Received: 9 November 2016 | Revised: 21 December 2016 | Accepted: 22 December 2016

DOI: 10.1002/pbc.26457

RESEARCH ARTICLE

WILEY Pediatric Blood & Cancer

SIOP INTERNATIONAL SOCIETY OF PAEDIATRIC ONCOLOGY

aspho The American Society of Pediatric Hematology/Oncology

Investigating the heterogeneity of alkylating agents' efficacy and toxicity between sexes: A systematic review and meta-analysis of randomized trials comparing cyclophosphamide and ifosfamide (MAIAGE study)

Brice Fresneau[1] | A. Hackshaw[2] | D.S. Hawkins[3] | M. Paulussen[4] | J.R. Anderson[5] | I. Judson[6] | S. Litière[7] | U. Dirksen[8] | I. Lewis[9] | H. van den Berg[10] | N. Gaspar[1] | H. Gelderblom[11] | J. Whelan[12] | A.V. Boddy[13] | K. Wheatley[14] | J.P. Pignon[15,16,17] | F. De Vathaire[18] | M.C. Le Deley[15,16] | G. Le Teuff[15,16,17]

[1]Department of Pediatric oncology, Gustave Roussy, Université Paris-Saclay, Villejuif, France

[2]Cancer Research UK & UCL Cancer Trials Centre, University College London, London, United Kingdom

[3]Division of Hematology/Oncology, Department of Pediatrics, Seattle Children's Hospital, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington

[4]Vestische Kinder-und Jugendklinik Datteln, Witten/Herdecke University, Datteln, Germany

[5]Merck Research Laboratories—Oncology, North Wales, Pennsylvania

[6]The Royal Marsden NHS Foundation Trust, London, United Kingdom

[7]Statistics Department, EORTC Headquarters, Brussels, Belgium

[8]Department of Pediatric Hematology and Oncology, University Hospital, Muenster, Germany

[9]Alder Hey Children's NHS Foundation Trust, Liverpool, United Kingdom

[10]Emma Children's Hospital/Academic Medical Center, Amsterdam, The Netherlands

[11]Department of Medical Oncology, Leiden University Medical CenterLeiden, The Netherlands

[12]Cancer Medicine and Consultant Medical Oncologist, The London Sarcoma Service, University College Hospital, London, United Kingdom

[13]Faculty of Pharmacy, University of Sydney, Sydney, Australia

[14]Cancer Research UK, Cancer Trials Unit, University of Birmingham, Birmingham, United Kingdom

[15]Departments of Biostatistics and Epidemiology, Gustave-Roussy, Paris, France

[16]Paris-Saclay and Paris-SudUniversities, CESP, INSERM, Villejuif, France

[17]Gustave Roussy, Ligue Nationale Contre le Cancer Meta-analysis Platform, Villejuif, France

[18]Radiation EpidemiologyGroup, INSERM, UMR1018, Villejuif, France

Correspondence
Brice Fresneau, Department of Pediatric Oncology, Gustave Roussy, Université Paris-Saclay, 114 rue Edouard Vaillant, 94805 Villejuif, France.
Email: brice.fresneau@gustaveroussy.fr

Abstract

Background: A marginal interaction between sex and the type of alkylating agent was observed for event-free survival in the Euro-EWING99-R1 randomized controlled trial (RCT) comparing cyclophosphamide and ifosfamide in Ewing sarcoma. To further evaluate this interaction, we performed an individual patient data meta-analysis of RCTs assessing cyclophosphamide versus ifosfamide in any type of cancer.

Methods: A literature search produced two more eligible RCTs (EICESS92 and IRS-IV). The endpoints were progression-free survival (PFS, main endpoint) and overall survival (OS). The hazard ratios (HRs) of the treatment-by-sex interaction and their 95% confidence interval (95% CI) were assessed using stratified multivariable Cox models. Heterogeneity of the interaction across age

Abbreviations: EFS, event-free survival; HR, hazard ratio; OR, odds ratio; OS, overall survival; PFS, progression-free survival; RCT, randomized controlled trial; VAC, vincristine dactinomycin cyclophosphamide; VAI, vincristine dactinomycin ifosfamide; 95% CI, 95% confidence interval

categories and trials was explored. We also assessed this interaction for severe acute toxicity using logistic models.

Results: The meta-analysis comprised 1,528 pediatric and young adult sarcoma patients from three RCTs: Euro-EWING99-R1 (n = 856), EICESS92 (n = 155), and IRS-IV (n = 517). There were 224 PFS events in Euro-EWING99-R1 and 200 in the validation set (EICESS92 + IRS-IV), and 171 and 154 deaths in each dataset, respectively. The estimated treatment-by-sex interaction for PFS in Euro-EWING99-R1 (HR = 1.73, 95% CI = 1.00–3.00) was not replicated in the validation set (HR = 0.97, 95% CI = 0.55–1.72), without heterogeneity across trials ($P = 0.62$). In the pooled analysis, the treatment-by-sex interaction was not significant (HR = 1.31, 95% CI = 0.89–1.95, $P = 0.17$), without heterogeneity across age categories ($P = 0.88$) and trials ($P = 0.36$). Similar results were observed for OS. No significant treatment-by-sex interaction was observed for leucopenia/neutropenia ($P = 0.45$), infection ($P = 0.64$), or renal toxicity ($P = 0.20$).

Conclusion: Our meta-analysis did not confirm the hypothesis of a treatment-by-sex interaction on efficacy or toxicity outcomes.

