Accepted Manuscript

Title: A Comparison of the Conditioning Regimens BEAM and FEAM for Autologous Hematopoietic Stem Cell Transplantation in Lymphoma: an Observational Study on Patients From Fondazione Italiana Linfomi (Fil).

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PII: S1083-8791(18)30270-2
DOI: https://doi.org/10.1016/j.bbmt.2018.05.018
Reference: YBBMT 55134

To appear in: *Biology of Blood and Marrow Transplantation*

Received date: 27-3-2018
Accepted date: 14-5-2018

Please cite this article as: Jacopo Olivieri, Federico Mosna, Matteo Pelosini, Angelo Fama, Sara Rattotti, Margherita Giannoccaro, Giuseppe Carli, Maria Chiara Tisi, Simone Ferrero, Nicola Sgherza, Anna Maria Mazzone, Dario Marino, Teresa Calimeri, Giacomo Loseto, Francesco Saraceni, Gabriella Tomei, Simona Sica, Giulia Perali, Katia Codeluppi, Atto Billio, Attilio Olivieri, Enrico Orciuolo, Rossella Matera, Piero Maria Stefani, Carlo Borghero, Paola Ghione, Nicola Cascavilla, Francesco Lanza, Patrizia Chiusolo, Silvia Finotto, Irene Federici, Filippo Gherlinzoni, Riccardo Centurioni, Renato Fanin, Francesco Zaja, Fondazione Italiana Linfomi Postgraduate Master course, A Comparison of the Conditioning Regimens BEAM and FEAM for Autologous Hematopoietic Stem Cell Transplantation in Lymphoma: an Observational Study on Patients From Fondazione Italiana Linfomi (Fil)., *Biology of Blood and Marrow Transplantation* (2018), https://doi.org/10.1016/j.bbmt.2018.05.018.

TITLE: A COMPARISON OF THE CONDITIONING REGIMENS BEAM AND FEAM FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN LYMPHOMA: AN OBSERVATIONAL STUDY ON PATIENTS FROM FONDAZIONE ITALIANA LINFOMI (FIL).

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RUNNING HEAD: **BEAM vs FEAM**

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MANUSCRIPT METRICS:

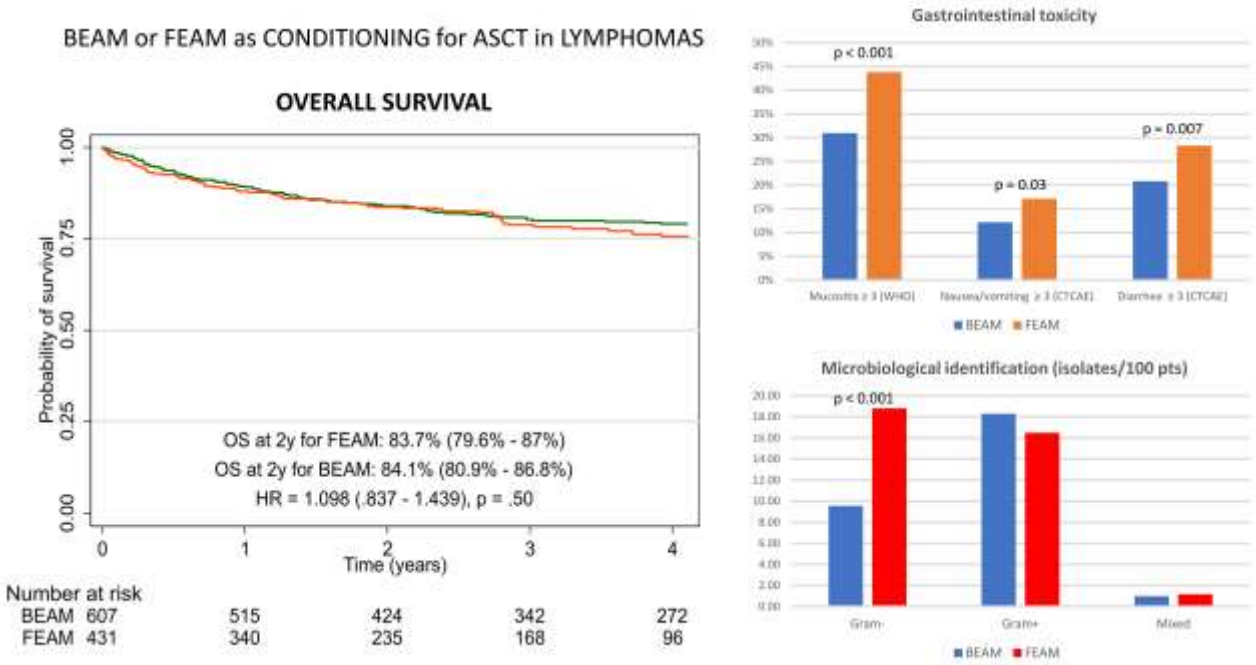
ABSTRACT WORDS: 248
ARTICLE WORDS: 3991
REFERENCES: 37
TABLES AND FIGURES: 5 (4 tables and 1 figure)
SUPPLEMENTARY FILE: 1

ACKNOWLEDGMENTS

This work has been conducted in the framework of the Postgraduate Master Course on Lymphoproliferative disorders promoted by the University of Udine, the University of Piemonte Orientale and the FIL (Fondazione Italiana Linfomi).

Keywords: Transplantation Conditioning; LYMPHOMA; TRANSPLANTATION, AUTOLOGOUS; BEAM regimen; Fotemustine

Graphical Abstract



HIGHLIGHTS

- We studied the effects of Fotemustine substitution in BEAM, prompted by BCNU shortage
- BEAM and FEAM did not appear different in terms of survival and disease control
- FEAM resulted in higher rates of gastrointestinal and infectious toxicities
- Mortality from infection was higher with FEAM, due to more sepsis from Gram- bacteria
- FEAM is an acceptable alternative to BEAM but is not justified when BCNU is available

ABSTRACT

BACKGROUND: Carmustine (BCNU)-Etoposide-Citarabine-Melphalan (BEAM) chemotherapy is the standard conditioning regimen for autologous stem cell transplantation (ASCT) in lymphomas. Owing to BCNU shortages, many centers switched to Fotemustine-substituted BEAM (FEAM), lacking proof of equivalence.

