory HL. Of the 33 patients in the NHL subgroup, at the time of transplantation, 12 patients, 14 patients, and 7 patients had relapsed, primary refractory, and high-risk remission status, respectively. Of the 22 HL and T cell lymphoma patients at the time of transplantation, 15 patients, 6 patients, and 1 patient had relapsed, primary refractory, and high-risk remission status, respectively, at the time of transplantation. The 2-year EFS rates were 70% (95% CI, 53% to 94%) and 30% (95% CI, 18% to 53%) for the HL/T cell lymphoma and NHL subgroups, respectively. The 2-year OS rates were 85% (95% CI, 71% to 100%) and 35% (95% CI, 21% to 58%) for the HL/T cell lymphoma and NHL subgroups, respectively (Table 5) [41].



*Each gemcitabine dose: 75 mg/m² bolus, targeting a C_{ss} of 15 μmol/L, followed by FDR and corresponding doses of busulfan and/or melphalan

Figure 4. Treatment Regimen [10].

GEMCITABINE USE IN ALLOGENEIC HSCT

Gemcitabine/Fludarabine/Melphalan

Gemcitabine has also been studied for inclusion in allogeneic HSCT conditioning regimens. Anderlini et al. combined gemcitabine with a standard regimen of fludarabine and melphalan in 15 patients with relapsed and refractory HL [42]. They had received a median of 4 previous therapies (range, 2 to 9 therapies), including a prior autologous HSCT in 7 patients, with a median time to progressive disease of 10 months (range, 3 to 19 months) after autologous HSCT. At a median follow-up time of 18 months (range, 3 to 33 months), EFS and OS were 49% (95% CI, 18% to 74%) and 87% (95% CI, 56% to 96%), respectively (Table 5) [42].

RRT such as pulmonary toxicity occurred in 4 patients (26%), cutaneous toxicities in 5 patients (33%), mucositis in 9 patients (60%), and grades II to IV cardiac toxicity in 5 patients (33%). Of note, 1 of 2 patients who received higher-dosed gemcitabine (1000 mg/m^2 for 2 doses) experienced severe multiorgan toxicities and graft rejection, and thus, the dosing strategy was no longer pursued [42].

Table 6
Gem/Bu/Mel Toxicities [10]

Toxicities	Patients, n (%)	Median Onset (HSCT day)	Median Duration, d
Diarrhea			
Grade I-II	8 (6.2)	-	-
Grade III-IV	-	-	-
Erythematous Rashes			
Grade I-II	23 (17.8)	-	-
Grade III-IV	3 (2.3)	-	-
Mucositis			
Grade I-II	75 (58.1)	Day +4	2
Grade III-IV	29 (22.5)		
Pneumonitis			
Grade I-II	2 (1.6)	-	-
Grade III-IV	-	-	-
Renal toxicity			
Grade I-II	1 (0.8)	-	-
Grade III-IV	-	-	-
Transaminitis			
Grade I-II	97 (75)	Day +1	7
Grade III-IV			

Preclinical experiments by Valdez et al. have additionally shown marked synergy of double nucleoside analog combinations, using gemcitabine and clofarabine or fludarabine, with busulfan [31,32]. These observations have led to ongoing phase 2 studies of gemcitabine, clofarabine, and busulfan in allogeneic HSCT for refractory aggressive lymphomas [43] and chronic lymphocytic leukemia [44], seeking to develop an active, high-dose, reduced-toxicity conditioning regimen for this difficult to treat population.

FUTURE DIRECTIONS

In an attempt to further develop Gem/Bu/Mel in the autologous HSCT setting, the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) has been added to this regimen. The benefit of SAHA is its ability to induce chromatin structural alterations to further facilitate DNA access for alkylating agents, such as busulfan and melphalan. Valdez et al. have tested various combinations of these drugs in T and B lymphoma cell lines that were previously exposed to the individual agents of gemcitabine, busulfan, and melphalan, without significant effects on cell proliferation or proportion of apoptotic cells. Confirming their prior experiments, exposure to triple therapy resulted in 52% inhibition of proliferation rate and 39% of cells in apoptosis. Furthermore, SAHA increased the cytotoxicity of Gem/Bu/Mel, increasing inhibition of cell proliferation by 65% [30]. A study testing the concurrent addition of escalating doses of SAHA to Gem/Bu/Mel (Clinical Trial: NCT01421173) is approaching completion [45]. Preliminary analysis conducted on 66 patients who were enrolled between October 2011 and June 2013 suggest a SAHA dose of 1000 mg with Gem/Bu/Mel without encountering additional dose-limiting toxicities, as previously reported with Gem/Bu/Mel alone. Preliminary efficacy results also demonstrated 1-year EFS of 72%, 71%, and 100% and 1-year OS of 94%, 91%, and 100% in patients with HL, B-DLCL, and T-NHL, respectively [46].

QUESTIONS STILL UNANSWERED

Gemcitabine is a unique nucleoside analog that demonstrates an additional cytotoxic mechanism of inhibiting ribonucleotide reductase, which results in a masking phenomenon

upon dFdCTP DNA incorporation. The addition of this agent to standard conditioning regimens such as Bu/Mel has demonstrated high clinical activity by eliciting higher CR rates in refractory or poor-risk relapsed NHL patients in the autologous HSCT setting [10,37].

Taken together, the studies above have not only established the efficacy of gemcitabine-containing conditioning regimens, but also their favorable toxicity profile of self-limiting mucositis, rash, and transient elevations in transaminases [10,33]. Identifying predictive markers for severe toxicity to conditioning regimens and/or outcomes in HSCT candidates would be beneficial. Analyses of pretransplantation serum ferritin, C-reactive protein, brain natriuretic peptide, and haptoglobin have, thus far, failed to show a correlation with the incidence of severe extramedullary side effects. Ongoing research is analyzing the correlation of common single nucleotide polymorphisms of the most important enzymes involved in the metabolism of gemcitabine, detoxification of busulfan, and DNA damage repair with toxicity and outcome endpoints.

In summary, gemcitabine is rapidly emerging as a major agent in HSCT. Phase 2 and, ultimately, phase 3 trial data will be necessary to further define the role of gemcitabine combinations, particularly, Gem/Bu/Mel, in the management of these malignancies.

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