KEYWORDS

acute toxicity, alkylating agent, cyclophosphamide, efficacy, ifosfamide, individual patient data, meta-analysis, sarcoma, systematic review, treatment-by sex interaction

1 | INTRODUCTION

The Euro-E.W.I.N.G.99-R1 randomized trial (EE99-R1, NCT00020566)[1] compared the efficacy of cyclophosphamide and ifosfamide combined with vincristine and dactinomycin (Vincristine dactinomycin cyclophosphamide [VAC] vs. vincristine dactinomycin ifosfamide [VAI]) as maintenance treatment in localized standard-risk Ewing sarcoma. We observed that sex marginally modified the treatment effect on event-free survival (EFS, interaction test, $P = 0.083$): in males, VAC was associated with poorer EFS than VAI with a hazard ratio (HR) (VAC/VAI) = 1.34 (95% CI = 0.96–1.86), whereas VAC was slightly better than VAI in females with an HR = 0.83 (95% CI = 0.54–1.28).[2]

Epidemiological studies have reported a higher incidence and mortality among men than women.[3,4] Registry-based survival analyses adjusted for age and disease stage have also shown that survival tends to be worse in males in various cancers.[4,5] Moreover, numerous clinical trials of cancer patients report a worse prognosis in males in most studies.[6–10] There are also sex differences in chemotherapy-related toxicity, especially with alkylating-based chemotherapy, with higher toxicity rates in females, especially hematological toxicity.[2,10–14] Some of these findings regarding efficacy and toxicity can be explained by pharmacokinetic differences in drug metabolism (e.g., different expression of liver metabolizing enzymes according to sex), leading some authors to propose sex-based dose adaptations.[15–18]

However, no interaction between the type of alkylating agent (cyclophosphamide or ifosfamide) and sex on efficacy and acute toxicity outcomes was reported before the EE99-R1 trial. In an attempt to confirm the EE99-R1 observation, we conducted a Meta-Analysis on Interaction between Alkylating agents and GEnder (MAIAGE) of randomized controlled trials (RCTs) comparing cyclophosphamide versus ifosfamide to confirm whether or not the effect of these two treatments differs between males and females.

2 | MATERIALS AND METHODS

2.1 | Trial selection

To identify an independent validation set for the EE99-R1 data, we undertook a bibliographic search of clinical trials randomizing cyclophosphamide versus ifosfamide (possibly in addition to other drugs but these drugs had to be identical in both arms) in both sex, without restriction on patient age and type of cancer. We searched PubMed and The Cochrane Library for articles published between 1980 and 2013 (any language), and the National Institute of Health clinical trials register (https://clinicaltrials.gov/). In addition, all participating trialists were asked to review and supplement a provisional list of trials. Trial selection was accomplished by two authors (BF and GLT) and all relevant articles were reviewed by a third author (MCLD).

Cyclophosphamide and ifosfamide could have been administered either as a single drug or combined with other drugs, but in the latter case, the only difference between the two arms had to be cyclophosphamide and ifosfamide. Differences in the dosage and infusion duration of cyclophosphamide and ifosfamide were allowed across studies. RCTs comparing only one course of cyclophosphamide or ifosfamide were not eligible. Moreover, RCTs for which individual patient data concerning survival and toxicity were not available were excluded.

2.2 | Data extraction and trial quality assessment

Individual patient data were collected for each trial: sex, date of birth, allocated treatment, date of randomization, date of first event, type of first event (progression, relapse, secondary malignancy, death), date of last follow-up or death, survival status, and cause of death (if applicable). We also collected acute toxicity data for leucopenia/neutropenia, thrombocytopenia, infection, mucositis and diarrhea, renal, liver, cardiac, skin, and central and peripheral neurologic toxicities during the randomized period with the grade according to the NCI-CTCAE (Common Terminology Criteria for Adverse Events) grading system. Individual anonymous data were centrally collected (BF and MCLD) and checked using a standard procedure (See Supplementary Methods S1). We noted missing data, data validity, randomization integrity, and follow-up of patients between the two arms.[19]

2.3 | Statistical analysis

The primary endpoint was progression-free survival (PFS), defined as the time from randomization to progression, recurrence or death from any cause, whichever occurred first. The secondary endpoint was overall survival (OS), defined as the time from randomization to death from any cause. Patients who had no events were censored at the date of the last follow-up. Analyses were performed on an intention-to-treat basis.

The validation set was analyzed using a multivariable Cox model, stratified by trial and sex, and including treatment (cyclophosphamide vs. ifosfamide) and age as main fixed effects. Age was divided into three categories (<12,[12–18] and >18 years) with selected cut-offs close to those defining the different pubertal status for males and females. The HR of the treatment effect by sex was measured by an interaction term ("one-stage" model).[20] Sensitivity analyses were also performed (see Supplementary Methods S2).

The heterogeneity test was assessed by Cochran's Q-statistics and I^2.[21,22] In addition, we performed an exploratory analysis on all RCTs, that is, EE99-R1 and the validation set. Stratified PFS curves were used to calculate the absolute difference at 5 years.[23] All statistical analyses performed for the validation set were also repeated on the pooled dataset. To explore heterogeneity of the treatment-by-sex interaction term across all trials and age categories, a three-order interaction term was included, with the relative two-order interactions terms.

For each type of acute toxicity, the maximum grade was computed for each patient and dichotomized as follows: hematologic toxicity (<, ≥grade-4); mucositis (<, ≥grade-3); diarrhea (<, ≥grade-3); and infection, renal, liver, cardiac, skin, central, and peripheral neurologic toxicities (<, ≥grade-2). The main safety analysis included toxicities that had occurred in at least five males and females in each trial arm to allow interaction analyses: leucopenia/neutropenia, infection, renal toxicity. For each type of toxicity, we estimated the treatment-by-sex interaction term using a logistic regression model stratified by trial and including age category, sex, treatment (main fixed effects), and treatment-by-sex interaction. We assessed the heterogeneity of the interaction across trials using a three-order interaction term between treatment, sex, and trial.