METHODS: We conducted a retrospective cohort study in 18 Italian centers to compare safety and efficacy of BEAM and FEAM regimens for ASCT in lymphomas performed from 2008 to 2015.

RESULTS: We enrolled 1038 patients (BEAM n=607, FEAM n=431), of which 27% had Hodgkin's lymphoma (HL), 14% indolent Non-Hodgkin's lymphoma (iNHL) and 59% aggressive NHL (aNHL). Baseline characteristics including age, sex, stage, B-symptoms, extranodal involvement, previous treatments, response before ASCT, overall conditioning intensity, were well balanced between BEAM and FEAM; notable exceptions were: ASCT year (median: BEAM=2011 vs FEAM=2013, p<0.001), Sorror score (≥3: BEAM=15% vs FEAM=10%, p=0.017), radiotherapy use (BEAM=18% vs FEAM=10%, p<0.001). FEAM conditioning resulted in higher rates of gastrointestinal and infectious toxicities, including severe oral mucositis (grade ≥3: BEAM=31% vs FEAM=44%, p<0.001), and sepsis from Gram-negative bacteria (mean isolates/patient: BEAM=0.1 vs FEAM=0.19, p<0.001). Response status at day 100 post-ASCT (overall response: BEAM=91% vs FEAM=88%, p=0.42), 2-years Overall Survival (83.9%, 95%CI:81.5%-86.1%) and Progression-free Survival (70.3%, 95%CI:67.4%–73.1%) were not different in the two groups. Mortality from infection was higher in the FEAM group (SHR 1.99; 95%CI:1.02–3.88, p=0.04).

CONCLUSIONS: BEAM and FEAM do not appear different in terms of survival and disease control. However, due to concerns of higher toxicity, Fotemustine substitution in BEAM does not seem justified, if not for easier supply.

INTRODUCTION

The first series of patients transplanted with autologous marrow for lymphomas was reported in 1978, using the BACT (Carmustine [BCNU]-Citarabine-Cyclophosphamide-Thioguanine) regimen.[1] Many variants were derived from the same chemotherapy backbone, among which the BCNU-Etoposide-Citarabine-Melphalan (BEAM) regimen, first reported in 1986.[2]

The BEAM regimen had strong conceptual points favouring its widespread application: it used readily available well-known drugs; it appeared highly effective in relapsed and refractory Hodgkin's (HL)[3] and non-Hodgkin's lymphomas (NHL)[4], while also having acceptable extra-hematologic toxicities. These consisted mostly in severe mucositis, chemotherapy-induced nausea and vomiting, diarrhea, hepatotoxicity and nephrotoxicity.4'[5,6,7]. Moreover, non-infective toxic pulmonary complications were reported in BCNU-containing regimens, involving 16-64% of patients among different studies.[8] Despite these limitations, in the last 40 years there have been few, if any, real alternatives to challenge BEAM as the standard conditioning regimen for lymphomas undergoing autologous stem cell transplantation (ASCT).[9]

Unexpectedly, though, after 2010 the oncological and hematological community faced the novel and unpredicted issue of shortage of some essential chemotherapy drugs, among which BCNU. Physician were thus forced to change their standards for those regimens in which a component was no longer available: a common solution was to replace the missing drug with a similar substitute molecule, trusting that the modified regimen would lead to similar results in terms of efficacy and toxicity.[10]

Although reasonable, such an approach was prone to dangerous risks: a national US survey in 2013 showed that use of surrogate drugs could have induced medication errors and increased unexpected toxicity;[11] reduced efficacy has also been reported when substituting one component of a consolidated regimen with a "similar" agent.[12]

BCNU shortage was reported in Italy in the same years:[13] Fotemustine, a third generation nitrosourea with thrombocytopenia as main dose-limiting toxicity, was chosen as a potential substitute. Since it was developed for treatment of brain tumours, Fotemustine had been engineered as a molecule with enhanced lipophilia, in order to ensure high cellular and central nervous system (CNS) penetration.[14] The first retrospective study to test the Fotemustine-substituted BEAM (FEAM) reported promising results in 84 patients with HL and NHL: in this series, the Overall Survival (OS), Progression-Free survival (PFS), and non-relapse mortality (NRM) at 2 years was 85%, 73% and 2.4%, respectively.[15] A prospective study focusing on HL had been recently reported by the same authors, with similar results[16]: in 122 patients, FEAM-conditioned ASCT yielded a 2-year PFS of 73.8%; the 100-day Treatment-related Morality (TRM) was 2.5%, in all cases attributable to multi-organ failure secondary to sepsis from Gram-negative bacteria.

These encouraging data, together with the persistent difficulties of supply for BCNU, contributed to ever increasing fortune in Italy of FEAM conditioning, even if comparative studies between Fotemustine and BCNU, especially in the context of ASCT, were missing.

The present study was therefore designed to fill this gap and to compare the efficacy, safety and toxicity of the BEAM and FEAM regimens. We chose to consider data retrospectively from the experience already available to 18 Italian ASCT Units up to now out of several issues. Although, theoretically, a randomized comparison would have been preferable, the main reason for BEAM to FEAM switching (i.e. BCNU shortage), would have threatened the feasibility of such a study. Moreover, we reasoned that the purely logistic and non-clinical choice of the treatment allocation between BEAM and FEAM, would reduce the selection bias attributable to a non-randomized comparison. Finally, we needed to achieve a prompt answer to concerns of toxicity regarding a widely used treatment, and that appeared more easily met by using retrospective data.

METHODS

An extended Methods section is reported in Supplementary file.

This is a cohort multicenter retrospective study enrolling all consecutive patients undergoing ASCT for lymphomas from January 1st, 2008 to December 31st, 2015, conditioned with BEAM or FEAM regimen4.