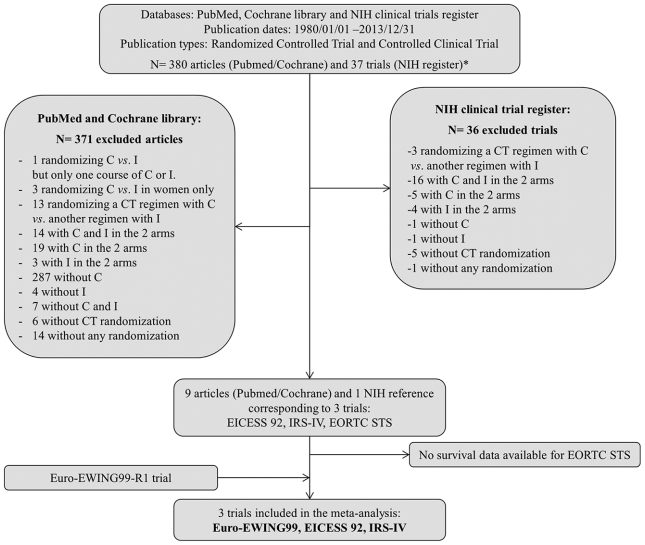


FIGURE 1 Flow chart of trial selection process. C, cyclophosphamide; I, ifosfamide; STS, soft tissue sarcoma.
*The search strategy used the following search terms: "ifosfamide" (Mesh) AND "cyclophosphamide" (Mesh) AND "randomized controlled trial" (Publication Type) OR "controlled clinical trial" (Publication Type) in PubMed, "ifosfamide" AND "cyclophosphamide" in the Cochrane Library, and "Ifosfamide" AND "cyclophosphamide" AND "randomized" in the NIH clinical trials register (http://www.clinicaltrials.gov). Note: Euro-EWING99-R1 trial was not yet published when we conducted the systematic review; that is why it does not appear in the initial systematic review box. Actualization of the literature search in November 2016 did not identify any other trial fulfilling the inclusion criteria

All estimates are given with 95% confidence intervals (95% CI) and two-sided *P*-values. Data collection and statistical analyses were performed using SAS Software 9.3. *Coxme* and *Meta* R packages for R version 3.0.2 (http://www.R-project.org) were used, respectively, to perform Cox regression models with random treatment effects and forest plots. The results are reported according to PRISMA-IPD recommendations.[24]

3 | RESULTS

3.1 | Trials description

In addition to the EE99-R1 trial,[1] we identified three trials (EICESS92,[25] IRS-IV,[26] and an EORTC randomized phase-II trial in soft tissue sarcomas[1]) among 380 references of published papers and 37 studies registered on ClinicalTrials.gov (Fig. 1). The EORTC trial was excluded because the individual patient data (survival and toxicity) were not available. We also excluded three randomized trials conducted exclusively in women (breast cancer,[28] ovarian epithelial cancer,[29] and endometrial adenocarcinoma[30]). Regarding the IRS-IV trial which compared three parallel groups, we considered the VAI and VAC arms, and excluded the third arm (vincristine–ifosfamide–etoposide arm). Actualization of the literature search in November 2016 did not identify any other trial fulfilling the inclusion criteria.

The three RCTs retained were high-quality phase III trials (See Supplementary Methods S1) comparing cyclophosphamide to ifosfamide in multidrug combinations administered as first-line treatment (Table 1). Sex was considered as a stratification variable in these three trials. The dose ratio of ifosfamide/cyclophosphamide ranged from 4 to 5. In total, 1,528 patients were included, 773 in the cyclophosphamide arm and 755 in the ifosfamide arm. The EE99-R1 trial represented 56% of the total number of patients. These trials were all conducted in sarcomas (Ewing sarcoma, rhabdomyosarcoma, and undifferentiated sarcomas). They included children, adolescents, and young adults, aged <15 years in 66% of the patients (Table 2).

3.2 | Survival analysis

With a median follow-up of 6.8 years (Q1–Q3, 4.5–8.9) (5.9 and 8.0 years in EE99-R1 and the validation set containing EICESS92 and IRS-IV, respectively), we observed 424 disease failures (i.e., PFS events: 224 and 200 in EE99-R1 and the validation set, respectively; progression or relapse in 395 patients and death as first event in 29, including six treatment-related deaths, nine from disease progression, nine other causes, and five unknown causes). There were 325 deaths overall (171 and 154 in EE99-R1 and the validation set, respectively). The estimated treatment-by-sex interaction on PFS in EE99-R1 (HR = 1.73, 95% CI = 1.00–3.00, *P*-value = 0.051) was not replicated in the validation set (n = 672) using the one-stage model (EICESS92+IRS-IV, HR = 0.97, 95% CI = 0.55–1.72, *P* = 0.93; Fig. 2), with no heterogeneity between both trials (*P* = 0.62). Interaction estimates were very similar in the sensitivity analyses (Table 3). In the same way, the estimated treatment-by-sex interaction in EE99-R1 for OS (HR = 1.85, 95% CI = 0.98–3.48, *P* = 0.056) was not replicated in the validation set (HR = 1.00, 95% CI 0.52–1.92, *P* = 0.99; Supplementary Fig. S1).