The study was approved by the institutional review board of the coordinating center and of all participating centers. The primary study endpoint was the frequency (intended as proportion of patients) of severe infectious events (grade 3 or 4 according to CTCAE 4.0) occurring in the first 100 days after transplantation. The secondary endpoints were: the overall response rate (ORR) evaluated 100 days after ASCT (defined according to standard lymphoma response criteria[17]); OS, PFS, cumulative incidence of Relapse (RI) and Non-relapse Mortality (NRM); engraftment of neutrophils (defined as the first of three consecutive days with a neutrophil count > 500 cell/μL) and platelets (defined as the first of three consecutive days with unsupported platelets count ≥ 20 000 cells/ μL); the frequency of severe adverse events of any type (grades 3 and 4 according to CTCAE 4.0); the frequency of mucositis according to the World Health Organization (WHO) criteria.[18]

Severe infectious events (SIE) were categorized as: SIE with microbiological identification (SIEM+); severe events of presumed infectious origin but without microbiological identification (SIEM-, e.g. pneumonia or neutropenic enterocolitis); febrile neutropenias (FN).[19]

TREATMENT PROTOCOLS

Patients were treated with either the BEAM regimen4, consisting of BCNU (300 mg/m^2, IV day − 7), etoposide (200 mg/m^2 days − 6 to − 3), cytarabine (400 mg/m^2 days − 6 to − 3), and melphalan (140 mg/m^2 day − 2) or the FEAM regimen, with substitution of BCNU with fotemustine 150 mg/m^2, IV days -7 and -6); variations in timing and fractionation of the drug doses were allowed, provided that the cumulative dose was maintained.

SUPPORTIVE MEASURES

Supportive measures were given per local policy and declared in a survey among participating centers. In general, post-transplant G-CSF (Filgrastim in most cases) was started shortly after reinfusion (day 3) and continued until neutrophil recovery; antimicrobial prophylaxis consisted of oral fluconazole, ciprofloxacin or levofloxacin and acyclovir, started on day 0; fluconazole and fluoroquinolones were generally stopped at hematologic recovery or 1 month after reinfusion; acyclovir was continued for 3 months after transplant. Cotrimoxazole was administered for Pneumocystis Jiroveci Pneumonia prophylaxis from hematologic recovery until 3 months after reinfusion (or when CD4 were ≥ 200/mmc). In case of fever and ANC < 0.5 x 10^9/l, empiric broad-spectrum intravenous antibiotics were administered (piperacillin/tazobactam in most cases). Packed red blood cells and platelet transfusions were administered in case of a hemoglobin level < 80 g/l and platelet count 15000 < 10^9/l.

STATISTICAL CONSIDERATIONS

We performed a power analysis to measure the minimum effect size of the primary endpoint likely to be detected with our planned sample: basing on an expected enrollment of 900 patients (BEAM, n=600; FEAM, n=300) and considering an expected frequency of SIE equal to 50%, and a Type I error set at 0.05, we estimated that such sample would allow to identify a 1.5 fold increased odd of SIE in FEAM with a power of 0.805[20].

Statistical tests were used to compare baseline characteristics or outcome measures between the BEAM and FEAM groups. OS and PFS were computed using the Kaplan-Meier method. Univariate and multivariate analyses were performed using the Cox proportional hazard method. The cumulative incidence method was applied to compute the incidence of relapse, NRM, cause-specific mortality in a competing risks setting. Predictive analyses for relapse and NRM were based on the proportional hazard model for subdistribution of competing risk. Univariate and multivariate analyses were performed using Gray's test and the proportional subdistribution hazard regression model developed by Fine and Gray.[21]

All tests were 2-sided. The type I error rate was fixed at .05. Analyses were performed using Stata 12.0 (Statacorp, College Station, Texas).

RESULTS

BASELINE CHARACTERISTICS

A total of 1038 patients (607 treated with BEAM and 431 treated with FEAM) were included in the study, enrolled from 18 Italian centers (Table 1). There were no differences in the baseline characteristics of the patients with respect to age (53.1 and 52.7 years in the BEAM and FEAM groups, respectively; p = 0.51), sex or disease distribution: the main indication for ASCT in both groups was aggressive NHL (BEAM 57% vs FEAM 61.7%), with DLBCL representing the largest disease category (BEAM 30.1% vs FEAM 30.4%) followed by MCL (BEAM 12.5% vs FEAM 16%); the remainder were HL (BEAM 27% vs FEAM 26.5%) and indolent NHL (BEAM 15.5% vs FEAM 11.6%). Also, disease characteristics at diagnosis were similar between the two groups: most patients were in advanced stage (BEAM 80.7% vs FEAM 77.7%) and about one third had BM involvement (BEAM 33.6% vs FEAM 34.6%); CNS involvement at diagnosis was rare (BEAM 1.7% vs FEAM 3.3%).

The therapeutic history of the patients did not differ in the two groups with respect to the number of previous chemotherapy courses (2 median lines of therapy for both groups); however, radiotherapy use was more frequent in the BEAM group, both at any site (BEAM 18.1% vs FEAM 9.7%, p < 0.001) and to the mediastinal region (BEAM 8.4% vs FEAM 2.1%, p < 0.001). The rate of refractoriness to 1st line treatment was not different in the two groups (BEAM 14.8% vs FEAM 13.9%), but there were more CR recorded in the BEAM group (BEAM 60.8% vs FEAM 50.3%, p = 0.001); likewise, overall response rate (ORR) before transplantation was similar in both groups (BEAM 92.7% vs FEAM 91%), but marginally more patients of the BEAM group accessed to transplant with a CR (BEAM 65.2% vs FEAM 59.2%, p = 0.05). However, in the patients evaluable for metabolic response before ASCT (BEAM: n = 471; FEAM: n = 288), the rate of PET positivity was not different in the two groups (BEAM 29.3% vs FEAM 33%).

The comorbidity burden measured by HCT-CI was significantly higher in BEAM-conditioned patients (HCT-CI ≥ 3: BEAM 15.3% vs FEAM 10.1%, p = 0.02), as it was the rate of pulmonary comorbidity (BEAM 19.5% vs FEAM 7.8%, p < 0.001). The timeframe of ASCT was not the same in the two groups, being 2011 the median year of transplant for BEAM patients and 2013 for FEAM. Overall dose intensity was similar for BEAM and FEAM conditioning (ratio ≥ 90% between delivered and standard dose: BEAM 80.7% vs FEAM 80.9%), as it was the addition of the anti-CD20 antibody Rituximab (BEAM 5.3% vs FEAM 7.4%). The number of reinfused CD34+ cells x 10^6/kg was slightly higher in the FEAM group (median 5.5 vs 5.3 in the BEAM group, p = 0.045).

TOXICITIES

Toxicities between two groups are summarized in table 2. FEAM patients had a higher gastrointestinal toxicity as shown by a higher rate of grade ≥3 mucositis (BEAM 31% vs FEAM 44%, p<0.001), grade ≥3

nausea and vomiting (BEAM 12% vs FEAM 17%, p=0.03) and grade ≥3 (BEAM 21% vs FEAM 28%, p=0.007) and ≥4 diarrhea (BEAM 2.4% vs FEAM 5%, p=0.03). No other statistically significant extra-hematological toxicities emerged.

Overall SIE (by definition of grade ≥3) did not differ between BEAM and FEAM patients (BEAM 71% vs FEAM 71%, p=0.94), but grade ≥4 SIE (BEAM 5% vs FEAM 11%, p<0.001) were higher in the FEAM group. In detail, in the FEAM group there were more grade ≥4 FN events (BEAM 1.5 % vs FEAM 6.3 %, p<0.001) and a higher rate of grade ≥3 and ≥4 SIEM+ (BEAM 30% vs FEAM 36%, p = 0.05; BEAM 2.6 % vs FEAM 5.6 %, p = 0.006). Among SIEM+, the FEAM group had higher incidence of infections with Gram-negative bacteria (mean isolates/patient: BEAM 0.10 vs FEAM 0.19, p < 0.001) or fungi (BEAM 0.015 vs FEAM 0.039, p=0.01).

Neutrophil engrafment was similar between the two groups, but there was a delayed platelet engrafment in FEAM patients (median: BEAM 12 day vs FEAM 13 days, p<0.001) with higher need of platelet trasfusions. Furthermore, also hospital stay (BEAM 21 days vs FEAM 23 days , p < 0,001) and need of total parenteral nutrition were higher in the FEAM group (BEAM 52% vs FEAM 64%, p = 0,001).

OUTCOME

Disease assessment at day 100 did not show any difference between FEAM and BEAM groups (CR + PR: BEAM 91% vs FEAM 88%, p=0.42). Furthermore, among CR patients, the rate of acquired CR (i.e. patients achieving post-transplant CR from pre-transplant PR or less) was similar (BEAM 22.6% vs FEAM 23.7%). Early death rate (for any cause, at day 100) was slightly higher in the FEAM group (BEAM 3.5 % vs FEAM 5.3 %, p = 0.14) without reaching statistical significance.

OS and PFS at 2 years in the whole cohort were 83.9% (95%CI: 81.5% - 86.1%) and 70.3% (95%CI: 67.4% – 73.1%), respectively, without significant differences between the BEAM and the FEAM group (Fig. 1A and 1B). Median follow-up was 42 months for both groups, and it was longer for BEAM-treated patients (BEAM 50 months vs FEAM 34 months, p<0.001).

The cumulative incidence of relapse (RI: BEAM 18.4% vs FEAM 20.7%, p=0.49) and non-relapse mortality (NRM: BEAM 2.6% vs FEAM 3.8%, p = 0.27) at 1 year did not differ between the two groups. Main death causes in the whole cohort were lymphoma relapse or progression in 138 patients, infection in 34, other treatment-related causes in 35, secondary malignancy in 8, other or unknown cause in 11. There were no differences in all death causes between the two groups, but mortality from infection was significantly higher in the FEAM group (SHR 1.99; 95%CI: 1.02 – 3.88, p=0.04).

Time-dependent outcomes were also evaluated according to major diagnostic categories (Supplementary file, Fig. S1-S3): when the two conditioning regimens were compared within aNHL, iNHL, and HL, there was no significant difference for OS, PFS, RI and NRM (Fig. S2). However, there was a trend for a worse outcome

of the FEAM group in PFS (HR 1.44, 95%CI 0.96 – 2.16, p=0.08) and RI (HR 1.50, 95%CI 1.00 – 2.27, p=0.051; Fig S2, panels B, C) in HL patients.

Multivariate analyses (MVA) for OS and PFS confirmed the negative roles of already known poor prognostic factors, such as older age, an increasing treatment burden, suboptimal quality of response before transplant (see Table 5). Interestingly, the category of aNHL was a poor independent predictor for OS but not for PFS; conversely, bone marrow involvement at diagnosis, primary refractory patients, a reduced BCNU/Fotemustine dose and transplantation in a FEAM-oriented center (BEAM/FEAM ratio < 25%) emerged as independent factors for PFS but not for OS.

The factors independently associated with a higher relapse occurrence faithfully reproduced those seen for PFS, with the exception of age. Finally, for NRM, the category of HL emerged as a strong protective factor, together with CR before ASCT and more recent time of transplantation; conversely, ASCT after 2 lines of treatment and use of FEAM conditioning independently associated with worse NRM.

DISCUSSION

Our results suggest a comparable efficacy of FEAM and BEAM conditioning in terms of survival and disease control for lymphoma patients treated with high-dose chemotherapy and ASCT. However, we also observed higher rates of severe gastrointestinal toxicities and of infectious events (mainly from Gram-negative bacteria) in patients transplanted after FEAM.