When the three RCTs were pooled, the estimated 5-year absolute PFS benefit associated with ifosfamide compared to cyclophosphamide was greater among males +6.0% (73.7% vs. 67.9%) than females (+0.2%, 75.2% vs. 75.0%; Fig. 3). However, the overall estimate of treatment-by-sex interaction was not statistically significant (HR = 1.31, 95% CI = 0.89–1.95, *P* = 0.17). Although a significant treatment-by-sex interaction was observed in EE99-R1 (*P* = 0.051), this interaction was not statistically different to interaction terms estimated in EICESS92 and IRS-IV trials (*P* = 0.36; Fig. 2). This interaction estimate did not vary across age categories (*P* = 0.88, Supplementary Fig. S2). The sensitivity analyses yielded similar results (last column, Table 3). For OS (Supplementary Fig. S3), the pooled estimate of the treatment-by-sex interaction was not statistically significant (HR = 1.37, 95% CI = 0.87–2.15, *P* = 0.17). We observed neither heterogeneity across trials (*P* = 0.35, Supplementary Fig. S4) nor across age categories (*P* = 0.64, Supplementary Fig. S4). Stable results were observed in the sensitivity analyses (Table 3).

3.3 | Toxicity analysis

The frequencies of severe acute toxicities by sex and treatment arm are shown in Supplementary Table S1. At least one episode of

TABLE 1 Characteristics of selected randomized clinical trials with regimens comparing cyclophosphamide versus ifosfamide

Trial	Accrual period	Type of trial and design	N	Median follow-up [Q1–Q3]	Inclusion criteria			Eligibility criteria for randomization	Randomized regimens		Primary endpoint	Results of ITT[a] analysis
					Pathology	Primary tumor site	Age (years)		Ifo (dose/3 w)	Cyclo (dose/3 w)		
EE99-R1[a]	2000–2010	Multicentric Phase III and noninferiority	856	5.9 [3.8–8.0]	EWS	Bone or soft tissue	<50	Localized tumors with a good response to preoperative CT[b]	7 VAI (3 g/m² × 2)	7 VAC (1.5 g/m² × 1)	3-y EFS	78% (VAI) 75% (VAC)
EICESS92[25]	1992–1999	Multicentric Phase III and noninferiority	155	8.3 [6.9–10.6]	ESFT	Bone	<35	Localized tumors of less than 100 ml	10 VAIA (2 g/m² × 3)	10 VACA (1.2 g/m² × 1)	3-y EFS	74% (VAIA) 73% (VACA)
IRS-IV[26]	1991–1997	Multicentric Phase III and superiority	517	8.0 [5.5–9.9]	RMS, undifferentiated sarcoma	Soft tissue	<21	Localized tumors[c]	8 VAI (1.8 g/m² × 5)	8 VAC (2.2 g/m² × 1)	3-y EFS	77% (VAI) 73% (VAC)

CT, chemotherapy; Cyclo, cyclophosphamide; Ifo, ifosfamide; EFS, event-free survival; ESFT, Ewing sarcoma family of tumors; EWS, Ewing sarcoma; N, number of randomized patients; Q1, first quartile; Q3, third quartile; RMS, rhabdomyosarcoma; VAC, vincristine, dactinomycin, cyclophosphamide; VACA, vincristine, dactinomycin, cyclophosphamide, adriamycin; VAI, vincristine, dactinomycin, ifosfamide; VAIA, vincristine, dactinomycin, ifosfamide, adriamycin; w, week; y, year.

[a] Intention to treat.

[b] Patients with either a good histologic response to preoperative treatment (<10% cells), or a small tumor (<200 mL) resected at diagnosis or with radiotherapy alone as local treatment.

[c] After exclusion of patients with completely resected paratesticular tumors, completely resected or microscopic residual disease of orbit or eyelid tumors, preexisting renal abnormalities.

TABLE 2 Characteristics of randomized patients in each trial included in the meta-analysis

	EE99-R1		EICESS92		IRS-IV		Pooled dataset	
	VAI (n = 425)	VAC (n = 431)	VAIA (n = 76)	VACA (n = 79)	VAI (n = 254)	VAC (n = 263)	Ifo arm (n = 755)	Cyclo arm (n = 773)
Sex								
Male	251	258	46	49	141	152	438	459
Female	174	173	30	30	113	111	317	314
Age (years)								
Median	14.0	14.6	15.4	13.8	6.0	5.0	11.8	12.0
[0–10]	120	99	17	18	172	190	309	307
[10–15]	127	127	19	31	54	39	200	197
[15–20]	88	107	23	17	28	32	139	156
≥20	90	98	17	13		2	107	113
Pathology								
ESFT	415	416	73	77			488	493
RMS					234	248	234	248
Other bone sarcoma	1	1	1				2	1
Other STS	10	14	2	2	20	15	32	31
Tumor stage								
Localized disease	425	430	72	78	244	253	741	761
Metastatic disease		1	3	1			3	2
NA			1		10	10	11	10
Number of events	106	118	28	28	62	82	196	228
Progression/relapse	102	115	27	27	55	69	184	211
Death as first event	4	3	1	1	7	13	12	17
Number of deaths	83	88	18	21	51	64	152	173

CT, chemotherapy; Cyclo, cyclophosphamide; Ifo, ifosfamide; ESFT, Ewing sarcoma family of tumors; RMS, rhabdomyosarcoma; NA, not applicable; STS, soft tissue sarcoma; VAC, vincristine, dactinomycin, cyclophosphamide; VACA, vincristine, dactinomycin, cyclophosphamide, adriamycin; VAI, vincristine, dactinomycin, ifosfamide; VAIA, vincristine, dactinomycin, ifosfamide, adriamycin.