The BEAM chemotherapy has become the standard conditioning regimen for lymphoproliferative diseases in the last decades. In fact, a large retrospective study from the CIBMTR reports BEAM as increasingly used in the last 20 years, from 13.4% of all conditionings regimens in 1995-1999 to 64.1% in 2005-2008, with CBV, BuCy and TBI-based regimens as main alternatives.[22]

A known concern related to BCNU is the development of pulmonary fibrosis, which has been reported especially in regimens with higher BCNU doses (i.e. CBV) than those scheduled in BEAM[22] or when BCNU was combined with cyclophosphamide. 8 Thus, substitution of this component with other drugs, namely Thiotepa (TEAM)[23], Lomustine (LEAM)[24] Fotemustine (FEAM)[15,16] and, most recently, Bendamustine (BeEAM)[25] has been proposed: all these alternatives to BEAM were reported to be apparently equally or more effective in controlling lymphoma and equally or less toxic than the original regimen. However, such claims were inferred from comparisons with historical cohorts or studies, done in different populations with different baseline risk factors, while no direct comparison in the same cohort has been conducted so far. A single exception, to our knowlegde, is represented by an ongoing prospective trial confronting BeEAM with BEAM regimens,[26] the results of which are still unavailable.

Despite belonging to the same drug class, one theoretical advantage of Fotemustine over BCNU is the apparent lack of pulmonary toxicity:[27] such a difference is explained by the reduced interference with the glutathione system, whose inhibition, driven by the carbamoylation activity of BCNU,[28] leads to unopposed production of reactive oxygen species and lung fibrosis. In the two studies testing FEAM,[15,16] no immediate or late pulmonary toxicity was reported. In our data, we observed a similarly low rate of pulmonary toxicity in both groups: considering that BEAM-conditioned patients had more lung comorbidities before ASCT and had a longer follow-up, it is unlikely that BCNU causes a significantly higher pulmonary toxicity.

Another compelling reason to search for alternatives to BEAM was the shortage of several chemotherapy drugs, a matter of the last decade.[10] As example, the shortage of Melphalan was routinarily managed by substitution with Cyclophosphamide (BEAC regimen); however, in 2016 four patients with lymphoma treated with BEAC faced severe complications in a single stem cell transplant center in France. This prompted a retrospective survey by EBMT on 383 patients treated with BEAC, which were matched to 766 BEAM-treated patients. Although the overall survival (OS) was similar (78% BEAC vs 77% BEAM), cardiac deaths were 32% in the BEAC group compared with 23% in BEAM;[29] however, this difference was not statistically significant and the authors concluded that BEAC was safe as a conditioning regimen.

For BCNU, the whole thing exploded from 2012 onwards,[13] when increasing difficulties to find BCNU determined a dramatic shift in the use of FEAM in Italy, forcing several Hematology Units to switch to the new regimen, even if evidence of equivalence was lacking. Moreover, BCNU shortage made it impossible to promote a prospective comparison with the new alternative; conversely, the growing experience with Fotemustine in Italy and the existence of good quality databases in many Italian transplant centers, suggested the feasibility and the opportunity of a retrospective comparative analysis of FEAM and BEAM. Finally, given the absence of direct comparisons, and nonetheless, the increasing use of Fotemustine in Italy, such a study was ethically due, aimed at least to exclude the possibility of a higher toxicity of the FEAM new regimen compared with the standard.

In our study, we recognized several signals of increased mucosal damage with FEAM: severe diarrhea resulting from intestinal mucositis (grade ≥3: BEAM 20.8% vs FEAM 28.3%, p=0.007) and oral mucositis (grade ≥3: BEAM 30.9% vs FEAM 43.8%, p<0.001) were in fact more frequent than in the BEAM group. Such a difference persists if we stratify our analysis by the attitude of centers (i.e. those using predominantly one of the two regimens and those switching intermittently between the two), making a measurement or performance bias (due to Fotemustine “novelty”) unlikely. The reason for an increased mucotoxicity of Fotemustine is not obvious: both nitrosoureas do not usually cause mucositis if used in monotherapy;[30,14] however, when used in combination, the occurrence of severe oral mucositis is relevant, with a reported occurrence of 42%[31] for BEAM and 15-30% for FEAM.[15,16] While the major determinant for mucositis severity is the type of chemotherapy regimen used,[32] yet there are no univocal pharmacological properties

predicting its mucotoxicity. Drug distribution in mucosal tissues has its role, given the established efficacy of cryotherapy in preventing oral mucositis,[33] by decreasing the exposure of mucosal tissue to cytotoxic agents through vasoconstriction. In this respect, the enhanced lipophilicity and tissue penetration of Fotemustine[14] may represent a drawback and contribute to the increased mucotoxicity observed with FEAM.

Mucositis is a complex phenomenon, originated by DNA damage induced by chemo- or radiotherapy, in which however proinflammatory cytokines play an important role in boosting local injury. In this respect, the new concept of "febrile mucositis" has emerged, highlighting the fact that chemotherapy-induced fever may also result from the inflammation arising in the context of mucositis, and not just from gut-derived bacteremia.[34] Thus, a significant proportion of prior labelled febrile neutropenias may represent epiphenomena of aseptic mucosal inflammation, carrying a different prognosis and requiring different treatments. In our study, we found a similar occurrence of SIE in patients treated with BEAM or FEAM. However, the rate of very severe FN (grade ≥4: BEAM 1.5% vs FEAM 6.3%) and SIEM+ (grade ≥4: BEAM 2.6% vs FEAM 6.0%) was higher in the FEAM group. This observation may be traced back to the higher mucotoxicity seen with the FEAM chemotherapy, and possibly related to a different damage determined by this regimen on the enteric mucosa, leading to enhanced disruption of the enteric/blood barrier and easier translocation to the bloodstream of Enterobacteriacae and resident anaerobes, resulting in bacteremia and sepsis. In fact, the increased occurrence of SIEM+ in the FEAM group is attributable to more frequent isolation of Gram- (mean isolates/patient: BEAM 0.1 vs FEAM 0.19, $p<0.001$), and in particular of Enterobacteriaceae (mean isolates/patient: BEAM 0.07 vs FEAM 0.13, $p=0.002$). This, in turn, may explain the higher mortality for infection found in the FEAM group.