severe acute neutropenia, infection, and renal toxicity had occurred in 69.8, 52.8, and 7.8% of patients, respectively. As illustrated in Supplementary Figs. S5–S7, no significant interaction was identified between sex and alkylating agent for leucopenia/neutropenia (odds ratio [OR] = 0.82, 95% CI = 0.49–1.36, $P = 0.43$), infection (OR = 1.11, 95% CI = 0.71–1.71, $P = 0.65$), or renal toxicity (OR = 1.71, 95% CI = 0.76–3.85, $P = 0.19$). These estimates did not significantly vary across trials (heterogeneity tests for leucopenia/neutropenia: $P = 0.81$, infection: $P = 0.12$, and renal toxicity: $P = 0.19$). The main effects were reported because no interaction was found between treatment and sex. Compared to ifosfamide, patients receiving cyclophosphamide experienced more severe leucopenia/neutropenia ($OR_{cyclo\ vs.\ ifo} = 1.47$, 95% CI = 1.14–1.88, $P = 0.003$) and infections ($OR_{cyclo\ vs.\ ifo} = 1.55$, 95% CI = 1.25–1.93, $P < 0.0001$), but less renal toxicity ($OR_{cyclo\ vs.\ ifo} = 0.71$, 95% CI = 0.48–1.06, $P = 0.098$). Regardless of treatment arm, females developed significantly more severe leucopenia/neutropenia ($OR_{female\ vs.\ male} = 1.39$, 95% CI = 1.08–1.79, $P = 0.013$) and infections ($OR_{female\ vs.\ male} = 1.25$, 95% CI = 1.01–1.56, $P = 0.041$) than males, but not significantly more severe renal toxicity ($OR_{female\ vs.\ male} = 1.22$, 95% CI = 0.83–1.82, $P = 0.32$).

4 | DISCUSSION

Using an independent validation set of two RCTs (EICESS92 and IRS-IV), we did not replicate the treatment-by-sex interactions observed in the EE99-R1 trial on PFS and OS. No significant interactions were observed when the three trials were pooled, with no significant heterogeneity across age and trials. Similarly, we did not identify any treatment-by-sex interaction on leucopenia/neutropenia, infection, and renal toxicity. Cyclophosphamide was significantly more hematotoxic (leucopenia/neutropenia and infections) than ifosfamide. We also observed more hematotoxicity in women than in males regardless of treatment arm.

This individual patient data meta-analysis is the first to assess a potential interaction between the type of alkylating agent and sex. Based on high-quality RCTs comparing cyclophosphamide to ifosfamide in both sex, with a total number of patients exceeding 1,500 and long follow-up, it provides an unbiased estimate of the treatment-by-sex interaction. Finally, even though the search was not restricted to age or to a specific type of cancer, these three trials included mainly pediatric and young adult patients, with Ewing sarcoma or rhabdomyosarcoma under first-line treatment. This probably reduces

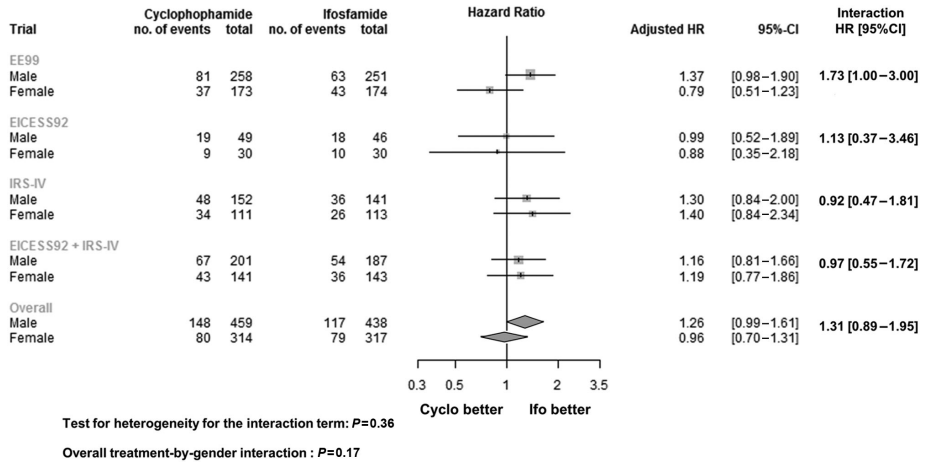


FIGURE 2 Forest plot of the hazard ratios (HRs) of progression-free survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex using fixed effects model. HRs given on the right side represent the HR of the treatment-by-sex interaction (HRCyclo/Ifo in males/HRCyclo/Ifo in females) estimated independently for each trial, in the validation set and in the pooled dataset, by the one-stage model, stratified by trial and sex, and including treatment (cyclophosphamide vs. ifosfamide) and age (<12, 12–18, and >18 years) as the main fixed effects. The heterogeneity of the interaction across trials was assessed using a three-order interaction term. The center of each square represents the HR for individual trials and for the validation set (EICESS92 + IRS-IV) and the corresponding horizontal line its 95% confidence interval (CI). The area of squares is proportional to the amount of information obtained from the trial. The center of the black diamond represents the overall HR and the extremities of the diamond represent its 95% CI, both estimated from the pooled dataset

TABLE 3 Estimate of the hazard ratio of the treatment-by-gender interaction term for progression-free survival and overall survival for EE99-R1 (training set), EICESS92 + IRS-IV (validation set), and the pooled dataset in the main and sensitivity analyses

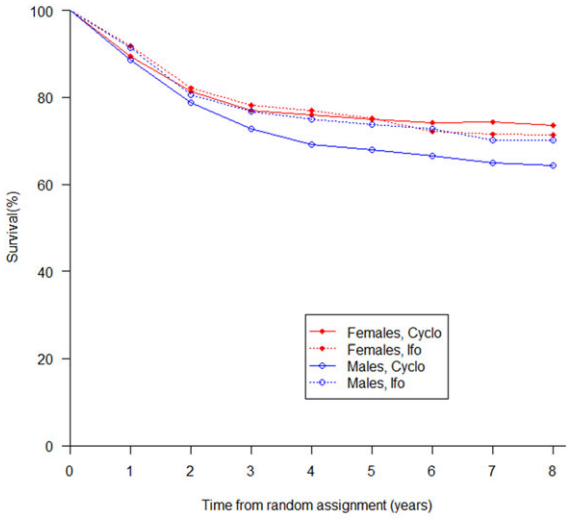
	Training set EE99-R1 (n = 856)	Validation set EICESS92 + IRS-IV (n = 672)	Pooled analysis EE99-R1 + EICESS92 + IRS-IV (n = 1528)
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Progression-free survival			
Main analysis: OSM, fixed effects, age category	1.73 (1.00–3.00), $P = 0.051$	0.97 (0.55–1.72), $P = 0.93$	1.31 (0.89–1.95), $P = 0.17$
Sensitivity analyses			
OSM, random effects, age category	1.73 (1.00–3.00), $P = 0.051$	0.98 (0.55–1.73), $P = 0.93$	1.32 (0.89–1.95), $P = 0.17$
OSM, fixed effects, age continuous	1.71 (0.98–2.96), $P = 0.057$	0.96 (0.55–1.71), $P = 0.90$	1.31 (0.89–1.95), $P = 0.17$
PWT, fixed effects, age category		0.97 (0.55–1.73), $P = 0.92$	1.32 (0.88–1.96), $P = 0.18$
Overall survival			
Main analysis: OSM, fixed effects, age category	1.85 (0.98–3.48), $P = 0.056$	1.00 (0.52–1.92), $P = 0.99$	1.37 (0.87–2.15), $P = 0.17$
Sensitivity analyses			
OSM, random effects, age category	1.85 (0.98–3.48), $P = 0.056$	1.00 (0.52–1.93), $P = 1.00$	1.37 (0.87–2.16), $P = 0.17$
OSM, fixed effects, age continuous	1.80 (0.96–3.38), $P = 0.068$	0.99 (0.51–1.91), $P = 0.98$	1.37 (0.87–2.16), $P = 0.17$
PWT, fixed effects, age category		0.99 (0.51–1.91), $P = 0.98$	1.37 (0.87–2.16), $P = 0.17$

OSM, one-stage model; PWT, pooling of within-trial covariate interactions model; age category: <12 [12–18] and >18 years.
HR: hazard ratio of the treatment-by-gender interaction term (HR Cyclo vs. Ifo in males/HR Cyclo vs. Ifo in females).

sources of heterogeneity across trials (e.g., pharmacodynamic differences and co-morbidity).

The EORTC trial[27] that randomized cyclophosphamide and ifosfamide as a single drug in advanced or metastatic soft-tissue sarcomas (n = 135 patients) was not included in the MAIAGE study due to the lack of availability of individual survival or toxicity data after contacting the principal investigator. This study reported lower response rates in the cyclophosphamide arm than in the ifosfamide arm, especially in males (observed response rate of 0% and 11% in males treated with cyclophosphamide and ifosfamide, respectively, and 17% and 23% in females). Based on these data, we did not observe any significant heterogeneity of the treatment effect between sexes (interaction test: $P = 0.12$). In the three other randomized trials excluded (because they were based on women only, see Supplementary Table S2),[28–30] a better prognosis was reported in two, in subgroups of women treated with ifosfamide,[29,30] whereas the difference was not significant in the third trial.[28]

Our study had some limitations. First, none of the trials analyzed were initially designed to study a treatment-by-sex interaction. Due to the observed number of events in each trial and when pooled, the



No. of deaths / person-years by period

Females-Ifo	25/295	29/260	11/223	3/192	4/172	6/138	1/110	0/87
Females-Cyclo	33/294	24/258	13/233	3/203	2/182	2/161	0/136	1/107
Males-Ifo	37/416	45/359	15/318	7/284	4/243	3/214	6/171	0/125
Males-Cyclo	51/424	43/360	25/311	14/269	4/238	4/201	4/160	1/119

FIGURE 3 Stratified progression-free survival (PFS) curves according to sex and alkylating agent (cyclophosphamide or ifosfamide) when the three RCTs were pooled (n = 1,528). The 5-year absolute PFS benefit associated with ifosfamide (Ifo) compared to cyclophosphamide (Cyclo) was estimated at 6% in males (73.7% vs. 67.9%), whereas females receiving ifosfamide or cyclophosphamide had similar PFS (75.2% vs. 75.0%, difference = 0.2%)

analyses could be underpowered to test the interaction with a standard statistical level ($P < 0.05$), let alone to detect heterogeneity of the treatment-by-sex interaction across trials (e.g., infection analysis with marginal heterogeneity across trials, $P = 0.12$). Although we did not validate a treatment-by-sex interaction on efficacy outcomes, our results do not conclusively rule out the existence of an interaction.

Second, in addition to the index trial, we identified only two other RCTs, which together contributed less than 50% of the total number of patients. We did not identify any other study comparing cyclophosphamide and ifosfamide, hence there is a paucity of independent trials. Finally, differences in population characteristics and in drug combinations in the backbone chemotherapy could impact the consistency of the estimates of treatment-by-sex interaction. Indeed, (i) rhabdomyosarcoma patients in IRS-IV were younger than Ewing sarcoma patients from the other two trials, and (ii) all IRS-IV patients received four additional courses with cyclophosphamide after the first eight courses allocated by randomization; in contrast, all patients also received ifosfamide as induction chemotherapy before randomization in both Ewing sarcoma trials.

Our findings concerning acute toxicity are consistent with previous reports in sarcoma and lymphoma patients treated with alkylating agents.[10–14] Differences in cytochrome P450 mediated drug metabolism between sexes could explain these results. Cyclophosphamide and ifosfamide are oxazaphosphorine alkylating prodrugs that are metabolized via different P450-catalyzed pathways: (i) 4-hydroxylation produces active alkylating agents and urotoxic acrolein via CYP2B6 for cyclophosphamide and CYP3A4 and CYP3A5 for ifosfamide, and (ii) N-dechloroethylation generates inactive metabolites and nephro- and neurotoxic chloroacetaldehyde via CYP3A4 for cyclophosphamide and, to a much greater extent, CYP3A4 and CYP2B6 for ifosfamide.[31–33] Greater activity of CYP3A4 and CYP2B6 has been reported in females resulting in higher concentrations of toxic chloroacetaldehyde after ifosfamide infusion and consequently in a possible higher risk of severe neurotoxicity in females.[34–36] However, no cytochrome P450 related difference in hematologic toxicity between sexes has previously been reported.

In conclusion, our meta-analysis did not show that the treatment effect of cyclophosphamide versus ifosfamide is influenced by sex for either efficacy or toxicity. Therefore, recommending the choice of alkylating agent should not be based on sex in children and young adults treated for sarcoma. Additional studies would be useful for long-term follow-up including fertility outcomes.

ACKNOWLEDGMENTS

This research was supported by The Gustave-Roussy Institute; The Fondation pour la Recherche Médicale; European Community's Seventh Framework Programme under grant agreements no. 261474 (project ENCCA) and no. 602856-2 (project EEC); Fédération Enfants et Santé, Société Française de Lutte Contre les Cancers et les Leucémies de l'Enfant et de l'Adolescent; Unicancer and the Ligue Nationale Contre le Cancer; Cancer Research UK (Grant No. CRUK/02/014); Deutsche Krebshilfe (Grants Nos. 50-2551-Jü3, 50-2551-Jü4, DKH-108128, 70-2551-Jue3, and 108128), Bundesministerium für Bildung und Forschung (TranSaRNet and Grants Nos. BMBF 01GM0869 and BMBF/Era-Net 01KT1310), and Deutsches Zentrum für Luft- und Raumfahrt e.V. 01GM0869; and the National Cancer Institute, Bethesda, MD (grant nos. U10CA180886, U10CA180899, U10CA098543, and U10CA098413). Special thanks for assistance to Joachim Boos for helpful discussions and Lorna Saint Ange for editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Le Deley M-C, Paulussen M, Lewis I, et al. Cyclophosphamide compared with ifosfamide in consolidation treatment of standard-risk Ewing sarcoma: Results of the randomized noninferiority Euro-EWING99-R1 trial. *J Clin Oncol*. 2014;32:2440–2448.
2. van den Berg H, Paulussen M, Le Teuff G, et al. Impact of gender on efficacy and acute toxicity of alkylating agent -based chemotherapy in Ewing sarcoma: Secondary analysis of the Euro-Ewing99-R1 trial. *Eur J Cancer*. 2015;51:2453–2464.
3. Cook MB, Dawsey SM, Freedman ND, et al. Sex disparities in cancer incidence by period and age. *Cancer Epidemiol Biomarkers Prev*. 2009;18:1174–82.
4. Cook MB, McGlynn KA, Devesa SS, Freedman ND, Anderson WF. Sex disparities in cancer mortality and survival. *Cancer Epidemiol Biomarkers Prev*. 2011;20:1629–1637.
5. Khamly KK, Thursfield VJ, Fay M, et al. Gender-specific activity of chemotherapy correlates with outcomes in chemosensitive cancers of young adulthood. *Int J Cancer*. 2009;125:426–431.
6. Molife R, Lorigan P, MacNeil S. Gender and survival in malignant tumours. *Cancer Treat Rev*. 2001;27:201–209.

7. Bartelink H, Roelofsen F, Eschwege F, et al. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: Results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Groups. *J Clin Oncol*. 1997;15:2040–2049.
8. Glynne-Jones R, Sebag-Montefiore D, Adams R, et al. Prognostic factors for recurrence and survival in anal cancer: Generating hypotheses from the mature outcomes of the first United Kingdom Coordinating Committee on Cancer Research Anal Cancer Trial (ACT I). *Cancer*. 2013;119:748–755.
9. Wakelee HA, Wang W, Schiller JH, et al. Survival differences by sex for patients with advanced non-small cell lung cancer on Eastern Cooperative Oncology Group trial 1594. *J Thorac Oncol*. 2006;1:441–446.
10. Collins M, Wilhelm M, Conyers R, et al. Benefits and adverse events in younger versus older patients receiving neoadjuvant chemotherapy for osteosarcoma: Findings from a meta-analysis. *J Clin Oncol*. 2013;31:2303–2312.
11. Ferrari S, Palmerini E, Staals E, et al. Sex- and age-related chemotherapy toxicity in patients with non-metastatic osteosarcoma. *J Chemother*. 2009;21:205–210.
12. Paioli A, Luksch R, Fagioli F, et al. Chemotherapy-related toxicity in patients with non-metastatic Ewing sarcoma: Influence of sex and age. *J Chemother*. 2014;26:49–56.
13. Wrobel G, Mauguen A, Rosolen A, Reiter A, Williams D, Horibe K, et al. Safety assessment of intensive induction therapy in childhood anaplastic large cell lymphoma: Report of the ALCL99 randomised trial. *Pediatr Blood Cancer*. 2011;56:1071–1077.
14. Juergens C, Weston C, Lewis I, et al. Safety assessment of intensive induction with vincristine, ifosfamide, doxorubicin, and etoposide (VIDE) in the treatment of Ewing tumors in the EURO-E.W.I.N.G. 99 clinical trial. *Pediatr Blood Cancer*. 2006;47:22–29.
15. Franconi F, Campesi I. Pharmacogenomics, pharmacokinetics and pharmacodynamics: Interaction with biological differences between men and women. *Br J Pharmacol*. 2014;171:580–594.
16. Anderson GD. Sex and racial differences in pharmacological response: Where is the evidence? Pharmacogenetics, pharmacokinetics, and pharmacodynamics. *J Womens Health*. 2005;14:19–29.
17. Waxman DJ, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol Pharmacol*. 2009;76:215–228.
18. Schmetzer O, Flörcken A. Sex differences in the drug therapy for oncologic diseases. *Handb Exp Pharmacol*. 2012:411–442.
19. Stewart LA, Clarke MJ. Practical methodology of meta-analyses (overviews) using updated individual patient data. Cochrane Working Group.*Stat Med*. 1995;14:2057–2079.
20. Fisher DJ, Copas AJ, Tierney JF, Parmar MKB. A critical review of methods for the assessment of patient-level interactions in individual participant data meta-analysis of randomized trials, and guidance for practitioners. *J Clin Epidemiol*. 2011;64:949–967.
21. Cochran WG. The combination of estimates from different experiments. *Biometrics*. 1954;10:101.
22. Higgins JPT. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327:557–560.
23. Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. *Lancet*. 1992;339:71–85.
24. Stewart LA, Clarke M, Rovers M, et al. Preferred reporting items for systematic review and meta-analyses of individual participant data: The PRISMA-IPD statement. *JAMA*. 2015;313:1657–1665.
25. Paulussen M, Craft AW, Lewis I, et al. Results of the EICESS-92 Study: Two randomized trials of Ewing's sarcoma treatment—Cyclophosphamide compared with ifosfamide in standard-risk patients and assessment of benefit of etoposide added to standard treatment in high-risk patients. *J Clin Oncol*. 2008;26:4385–4393.
26. Crist WM, Anderson JR, Meza JL, et al. Intergroup rhabdomyosarcoma study-IV: Results for patients with nonmetastatic disease. *J Clin Oncol*. 2001;19:3091–3102.
27. Bramwell VH, Mouridsen HT, Santoro A, et al. Cyclophosphamide versus ifosfamide: Final report of a randomized phase II trial in adult soft tissue sarcomas. *Eur J Cancer Clin Oncol*. 1987;23:311–321.
28. Buzdar AU, Legha SS, Tashima CK, et al. Ifosfamide versus cyclophosphamide in combination drug therapy for metastatic breast cancer. *Cancer Treat Rep*. 1979;63:115–120.
29. Nishida T, Sugiyama T, Yakushiji M. Cisplatin, epirubicin, and ifosfamide versus cisplatin, epirubicin, and cyclophosphamide in clear cell carcinoma of the ovary. *Gynecol Oncol*. 1997;67:230.
30. Pawinski A, Tumolo S, Hoesel G, et al. Cyclophosphamide or ifosfamide in patients with advanced and/or recurrent endometrial carcinoma: A randomized phase II study of the EORTC Gynecological Cancer Cooperative Group. *Eur J Obstet Gynecol Reprod Biol*. 1999;86:179–183.
31. Walker D, Flinois JP, Monkman SC, et al. Identification of the major human hepatic cytochrome P450 involved in activation and N-dechloroethylation of ifosfamide. *Biochem. Pharmacol*. 1994;47:1157–1163.
32. Roy P, Tretyakov O, Wright J, Waxman DJ. Stereoselective metabolism of ifosfamide by human P-450s 3A4 and 2B6. Favorable metabolic properties of R-enantiomer. *Drug Metab Dispos Biol Fate Chem*. 1999;27:1309–1318.
33. Huang Z, Roy P, Waxman DJ. Role of human liver microsomal CYP3A4 and CYP2B6 in catalyzing N-dechloroethylation of cyclophosphamide and ifosfamide. *Biochem Pharmacol*. 2000;59:961–972.
34. Schmidt R, Baumann F, Hanschmann H, Geissler F, Preiss R. Gender difference in ifosfamide metabolism by human liver microsomes. *Eur J Drug Metab Pharmacokinet*. 2001;26:193–200.
35. Wolbold R, Klein K, Burk O, et al. Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology*. 2003;38:978–988.
36. Lamba V, Lamba J, Yasuda K, et al. Hepatic CYP2B6 expression: Gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. *J Pharmacol Exp Ther*. 2003;307:906–922.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Fresneau B, Hackshaw A, Hawkins DS, Paulussen M, Anderson JR, Judson I, Litière S, Dirksen U, Lewis I, van den Berg H, Gaspar N, Gelderblom H, Whelan J, Boddy AV, Wheatley K, Pignon JP, De Vathaire F, Le Deley MC, and Le Teuff G. Investigating the heterogeneity of alkylating agents' efficacy and toxicity between genders: a systematic review and meta-analysis of randomized trials comparing cyclophosphamide and ifosfamide (MAIAGE study). *Pediatr Blood Cancer*. 2017;64:e26457. doi: 10.1002/pbc.26457