In favour of this hypothesis, an association between transplant-related mortality and Gram-negative infections has also been described in a previous experience with FEAM.[16] Interestingly, an increased occurrence of bacteremias, but with similar rate of overall infectious events, has been reported for another more lipophilic substitute of BCNU, i.e. Thiotepa (BEAM n=75; TEAM n=47; rate of infectious complications: BEAM 47% vs TEAM 53%; rate of sepsis/bacteriemia: BEAM 13% vs TEAM 32%).[23]

An alternative explanation could be the spread of mutiresistant Gram-negative bacteria in Italian transplant centers in more recent years.[35] However, in our data, the excess isolates of Enterobacteriaceae observed with FEAM were confirmed restricting the analysis after 2011 (mean isolates/patient: BEAM 0.07 vs FEAM 0.13, $p=0.009$).

Our study has several limitations, the main one being related to its design: retrospective cohort studies are considered at the lowest level of evidence in the hierarchy of comparative research, with randomized controlled trials (RCT) being at the opposite end.[36] However, RCT may not be feasible in several situations

and owing to their interventional nature, they are often restricted to a subset of the population of interest, thereby affecting their external validity.[36]

On the contrary, the main threaten for evidence gathered from cohort studies is related to their internal validity, due to risk of selection bias.[37] Although statistical techniques have been developed to control for known imbalances between the groups that are compared, they cannot obviate for unknown factors which are neutralized by randomization. However, if treatment allocation results from factors independent of clinical decision, one might expect more easily two prognostically homogeneous groups, thereby allowing a more reliable comparison between them. In our study, the choice between BEAM and FEAM regimen resulted from random variability of BCNU supply, differing among centers and time periods. Such variability mirrored the logistic ability of the centers to procure themselves with BCNU, which on turn generally hindered on the pharmacy attitude to find alternative ways to get BCNU (i.e. foreign import). Indeed, in our study, centers' attitude was pretty evenly distributed between those who were able to get always BCNU without major interruptions using alternative channels (BEAM/FEAM ratio >75%, n=8), those who started to use steadily Fotemustine since they experienced the first difficulties to get BCNU (BEAM/FEAM ratio <25%, n=5) and those who switched between the two owing to intermittent BCNU shortage (BEAM/FEAM ratio 25-75%, n=5). Therefore, most of the basal characteristics were balanced between BEAM and FEAM, without use of matching or other statistical techniques. The only significant differences were year of transplant (later for FEAM), Sorror score (higher in BEAM), use of radiotherapy (more for BEAM), dose intensity of BCNU/Fotemustine and Etoposide (higher in FEAM) and number of reinfused CD34+ cells x 10^6/kg (more for FEAM). Although it is expected that some (5%) statistical tests will result significant owing to chance, the observed imbalances likely reflect different policies for transplantation in use in different centers, rather than preferential allocation to one of the two groups. Center disparities may confound results even in RCTs if the randomization procedure does not account for center stratification. In our analysis, we accounted for center effect by adding to the MVA a variable coding for center attitude toward the two conditioning regimens. Interestingly, in the MVA for PFS and RI, the variable coding for center attitude was more informative than type of conditioning regimen and thus, in the final model, the worse outcome related to FEAM conditioning appears to be limited to FEAM-oriented centers (BEAM/FEAM ratio <25%). Conversely, in the MVA for NRM, type of conditioning emerged as a significant independent predictor, while center attitude was not: thus it is likely that FEAM conditioning itself contributes to higher TRM. In our opinion, such interpretation appears credible and is consistent with the other data suggesting a higher toxicity induced by substitution of Fotemustine in the BEAM regimen. However, given the discussed caveats and the limited size effect observed, there is no absolute confidence about this finding.

In conclusion, we compared two groups belonging to the same cohort of patients and differing for one treatment variable, aiming to add evidence to the increasing trend of Fotemustine substitution in the BEAM conditioning regimens in lymphomas: our results exclude substantial differences between the two

treatments in terms of survival and disease control. However, considering that no advantages of FEAM over BEAM emerged but rather concerns of higher toxicity, Fotemustine substitution in BEAM may not be completely neutral and thus its use in conditioning does not appear justified when BCNU is available.

CONTRIBUTIONS: JO, FM, MP and FZ conceived the study and wrote the study protocol; JO performed the statistical analyses; JO, FM, MP wrote the final draft; JO and FZ reviewed and approved the final manuscript; JO, FM, MP, AF, SF, GC, MG, NS, SR, DM, GL, AMM, TC, MCT, GT, FS, SS, AB, EO, PMS, PG, FG, AO, IR, CB, RC, SF, FL, RM, NC, RF, FZ provided patients' data.

Conflict-of-interest disclosure: the authors declare no competing financial interests.

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Fig. 1: Overall Survival (OS, panel A), Progression-Free Survival (PFS, panel B), cumulative incidence of relapse (panel C) and non-relapse mortality (panel D), according to type of conditioning.

TABLES and FIGURES

Tab. 1: Basal characteristics of the enrolled patients

Basal characteristics	**BEAM, n (%)**	**FEAM, n (%)**	**P-value**
Evaluable patients	n = 607	n = 431	
Sex (Female)	244 (40.2%)	169 (39.2%)	0.75
Age (median, range)	53.1 (16.5-79.5)	52.7 (17.2-77.8)	0.51
Disease	n = 607	n = 431	0.16[1]
Hodgkin lymphoma	164 (27.0%)	114 (26.5%)	
Indolent NHL	94 (15.5%)	50 (11.6%)	
Aggressive NHL	346 (57.0%)	266 (61.7%)	
DLBCL	183 (30.1%)	131 (30.4%)	
MCL	76 (12.5%)	69 (16.0%)	
PTCL	52 (8.6%)	39 (9.0%)	
Other	35 (5.8%)	27 (6.3%)	
Disease characteristics at diagnosis	n = 607	n = 431	
Advanced stage (Ann Arbor III-IV)	490 (80.7%)	335 (77.7%)	0.24
B symptoms	235 (38.7%)	157 (36.4%)	0.45
BM involvement	204 (33.6%)	149 (34.6%)	0.75
CNS involvement	10 (1.7%)	14 (3.3%)	0.09
Pre-transplant evaluation			
Sorror score	n = 603	n = 424	
0	372 (61.7%)	290 (68.4%)	**0.017**2
1 - 2	139 (23.1%)	91 (21.5)%	
≥ 3	92 (15.3)%	43 (10.1)%	
Lung comorbidity	n = 603	n = 424	
Mild	66 (11.0%)	30 (7.1%)	**<0.001**3
Moderate-severe	51 (8.5%)	3 (0.7%)	
Therapeutic history	n = 607	n = 431	
Previous chemotherapy courses (median)	2	2	0.43
Line of treatment	n = 607	n = 431	
Upfront ASCT (1° line)	180 (29.7%)	121 (28.1%)	
After salvage (2° line)	339 (55.9%)	242 (56.2%)	0.584
After ≥3 lines of treatment	88 (14.5%)	68 (15.8%)	
Radiotherapy before transplant	n = 607	n = 431	
Yes, any site	110 (18.1%)	42 (9.7%)	**<0.001**
Mediastinal	51 (8.4%)	12 (2.8%)	**<0.001**
Response to 1° line:	n = 607	n = 431	
CR	369 (60.8%)	217 (50.3%)	0.705
PR	147 (24.2%)	152 (35.3%)	
RD	90 (14.8%)	60 (13.9%)	
Response before ASCT:	n = 607	n = 431	
CR	396 (65.2%)	255 (59.2%)	
PR	167 (27.5%)	137 (31.8%)	0.555
RD	41 (6.8%)	33 (7.7%)	
ND	3 (0.5%)	6 (1.4%)	
Metabolic response before ASCT	n = 471	n = 288	
PET positive	138 (29.3%)	95 (33.0%)	0.29
Interval diagnosis – transplant (months)	n = 607	n = 431	
Median (range)	13.0 (2 – 223)	13.5 (2.8 – 185)	0.80

Conditioning and transplant	n = 607	n = 431	
Year of transplant (median)	2011	2013	**<0.001**
Full dose (≥ 90% delivered/standard)	n = 471	n = 288	
BCNU / Fotemustine	476 (79.1%)	374 (87%)	**0.001**
Etoposide	490 (81.4%)	380 (88.6%)	**0.002**
Citarabine	498 (82.7%)	350 (81.6%)	0.64
Melphalan	497 (82.6%)	368 (85.6%)	0.17
Overall	486 (80.7%)	347 (80.9%)	0.95
Rituximab addition to conditioning regimen	n = 607	n = 431	
Yes	32 (5.3%)	32 (7.4%)	0.16
Stem cell source	n = 607	n = 431	
Peripheral blood	598 (98.5%)	430 (99.8%)	0.05
Reinfused CD34+ x 10^6/kg (median, range)	5.3 (1.1 – 22)	5.5 (2.0 – 27)	**0.045**

Legend: Non-Hodgkin Lymphoma (NHL); Hodgkin Lymphoma (HL); Diffuse Large B-Cell Lymphoma (DLBCL); Mantle Cell Lymphoma (MCL); Peripheral T-Cell Lymphoma (PTCL); Bone Marrow (BM); Central Nervous System (CNS); Autologous Stem Cell Transplant (ASCT); Complete Response (CR); Partial Response (PR); Resistant Disease (RD); Not Done (ND). Bold p-values denote statistical significance (< 0.05). P-values refer to comparisons:

1. Among major disease categories (Hodgkin's lymphoma, Indolent NHL, Aggressive NHL)
2. Sorror score < 3 vs ≥ 3
3. No lung comorbidity vs mild + moderate + severe
4. Upfront ASCT vs after ≥ 2 lines of treatment
5. CR + PR vs RD

Tab. 2: Main extra-hematological toxicities according to CTCAE v 4.0

TOXICITY	BEAM	FEAM	P-value
Mucositis (WHO scale)	n = 591	n = 388	
Grade 1 (% pts)	16.9%	9.3%	
Grade 2 (% pts)	34.5%	23.7%	
Grade 3 (% pts)	21.1%	34.8%	**<0.001**
Grade 4 (% pts)	9.8%	9.0%	0.68
Nausea and vomiting (CTCAE 4.0)	n = 591	n = 387	
Grade 3 (% pts)	10.7%	16.1%	**0.03**
Grade 4 (% pts)	1.5%	1.0%	0.58
Diarrhea (CTCAE 4.0)	n = 591	n = 403	
Grade 3 (% pts)	18.4%	23.3%	**0.007**
Grade 4 (% pts)	2.4%	5.0%	**0.03**
Other toxicities (CTCAE 4.0)	n = 607	n = 431	
Pulmonary (% pts with grade ≥ 3)	0.7%	0.7%	1
Renal (% pts with grade ≥ 3)	1.3%	0.7%	0.38
Hepatic (% pts with grade ≥ 3)	2.0%	3.0%	0.31
Cardiac (% pts with grade ≥ 3)	3.1%	1.6%	0.16
Cutaneous (% pts with grade ≥ 3)	0.7%	1.2%	0.5
Other GI (% pts with grade ≥ 3)	1.2%	1.2%	1
Neurological (% pts with grade ≥ 3)	0.5%	0.5%	1
Vascular (% pts with grade ≥ 3)	0.2%	0.7%	0.31
Other (% pts with grade ≥ 3)	0.8%	1.2%	0.75
All toxicities, excluding infectious and major GI (% pts with grade ≥ 3)	9.2%	9.5%	0.88

Tab. 3: Transplant outcomes in the first 100 days after reinfusion

	BEAM	FEAM	P-VALUE
INFECTIOUS EVENTS			
Febrile neutropenia	n = 607	n = 431	
Grade 3 (% pts)	45.1%	40.8%	0.861
Grade 4 (% pts)	1.5%	6.3%	**<0.001**2
Grade 5 (% pts)	0.16%	0.23%	
N. of episodes (mean)	0.57	0.54	0.86
WITHOUT microbiological identification	n = 607	n = 431	
Grade 3 (% pts)	8.2%	8.6%	0.971
Grade 4 (% pts)	1.7%	1.4%	0.812
Grade 5 (% pts)	0.16%	0%	
N. of episodes (mean)	0.10	0.11	0.99
WITH microbiological identification	n = 607	n = 431	
Grade 3 (% pts)	27.4%	29.7%	**0.05**1
Grade 4 (% pts)	2.6%	5.6%	**0.006**2
Grade 5 (% pts)	0%	0.46%	
N. of isolates (mean)	0.34	0.46	**0.02**
Gram-negative bacteria	0.097	0.190	**<0.001**
Gram-positive bacteria	0.183	0.165	0.48
Fungal	0.015	0.039	**0.01**
Viral	0.035	0.051	0.25
Other (intracellular, parasites, etc.)	0.015	0.016	0.86
Any infectious event	n = 607	n = 431	
Grade 3 (% pts)	65.7%	59.2%	0.941
Grade 4 (% pts)	4.9%	11.4%	**<0.001**2
Grade 5 (% pts)	0.33%	0.70%	
N. of episodes (mean)	1.02	1.10	0.15
ENGRAFTMENT			
Days to neutrophils > 0.5 x 10^3/L	n = 600	n = 429	
Median (range)	10 (5 – NR)	10 (6 – NR)	0.09
Days to platelets > 0.5 x 10^3/L	n = 553	n = 400	
Median (range)	12 (3 – NR)	13 (7 – NR)	**<0.001**
TRANSFUSIONAL SUPPORT	n = 595	n = 375	
Median RBC units (range)	2	2	0.74
Median Platelets units (range)	2	3	**0.018**
USE OF TOTAL PARENTERAL NUTRITION	n = 582	n = 365	
Yes	52.2%	63.6%	**0.001**
NEED FOR INTENSIVE CARE UNIT	n = 604	n = 430	
Yes	1.5%	2.3%	0.32
LENGTH OF STAY IN HOSPITAL	n = 602	n = 420	
Median days (range)	21 (1 – 82)	23 (1 – 71)	**<0.001**
Response at 100 days after ASCT:	n = 607	n = 431	0.423
CR	84.2%	79.8%	
PR	6.4%	7.7%	
RD	5.4%	5.8%	
ND	0.5%	1.2%	

LEGEND: P-values refer to comparisons:

1. Experiencing ≥ 1 event of grade ≥ 3 vs not
2. Experiencing ≥ 1 event of grade ≥ 4 vs not
3. CR + PR vs RD

Tab. 4: Multivariate analysis for Overall Survival, Progression-Free Survival, cumulative incidence of Relapse and Non-Relapse Mortality

OVERALL SURVIVAL		HR (95% CI)	P-VALUE
AGE AT TRANSPLANT	Each year more	1.02 (1.009 - 1.03)	<0.001
AGGRESSIVE NHL	vs iNHL and HL	1.85 (1.346 - 2.543)	<0.001
ASCT AFTER 1 SALVAGE	vs upfront ASCT	1.495 (1.035 - 2.158)	0.032
ASCT AFTER >1 SALVAGE	vs upfront ASCT	2.89 (1.835 - 4.553)	<0.001
PR PRE-ASCT	vs RD	0.374 (0.256 - 0.547)	<0.001
CR PRE-ASCT	vs RD	0.152 (0.102 - 0.225)	<0.001

PROGRESSION-FREE SURVIVAL		HR (95% CI)	P-VALUE
AGE AT TRANSPLANT	Each year more	1.012 (1.004 - 1.02)	0.003
BM INVOLVEMENT AT DIAGNOSIS	vs not	1.293 (1.039 - 1.61)	0.022
ASCT AFTER >1 SALVAGE	vs upfront/only 1 salvage	1.819 (1.401 - 2.362)	<0.001
PRIMARY REFRACTORY	vs response at 1st line	1.478 (1.115 - 1.959)	0.007
PR PRE-ASCT	vs RD	0.45 (0.32 - 0.631)	<0.001
CR PRE-ASCT	vs RD	0.225 (0.159 - 0.317)	<0.001
FULL DOSE BCNU/FOTEMUSTINE	vs reduced dose	0.757 (0.581 - 0.987)	0.04
FEAM-ORIENTED CENTER	vs BEAM-oriented or equally oriented	1.312 (1.039 - 1.656)	0.022

RELAPSE INCIDENCE		SHR (95% CI)	P-VALUE
BM INVOLVEMENT AT DIAGNOSIS	vs not	1.348 (1.071 - 1.696)	0.011
ASCT AFTER >1 SALVAGE	vs upfront/only 1 salvage	1.732 (1.296 - 2.315)	<0.001
PRIMARY REFRACTORY	vs response at 1st line	0.718 (0.523 - 0.987)	0.041
PR PRE-ASCT	vs RD	0.501 (0.328 - 0.767)	0.001
CR PRE-ASCT	vs RD	0.28 (0.185 - 0.423)	<0.001
REDUCED (<70%) BCNU/FOTEMUSTINE DOSE	vs dose >70%	2.125 (1.488 - 3.034)	<0.001
FEAM-ORIENTED CENTER	vs BEAM-oriented or equally oriented	1.308 (1.018 - 1.679)	0.035

NON-RELAPSE MORTALITY		SHR (95% CI)	P-VALUE
HODGKIN'S LYMPHOMA	vs others	0.266 (0.106 - 0.67)	0.005
ASCT AFTER >1 SALVAGE	vs upfront/only 1 salvage	2.293 (1.174 - 4.478)	0.015
YEAR OF TRANSPLANT	Each year later	0.805 (0.699 - 0.927)	0.003
CR PRE-ASCT	vs RD	0.313 (0.167 - 0.585)	<0.001
FEAM CONDITIONING	vs BEAM	1.861 (1.023 - 3.385)	0.042

Legend: Non-Hodgkin Lymphoma (NHL); Hodgkin Lymphoma (HL); aggressive NHL (aNHL); indolent NHL (iNHL); Autologous Stem Cell Transplant (ASCT); Complete Response (CR); Partial Response (PR); Resistant Disease (RD); Hazard Ratio (HR); Subhazard Ratio (SHR).
Center orientation: BEAM-oriented center (BEAM/FEAM ratio >75%); FEAM-oriented center (BEAM/FEAM ratio <25%); Equally oriented center (BEAM/FEAM ratio 25-75.

Fig. 1: