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Investigating the heterogeneity of alkylating agents' efficacy and toxicity between sexes

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1 **Investigating the heterogeneity of alkylating agents' efficacy and toxicity between**
2 **genders: a systematic review and meta-analysis of randomized trials comparing**
3 **cyclophosphamide and ifosfamide (MAIAGE study)**

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63

64   Abbreviations

RCT	Randomized controlled trial
EFS	Event-free survival
PFS	Progression-free survival
OS	Overall survival
HR	Hazard ratio
OR	Odds ratio
95%CI	95%-confidence interval
VAC	Vincristine dactinomycin cyclophosphamide
VAI	Vincristine dactinomycin ifosfamide

65

66 **ABSTRACT**

67 **Background**: A marginal interaction between sex and the type of alkylating agent was

68 observed for event-free survival in the Euro-EWING99-R1 randomized controlled trial (RCT)

69 comparing cyclophosphamide and ifosfamide in Ewing sarcoma. To further evaluate this

70 interaction, we performed an individual patient data meta-analysis of RCTs assessing

71 cyclophosphamide *vs*. ifosfamide in any type of cancer. **Methods**: A literature search

72 produced two more eligible RCTs (EICESS92 and IRS-IV). The endpoints were progression-

73 free survival (PFS, main endpoint) and overall survival (OS). The hazard ratios (HR) of the

74 treatment-by-sex interaction and their 95%-confidence interval (95%CI) were assessed using

75 stratified multivariable Cox models. Heterogeneity of the interaction across age categories

76 and trials was explored. We also assessed this interaction for severe acute toxicity using

77 logistic models. **Results**: The meta-analysis comprised 1528 pediatric and young adult

78 sarcoma patients from three RCTs: Euro-EWING99-R1 (n=856), EICESS92 (n=155) and

79 IRS-IV (n=517). There were 224 PFS events in Euro- EWING99-R1 and 200 in the validation

80 set (EICESS92+IRS-IV); and 171 and 154 deaths in each dataset respectively. The estimated

81 treatment-by-sex interaction for PFS in Euro-EWING99-R1 (HR=1.73, 95%CI=1.00-3.00)

82 was not replicated in the validation set (HR=0.97, 95%CI=0.55-1.72), without heterogeneity

83 across trials (p=0.62). In the pooled analysis, the treatment-by-sex interaction was not

84 significant (HR=1.31, 95%CI=0.89-1.95, p=0.17), without heterogeneity across age

85 categories (p=0.88) and trials (p=0.36). Similar results were observed for OS. No significant

86 treatment-by-sex interaction was observed for leucopenia/neutropenia (p=0.45), infection

87 (p=0.64) or renal toxicity (p=0.20). **Conclusion**: Our meta-analysis did not confirm the

88 hypothesis of a treatment-by-sex interaction on efficacy or toxicity outcomes.

89   **INTRODUCTION**

90   The Euro-E.W.I.N.G.99-R1 randomized trial (EE99-R1, NCT00020566)[1] compared the

91   efficacy of cyclophosphamide and ifosfamide combined with vincristine and dactinomycin

92   (VAC vs. VAI) as maintenance treatment in localized standard-risk Ewing sarcoma. We

93   observed that sex marginally modified the treatment effect on event-free survival (EFS,

94   interaction test, p=0.083): in males, VAC was associated with poorer EFS than VAI with a

95   hazard ratio (HR) (VAC/VAI) =1.34 (95%CI, 0.96-1.86), whereas VAC was slightly better

96   than VAI in females with a HR=0.83 (95%CI, 0.54-1.28).[2]

97   Epidemiological studies have reported a higher incidence and mortality among men than

98   women.[3,4] Registry-based survival analyses adjusted for age and disease stage have also

99   shown that survival tends to be worse in males in various cancers.[4,5] Moreover, numerous

100   clinical trials of cancer patients report a worse prognosis in males in most studies.[6–10]

101   There are also sex differences in chemotherapy-related toxicity, especially with alkylating-

102   based chemotherapy, with higher toxicity rates in females, especially hematological

103   toxicity.[2,10–14] Some of these findings regarding efficacy and toxicity can be explained by

104   pharmacokinetic differences in drug metabolism (e.g. different expression of liver

105   metabolizing enzymes according to sex), leading some authors to propose sex-based dose

106   adaptations.[15–18]

107   However, no interaction between the type of alkylating agent (cyclophosphamide or

108   ifosfamide) and sex on efficacy and acute toxicity outcomes was reported before the EE99-R1

109   trial. In an attempt to confirm the EE99-R1 observation, we conducted a Meta-Analysis on

110   Interaction between Alkylating agents and GEnder (MAIAGE) of randomized controlled

111   trials (RCT) comparing cyclophosphamide versus ifosfamide, to confirm whether or not the

112   effect of these two treatments differs between males and females.

113

114   **MATERIALS and METHODS**

115   **Trial selection**

116   To identify an independent validation set for the EE99-R1 data, we undertook a bibliographic

117   search of clinical trials randomizing cyclophosphamide *vs*. ifosfamide (possibly in addition to

118   other drugs but these drugs had to be identical in both arms) in both sex, without restriction on

119   patient age and type of cancer. We searched PubMed and The Cochrane Library for articles

120   published between 1980 and 2013 (any language), and the National Institute of Health clinical

121   trials register (https://clinicaltrials.gov/). In addition, all participating trialists were asked to

122   review and supplement a provisional list of trials. Trial selection was accomplished by two

123   authors (BF, GLT) and all relevant articles were reviewed by a third (MCLD).

124   Cyclophosphamide and ifosfamide could have been administered either as a single drug or

125   combined with other drugs, but in the latter case, the only difference between the two arms

126   had to be cyclophosphamide and ifosfamide. Differences in the dosage and infusion duration

127   of cyclophosphamide and ifosfamide were allowed across studies. RCTs comparing only one

128   course of cyclophosphamide or ifosfamide were not eligible. Moreover RCTs for which

129   individual patient data concerning survival and toxicity were not available, were excluded.

130

131   **Data extraction and trial quality assessment**

132   Individual patient data were collected for each trial: sex, date of birth, allocated treatment,

133   date of randomization, date of first event, type of first event (progression, relapse, secondary

134   malignancy, death), date of last follow-up or death, survival status and cause of death (if

135   applicable). We also collected acute toxicity data for leucopenia/neutropenia,

136   thrombocytopenia, infection, mucositis and diarrhea, renal, liver, cardiac, skin, central and

137   peripheral neurologic toxicities during the randomized period with the grade according to the

138   NCI-CTCAE (Common Terminology Criteria for Adverse Events) grading system. Individual

139   anonymous data were centrally collected (BF, MCLD) and checked using a standard

140   procedure (See Supplemental Methods S1). We noted missing data, data validity,

141   randomization integrity and follow-up of patients between the two arms.[19]

142

143   **Statistical analysis**

144   The primary endpoint was progression-free survival (PFS), defined as the time from

145   randomization to progression, recurrence or death from any cause, whichever occurred first.

146   The secondary endpoint was overall survival (OS), defined as the time from randomization to

147   death from any cause. Patients who had no events were censored at the date of the last follow-

148   up. Analyses were performed on an intention-to-treat basis.

149   The validation set was analyzed using a multivariable Cox model, stratified by trial and sex,

150   and including treatment (cyclophosphamide *vs.* ifosfamide) and age as main fixed effects.

151   Age was divided into 3 categories (< 12, [12-18] and > 18 years) with selected cut-offs close

152   to those defining the different pubertal status for males and females. The hazard ratio (HR) of

153   the treatment effect by sex was measured by an interaction term ("one-stage" model).[20]

154   Sensitivity analyses were also performed (see Supplemental Methods S2).

155   The heterogeneity test was assessed by Cochran's Q-statistics and I^2.[21,22] In addition, we

156   performed an exploratory analysis on all RCTs, i.e. EE99-R1 and the validation set. Stratified

157   PFS curves were used to calculate the absolute difference at 5 years.[23] All statistical

158   analyses performed for the validation set were also repeated on the pooled dataset. To explore

159   heterogeneity of the treatment-by-sex interaction term across all trials and age categories, a 3-

160   order interaction term was included, with the relative 2-order interactions terms.

161   For each type of acute toxicity, the maximum grade was computed for each patient and

162   dichotomized as follows: hematologic toxicity (<, ≥grade-4), mucositis (<, ≥grade-3),

163   diarrhea (<, ≥grade-3) and infection, renal, liver, cardiac, skin, central and peripheral

164   neurologic toxicities (<, ≥grade-2). The main safety analysis included toxicities which had

165   occurred in at least five males and females in each trial arm to allow interaction analyses:

166 leucopenia/neutropenia, infection, renal toxicity. For each type of toxicity, we estimated the

167 treatment-by-sex interaction term using a logistic regression model stratified by trial and

168 including age category, sex, treatment (main fixed effects) and treatment-by-sex interaction.

169 We assessed the heterogeneity of the interaction across trials using a 3-order interaction term

170 between treatment, sex and trial.

171 All estimates are given with 95% confidence intervals (95%CI) and two-sided p-values. Data

172 collection and statistical analyses were performed using SAS Software 9.3. *Coxme* and *Meta*

173 R packages for R version 3.0.2 (http://www.R-project.org) were used respectively to perform

174 Cox regression models with random treatment effects and forest plots. The results are

175 reported according to PRISMA-IPD recommendations.[24]

176

177 **RESULTS**

178 **Trials description**

179 In addition to the EE99-R1 trial[1], we identified three trials (EICESS92[25], IRS-IV[26] and

180 an EORTC randomized phase-II trial in soft tissue sarcomas[27]) among 380 references of

181 published papers and 37 studies registered on ClinicalTrials.gov (Figure-1). The EORTC trial

182 was excluded because the individual patient data (survival and toxicity) were not available.

183 We also excluded three randomized trials conducted exclusively in women (breast cancer[28],

184 ovarian epithelial cancer[29] and endometrial adenocarcinoma[30]). Regarding the IRS-IV

185 trial which compared three parallel groups, we considered the VAI and VAC arms, and

186 excluded the third arm (vincristine-ifosfamide-etoposide arm). Actualization of the literature

187 search in November 2016 did not identify any other trial fulfilling the inclusion criteria.

188 The three RCTs retained were high-quality phase III trials (See Supplemental Methods S1)

189 comparing cyclophosphamide to ifosfamide in multi-drug combinations administered as first-

190 line treatment (Table-1). Sex was considered as a stratification variable in these three trials.

191   The dose ratio of ifosfamide/cyclophosphamide ranged from 4 to 5. In total, 1528 patients

192   were included, 773 in the cyclophosphamide arm and 755 in the ifosfamide arm. The EE99-

193   R1 trial represented 56% of the total number of patients. These trials were all conducted in

194   sarcomas (Ewing sarcoma, rhabdomyosarcoma and undifferentiated sarcomas). They included

195   children, adolescents and young adults, aged <15 years in 66% of the patients (Table-2).

196

197   **Survival analysis**

198   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

199   validation set containing EICESS92 and IRS-IV, respectively), we observed 424 disease

200   failures (i.e. PFS events: 224 and 200 in EE99-R1 and the validation set, respectively;

201   progression or relapse in 395 patients and death as first event in 29, including 6 treatment-

202   related deaths, 9 from disease progression, 9 other causes and 5 unknown causes). There were

203   325 deaths overall (171 and 154 in EE99-R1 and the validation set, respectively). The

204   estimated treatment-by-sex interaction on PFS in EE99-R1 (HR=1.73, 95%CI 1.00-3.00, p-

205   value=0.051) was not replicated in the validation set (n=672) using the one-stage model

206   (EICESS92+IRS-IV, HR=0.97, 95%CI 0.55-1.72, p=0.93, Figure-2), with no heterogeneity

207   between both trials (p=0.62). Interaction estimates were very similar in the sensitivity

208   analyses (Table-3). In the same way, the estimated treatment-by-sex interaction in EE99-R1

209   for OS (HR=1.85, 95%CI 0.98-3.48, p=0.056) was not replicated in the validation set

210   (HR=1.00, 95%CI 0.52-1.92, p=0.99, Supplemental Figure-1).

211   When the three RCTs were pooled, the estimated 5-year absolute PFS benefit associated with

212   ifosfamide compared to cyclophosphamide was greater among males +6.0% (73.7% vs

213   67.9%), than females (+0.2%, 75.2% vs 75.0%, Figure-3). However, the overall estimate of

214   treatment-by-sex interaction was not statistically significant (HR=1.31, 95%CI 0.89-1.95,

215   p=0.17). Although a significant treatment-by-sex interaction was observed in EE99-R1

216   (p=0.051), this interaction was not statistically different to interaction terms estimated in

217   EICESS92 and IRS-IV trials (p=0.36, Figure-2). This interaction estimate did not vary across

218   age categories (p=0.88, Supplemental Figure S2). The sensitivity analyses yielded similar

219   results (last column, Table-3). For OS (Supplemental Figure S3), the pooled estimate of the

220   treatment-by-sex interaction was not statistically significant (HR=1.37, 95%CI 0.87-2.15,

221   p=0.17). We observed neither heterogeneity across trials (p=0.35, Figure-4) nor across age

222   categories (p=0.64, Supplemental Figure S4). Stable results were observed in the sensitivity

223   analyses (Table-3).

224

225   **Toxicity analysis**

226   The frequencies of severe acute toxicities by sex and treatment arm are shown in

227   Supplemental Table S1. At least one episode of severe acute neutropenia, infection and renal

228   toxicity had occurred in 69.8%, 52.8% and 7.8% of patients, respectively. As illustrated in

229   Supplemental Figures S5-7, no significant interaction was identified between sex and

230   alkylating agent for leucopenia/neutropenia (OR=0.82, 95%CI 0.49-1.36, p=0.43), infection

231   (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).

232   These estimates did not significantly vary across trials (heterogeneity tests for

233   leucopenia/neutropenia: p=0.81, infection: p=0.12, and renal toxicity: p=0.19). The main

234   effects were reported because no interaction was found between treatment and sex. Compared

235   to ifosfamide, patients receiving cyclophosphamide experienced more severe

236   leucopenia/neutropenia ($OR_{cyclo\ vs\ ifo}$=1.47, 95%CI 1.14-1.88, p=0.003) and infections (OR_{cyclo}

237    $_{vs\ ifo}$=1.55, 95%CI 1.25-1.93, p<0.0001), but less renal toxicity ($OR_{cyclo\ vs\ ifo}$=0.71, 95%CI

238   0.48-1.06, p=0.098). Regardless of treatment arm, females developed significantly more

239   severe leucopenia/neutropenia ($OR_{female\ vs\ male}$=1.39, 95%CI 1.08-1.79, p=0.013) and

240   infections ($OR_{female\ vs\ male}$=1.25, 95%CI 1.01-1.56, p=0.041) than males, but not significantly

241   more severe renal toxicity ($OR_{female\ vs\ male}$=1.22, 95%CI 0.83-1.82, p=0.32).

242

243   **DISCUSSION**

244   Using an independent validation set of two RCTs (EICESS92 and IRS-IV), we did not

245   replicate the treatment-by-sex interactions observed in the EE99-R1 trial on PFS and OS. No

246   significant interactions were observed when the three trials were pooled, with no significant

247   heterogeneity across age and trials. Similarly, we did not identify any treatment-by-sex

248   interaction on leucopenia/neutropenia, infection and renal toxicity. Cyclophosphamide was

249   significantly more hemato-toxic (leucopenia/neutropenia and infections) than ifosfamide. We

250   also observed more hemato-toxicity in women than in males regardless of treatment arm.

251   This individual patient data meta-analysis is the first to assess a potential interaction between

252   the type of alkylating agent and sex. Based on high-quality RCTs comparing

253   cyclophosphamide to ifosfamide in both sex, with a total number of patients exceeding 1, 500

254   and long follow-up, it provides an unbiased estimate of the treatment-by-sex interaction.

255   Finally, even though the search was not restricted to age or to a specific type of cancer, these

256   three trials included mainly pediatric and young adult patients, with Ewing sarcoma or

257   rhabdomyosarcoma under first-line treatment. This probably reduces sources of heterogeneity

258   across trials (e.g. pharmacodynamic differences, co-morbidity, etc.).

259   The EORTC trial [27] which randomized cyclophosphamide and ifosfamide as a single drug

260   in advanced or metastatic soft-tissue sarcomas (n=135 patients) was not included in the

261   MAIAGE study due to the lack of availability of individual survival or toxicity data after

262   contacting the principal investigator. This study reported lower response rates in the

263   cyclophosphamide arm than in the ifosfamide arm, especially in males (observed response

264   rate of 0% and 11% in males treated with cyclophosphamide and ifosfamide, respectively,

265   and of 17% and 23% in females). Based on these data, we did not observe any significant

266   heterogeneity of the treatment effect between sex (interaction test: $p=0.12$). In the three other

267   randomized trials excluded (because they were based on women only, see Appendix) [28-30],

268   a better prognosis was reported in two, in subgroups of women treated with ifosfamide

269   [29,30] whereas the difference was not significant in the third trial.[28]

270   Our study had some limitations. First, none of the trials analyzed were initially designed to

271   study a treatment-by-sex interaction. Due to the observed number of events in each trial and

272   when pooled, the analyses could be underpowered to test the interaction with a standard

273   statistical level ($p<0.05$), let alone to detect heterogeneity of the treatment-by-sex interaction

274   across trials (e.g. infection analysis with marginal heterogeneity across trials, $p=0.12$).

275   Although we did not validate a treatment-by-sex interaction on efficacy outcomes, our results

276   do not conclusively rule out the existence of an interaction.

277   Second, in addition to the index trial, we identified only two other RCTs, which together

278   contributed less than 50% of the total number of patients. We did not identify any other study

279   comparing cyclophosphamide and ifosfamide, hence there is a paucity of independent trials.

280   Finally, differences in population characteristics and in drug combinations in the backbone

281   chemotherapy could impact the consistency of the estimates of treatment-by-sex interaction.

282   Indeed, (i) rhabdomyosarcoma patients in IRS-IV were younger than Ewing sarcoma patients

283   from the other two trials, and (ii) all IRS-IV patients received four additional courses with

284   cyclophosphamide after the first eight courses allocated by randomization; in contrast, all

285   patients also received ifosfamide as induction chemotherapy before randomization in both

286   Ewing sarcoma trials.

287   Our findings concerning acute toxicity are consistent with previous reports in sarcoma and

288   lymphoma patients treated with alkylating agents.[10–14] Differences in cytochrome P450-

289   mediated drug metabolism between sex could explain these results. Cyclophosphamide and

290   ifosfamide are oxazaphosphorine alkylating prodrugs that are metabolized via different P450-

291   catalyzed pathways: (i) 4-hydroxylation produces active alkylating agents and urotoxic

292   acrolein via CYP2B6 for cyclophosphamide and CYP3A4 and CYP3A5 for ifosfamide, and

293   (ii) N-dechloroethylation generates inactive metabolites and nephro- and neuro-toxic

294   chloroacetaldehyde via CYP3A4 for cyclophosphamide and, to a much greater extent,

295   CYP3A4 and CYP2B6 for ifosfamide.[31–33] Greater activity of CYP3A4 and CYP2B6 has

296   been reported in females resulting in higher concentrations of toxic chloroacetaldehyde after

297   ifosfamide infusion and consequently in a possible higher risk of severe neurotoxicity in

298   females.[34–36] However, no cytochrome P450-related difference in hematologic toxicity

299   between sex has previously been reported.

300   In conclusion, our meta-analysis did not show that the treatment effect of cyclophosphamide

301   versus ifosfamide is influenced by sex, for either efficacy or toxicity. Therefore,

302   recommending the choice of alkylating agent should not need be based on sex in children and

303   young adults treated for sarcoma. Additional studies would be useful for long-term follow-up

304   including fertility outcomes.

305

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441
442

443   **FIGURE LEGENDS**

444

445   **Figure 1: Flow chart of trial selection process.**

446

447   C=Cyclophosphamide, I=Ifosfamide, STS=Soft tissue sarcoma.

448   *The search strategy used the following search terms: "Ifosfamide"[Mesh] AND

449   "Cyclophosphamide"[Mesh] AND ("Randomized Controlled Trial" [Publication Type] OR

450   "Controlled Clinical Trial" [Publication Type]) in PubMed, "Ifosfamide" AND

451   "Cyclophosphamide" in the Cochrane Library, and "Ifosfamide" AND "Cyclophosphamide"

452   AND "Randomized" in the NIH clinical trials register (http://www.clinicaltrials.gov).

453   Notes: Euro-EWING99-R1 trial was not yet published when we conducted the systematic

454   review, that is why it does not appear in the initial systematic review box. Actualization of the

455   literature search in November 2016 did not identify any other trial fulfilling the inclusion

456   criteria.

457

458   **Figure 2: Forest plot of the hazard ratios (HR) of progression-free survival in the**

459   **cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex using fixed effects**

460   **model.**

461

462   The hazard ratios (HRs) given on the right side represent the HR of the treatment-by-sex

463   interaction (HRCyclo/Ifo in males/ HRCyclo/Ifo in females) estimated independently for each

464   trial, in the validation set and in the pooled dataset, by the one-stage model, stratified by trial

465   and sex, and including treatment (cyclophosphamide vs. ifosfamide) and age (< 12, 12-18,

466   and >18 years) as the main fixed effects. The heterogeneity of the interaction across trials was

467   assessed using a 3-order interaction term. The center of each square represents the HR for

468   individual trials and for the validation set (EICESS92 + IRS-IV) and the corresponding

469   horizontal line its 95% confidence interval (CI). The area of squares is proportional to the

470   amount of information obtained from the trial. The center of the black diamond represents the

471   overall HR and the extremities of the diamond represent its 95% CI, both estimated from the

472   pooled dataset.

473

474   **Figure 3: Stratified progression-free survival (PFS) curves according to sex and**

475   **alkylating agent (cyclophosphamide or ifosfamide) when the 3 RCTs were pooled**

476   **(n=1528).**

477

478   The 5-year absolute PFS benefit associated with ifosfamide (Ifo) compared to

479   cyclophosphamide (Cyclo) was estimated at 6% in males (73.7% vs. 67.9%), whereas females

480   receiving ifosfamide or cyclophosphamide had similar PFS (75.2% vs. 75.0%,

481   difference=0.2%).

482

483

484   **SUPPLEMENTAL MATERIAL LEGENDS**

485

486   **1.   Supplementary methods**

487   Supplemental Methods S1: Procedure of data checking

488   Supplemental Methods S2: Statistical methods for sensitivity analyses

489

490   **2.   Supplementary results of survival analyses**

491   Supplemental Figure S1: Forest plot of the hazard ratios (HR) of death (overall

492   survival) in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex

493   using fixed effects models.

494   Supplemental Figure S2: Forest plot of the hazard ratios (HR) of progression-free

495   survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex

496   for each age category (<12 years, 12-18 years, >18 years) using fixed effects models

497   when the 3 trials were pooled.

498   Supplemental Figure S3: Stratified overall survival (OS) curves according to sex and

499   alkylating agent (cyclophosphamide or ifosfamide) when the 3 trials were pooled.

500   Supplemental Figure S4: Forest plot of the hazard ratios (HR) of overall survival in the

501   cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex for each age

502   category (<12 years, 12-18 years, >18 years) using fixed effects models when the 3

503   trials were pooled.

504

505   **3.   Detailed results of toxicity analyses**

506   Supplemental Table S1: Number of patients in each trial who experienced at least one

507   episode of severe acute toxicity by sex and by treatment arm.

508   Supplemental Figure S5: Forest plot of the odd ratios (OR) of leucopenia/neutropenia

509   in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the

510   3 trials were pooled.

511   Supplemental Figure S6: Forest plot of the odd ratios (OR) of infection in the

512   cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3

513   trials were pooled.

514   Supplemental Figure S7: Forest plot of the odd ratios (OR) of renal toxicity in the

515   cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3

516   trials were pooled.

517

518   **4.   Description of the randomized controlled trials comparing alkylating agents, not**

519   **included in the meta-analysis**

520   Supplemental Table S2: Information extracted from the 3 randomized trials conducted

521   in women and not included in the meta-analysis

Databases: PubMed, Cochrane library and NIH clinical trials register
Publication dates: 1980/01/01 –2013/12/31
Publication types: Randomized Controlled Trial and Controlled Clinical Trial
N= 380 articles (Pubmed/Cochrane) and 37 trials (NIH register)*

PubMed and Cochrane library:

N= 371 excluded articles

- 1 randomizing C *vs.* I
  but only one course of C or I.
- 3 randomizing C *vs.* I in women only
- 13 randomizing a CT regimen with C
  vs. another regimen with I
- 14 with C and I in the 2 arms
- 19 with C in the 2 arms
- 3 with I in the 2 arms
- 287 without C
- 4 without I
- 7 without C and I
- 6 without CT randomization
- 14 without any randomization

NIH clinical trial register:

N= 36 excluded trials

-3 randomizing a CT regimen with C
  vs. another regimen with I
-16 with C and I in the 2 arms
-5 with C in the 2 arms
-4 with I in the 2 arms
-1 without C
-1 without I
-5 without CT randomization
-1 without any randomization

9 articles (Pubmed/Cochrane) and 1 NIH reference
corresponding to 3 trials:
EICESS 92, IRS-IV, EORTC STS

No survival data available for EORTC STS

Euro-EWING99-R1 trial

3 trials included in the meta-analysis:
Euro-EWING99, EICESS 92, IRS-IV

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). Notes: 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.

190x142mm (300 x 300 DPI)
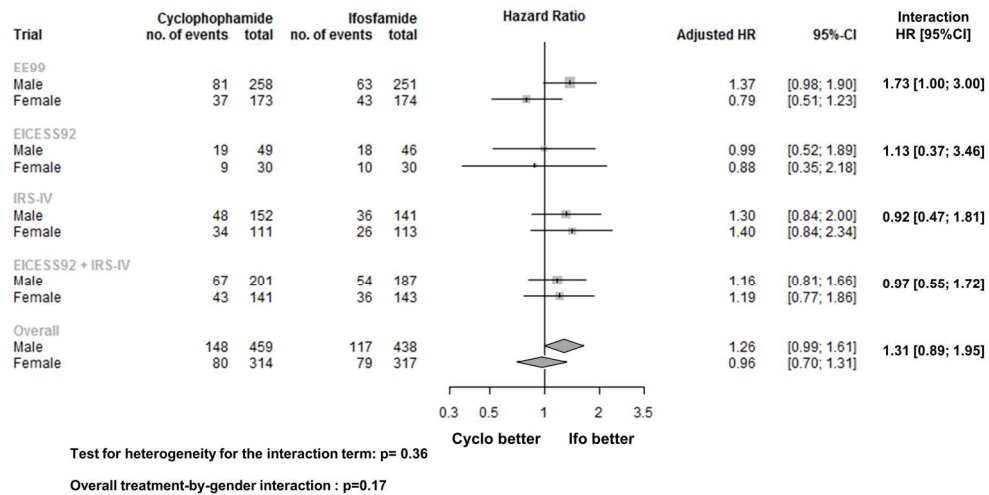
Figure 2: Forest plot of the hazard ratios (HR) of progression-free survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by gender using fixed effects model.

The hazard ratios (HRs) given on the right side represent the HR of the treatment-by-gender 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 gender, 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 3-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.

190x142mm (300 x 300 DPI)
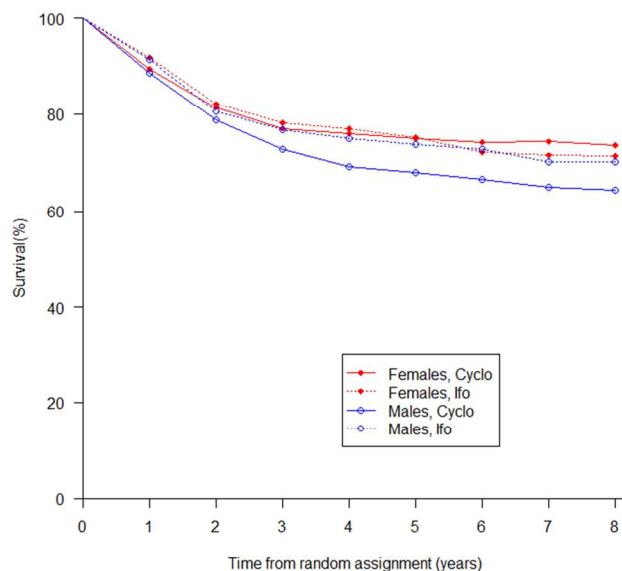
No. of deaths / person-years by period

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 gender and alkylating agent (cyclophosphamide or ifosfamide) when the 3 RCTs were pooled (n=1528).

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%).

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

Trial[ref]	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† analysis
					Pathology‡	Primary tumor site	Age (years)		Ifo (dose/3w)	Cyclo (dose/3w)		
EE99-R1[1]	2000-2010	Multicentric Phase III Non-inferiority	856	5.9 [3.8; 8.0]	EWS	Bone or soft tissue	< 50	Localized tumors With a good response to preoperative CT*	7 VAI (3 g/m²x2)	7 VAC (1.5 g/m²x1)	3y-EFS	78% (VAI) 75% (VAC)
EICESS92[25]	1992-1999	Multicentric Phase III Non-inferiority	155	8.3 [6.9; 10.6]	ESFT	Bone	< 35	Localized tumors of less than 100mL	10 VAIA (2 g/m²x3)	10 VACA (1.2 g/m²x1)	3y-EFS	74% (VAIA) 73% (VACA)
IRS-IV[26]	1991-1997	Multicentric Phase III Superiority	517	8.0 [5.5; 9.9]	RMS, undifferentiated sarcoma	Soft tissue	< 21	Localized tumors**	8 VAI ◊ (1.8 g/m²x5)	8 VAC (2.2 g/m²x1)	3y-EFS	77% (VAI) 73% (VAC)

N: number of randomized patients, Cyclo: cyclophosphamide, Ifo: Ifosfamide, CT : chemotherapy, VAI : vincristine, dactinomycin, ifosfamide, VAC: vincristine, dactinomycin, cyclophosphamide, VAIA: vincristine, dactinomycin, ifosfamide, adriamycin, VACA: vincristine, dactinomycin, cyclophosphamide, adriamycin, EFS: event-free survival, Q1: first quartile, Q3: third quartile.

‡: EWS: Ewing sarcoma, ESFT: Ewing sarcoma family of tumors, RMS: rhabdomyosarcoma.

†: Intention to Treat. , w: week, y: year

* 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.

** after exclusion of patients with completely resected paratesticular tumors, completely resected or microscopic residual disease of orbit or eyelid tumors, pre-existing 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

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

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

HR: hazard ratio of the treatment-by-gender interaction term (HR Cyclo vs. Ifo in males / HR Cyclo vs. Ifo in Females)

95%CI: 95% Confidence Interval

OSM: one-stage model; PWT: pooling of within-trial covariate interactions model; age category: <12 years, [12-18] years and >18 years

Supplementary material

1. **Supplementary methods**

   Supplemental Methods S1: Procedure of data checking

   Supplemental Methods S2: Statistical methods for sensitivity analyses

2. **Supplementary results of survival analyses**

3. **Detailed results of toxicity analyses**

4. **Description of the randomized controlled trials comparing alkylating agents, not included in the meta-analysis**

1. **Supplementary methods**

Supplemental Methods S1: Procedure of data checking

We have checked the data according to a standardized procedure[1]. Missing values and discrepancies were discussed with the trialists. Randomization validity was assessed by checking the patterns of treatment allocation and the balance in baseline characteristics between treatment groups. Definition of population set was evaluated for each trial to perform the meta-analysis according to the intention-to-treat principle. Patients follow-up was also compared between treatment groups. Each trial was then reanalyzed and the analyses were sent to the trialists for validation.

A. Randomization validity

Curves representing cumulative accrual were plotted and compared between treatment arms: no bias was observed. Among the selected trials, an imbalance between the baseline characteristics of the treatment arms was not detected (See Table 2).

B. Definition of the population sets

Respect of the intention-to-treat principle was requested for randomized trials even if some patients were excluded in the initial analyses of the trial. Overall, 65 randomized patients had been excluded in the initial trial publications, all in the IRS-IV trial. These 65 patients were included in the meta-analysis.

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[1] Stewart LA, Clarke MJ on behalf of the Cochrane Working Group on meta-analyses using individual patient data. Practical methodology of meta-analyses (overviews) using updated individual patients data. Stat Med 1995;14:2057-2079.

C. Follow-up

For each treatment arm, reverse Kaplan-Meier curves were plotted: no bias was observed. Median follow-up was 6.8 years [Q1:4.5; Q3:8.9] in the pooled dataset and there was no difference between treatment arms within each trial (EE99-R1: 5.9 and 6.0 for VAC and VAI, respectively. EICESS92: 8.2 and 8.3 for VAIA and VACA, respectively. IRS-IV: 7.7 and 8.1 for VAI and VAC, respectively).

Supplemental Methods S2: Statistical methods for sensitivity analyses

Several pre-specified sensitivity analyses were performed:

(i)   The addition of a study-specific random component for the treatment effect in the one-stage method (OSM);

(ii)   The impact of a misspecification of age was evaluated by including age as a continuous covariate in the OSM;

(iii)   We used the "two-stage" approach to assess the overall treatment-by-sex interaction ("pooling within-trial covariate interactions" method, PWT).[20] We estimated interaction coefficients independently within each trial using multivariable Cox regression models, and then pooled them using the inverse-variance technique with fixed effects.[37]

1. Supplementary results of survival analyses

Supplemental Figure S1: Forest plot of the hazard ratios (HR) of death (overall survival) in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex using fixed effects models.

Trial	Cyclophophamide no. of events	total	Ifosfamide no. of events	total	Hazard Ratio	Adjusted HR	95%-CI	Interaction HR [95%CI]
EE99								
Male	61	258	49	251		1.30	[0.89; 1.90]	1.85 [0.98; 3.48]
Female	27	173	34	174		0.70	[0.42; 1.17]	
EICESS92								
Male	15	49	12	46		1.24	[0.58; 2.65]	1.31 [0.33; 5.20]
Female	6	30	6	30		0.94	[0.30; 2.96]	
IRS-IV								
Male	38	152	31	141		1.20	[0.74; 1.93]	0.91 [0.43; 1.93]
Female	26	111	20	113		1.32	[0.74; 2.37]	
EICESS92 + IRS-IV								
Male	53	201	43	187		1.18	[0.79; 1.77]	1.00 [0.52; 1.92]
Female	32	141	26	143		1.18	[0.71; 1.99]	
Overall								
Male	114	459	92	438		1.24	[0.94; 1.63]	1.37 [0.87; 2.15]
Female	59	314	60	317		0.90	[0.63; 1.29]	

Hazard Ratio axis: 0.3 0.5 1 2 3.5
Cyclo better   Ifo better

Test for heterogeneity for the interaction term: p= 0.35

Overall treatment-by-gender interaction : p=0.17

Supplemental Figure S2: Forest plot of the hazard ratios (HR) of progression-free survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex for each age category (<12 years, 12-18 years, >18 years) using fixed effects models when the 3 trials were pooled.

Trial	Cyclophophamide no. of events	total	Ifosfamide no. of events	total	Adjusted HR	95%-CI	Interaction HR [95%CI]
<12 years							
Male	54	214	38	204	1.41	[0.93; 2.14]	1.30 [0.71; 2.36]
Female	42	162	41	177	1.09	[0.71; 1.68]	
12-18 years							
Male	51	143	42	135	1.20	[0.79; 1.80]	1.24 [0.61; 2.52]
Female	23	97	23	96	0.97	[0.54; 1.73]	
>18 years							
Male	43	102	37	99	1.18	[0.76; 1.83]	1.62 [0.70; 3.77]
Female	15	55	15	44	0.73	[0.35; 1.49]	

Hazard Ratio

0.3   0.5   1   2   3.5

Cyclo better   Ifo better

Test for heterogeneity for the interaction term: p= 0.88

5

Supplemental Figure S3: Stratified overall survival (OS) curves according to sex and alkylating agent (cyclophosphamide or ifosfamide) when the 3 trials were pooled.
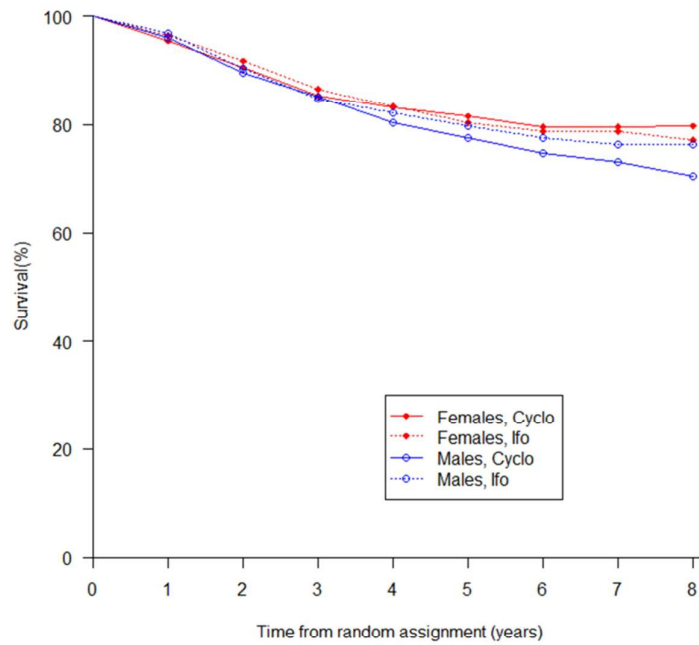
No. of deaths / person-years by period

Females-Ifo	11/303	14/281	14/248	8/211	7/184	3/150	0/123	2/97
Females-Cyclo	14/304	15/283	15/257	6/220	4/196	4/172	0/145	0/115
Males-Ifo	14/427	27/398	23/357	10/313	8/270	6/232	3/186	0/139
Males-Cyclo	18/439	28/402	18/358	18/314	10/273	8/230	4/184	5/137

The 5-year absolute OS benefit associated with ifosfamide (Ifo) compared to cyclophosphamide (Cyclo) was estimated at +2.2% in males (79.7% vs. 77.5%) and -1.1% in females (80.4% vs. 81.5%).

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Supplemental Figure S4: Forest plot of the hazard ratios (HR) of overall survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex for each age category (<12 years, 12-18 years, >18 years) using fixed effects models when the 3 trials were pooled.

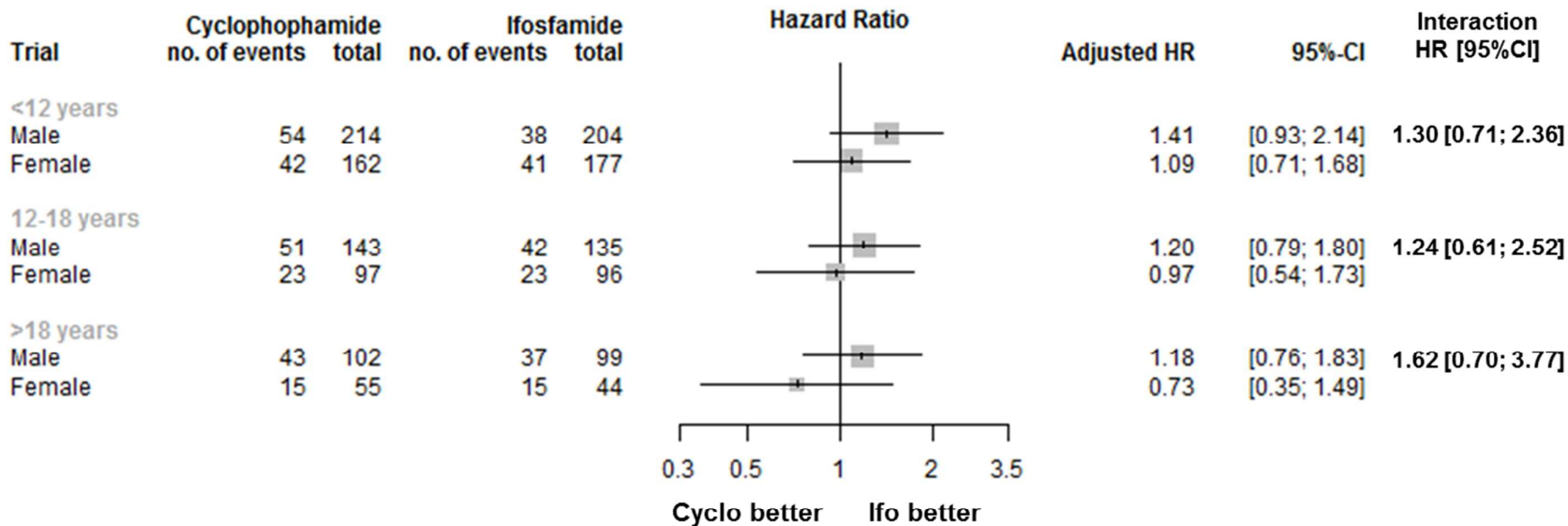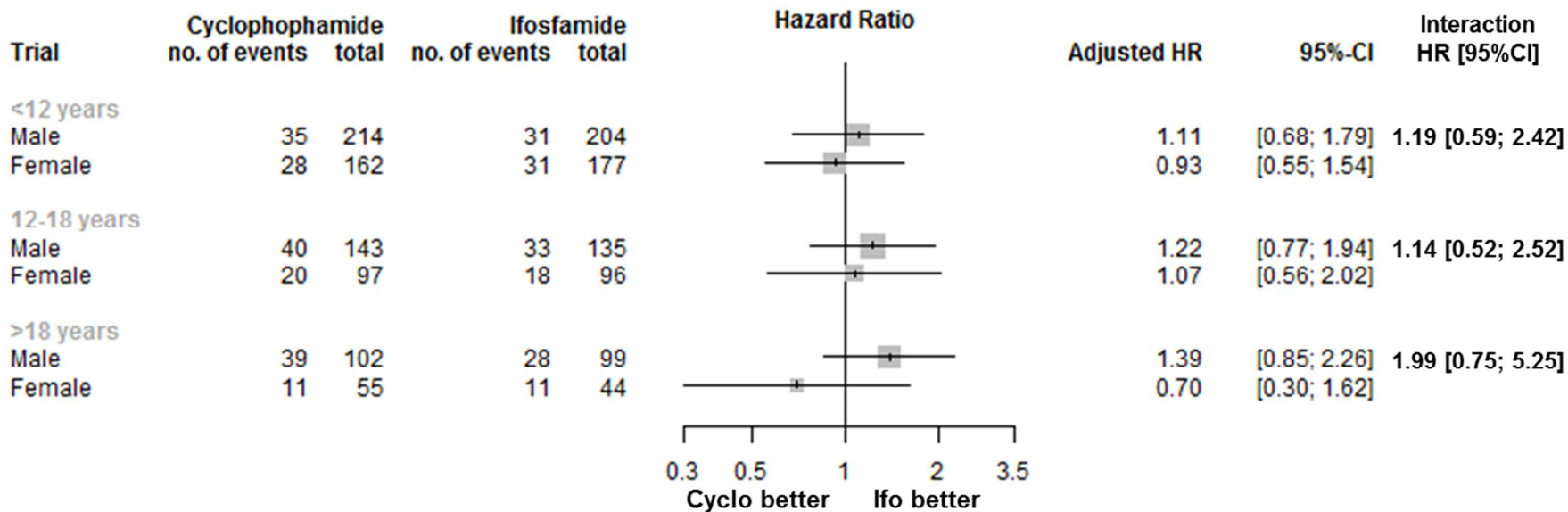Test for heterogeneity for the interaction term: p= 0.64

2. Detailed results of toxicity analyses

Supplemental Table S1: Number of patients in each trial who experienced at least one episode of severe acute toxicity by sex and by treatment arm.

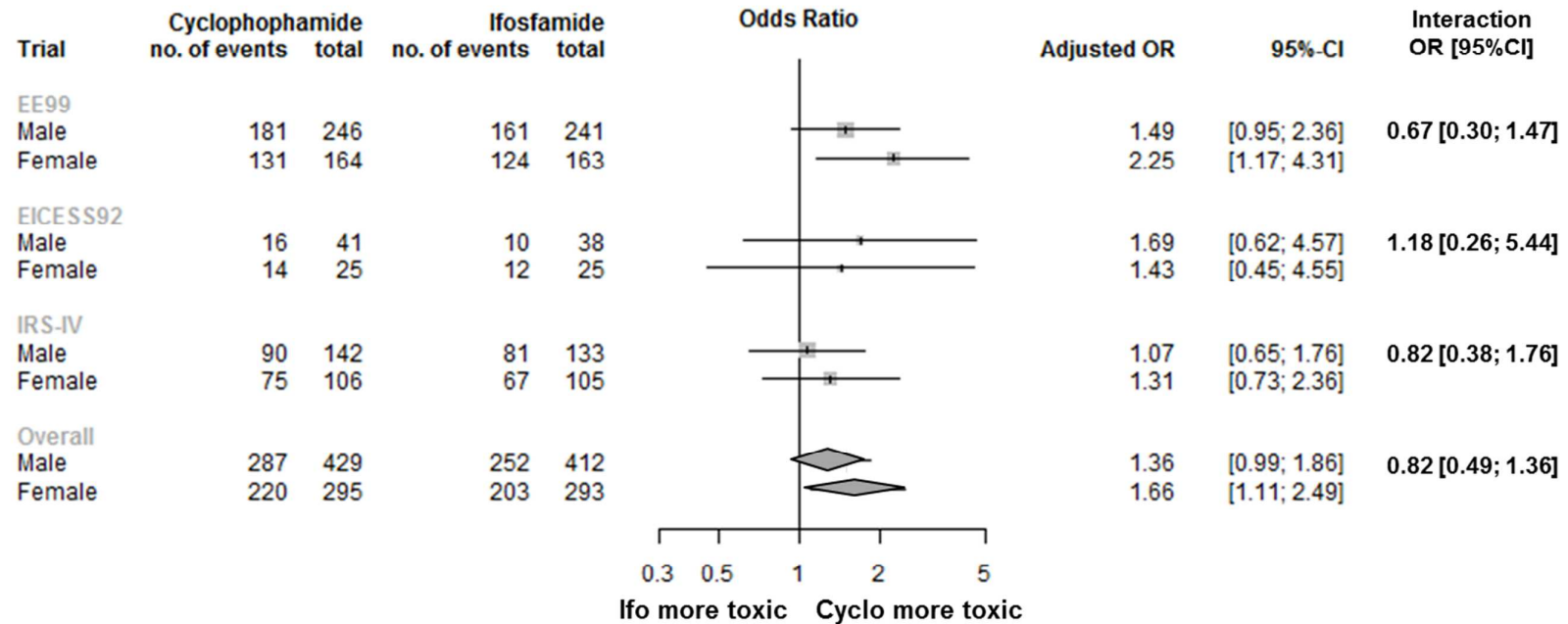| Acute toxicity | Sex | Treatment | Number of patients with acute toxicity / number of patients with available information (%) | | | | | | | | | | | |
			EE99-R1 (n=814)				EICESS92 (n=129)				IRS-IV (n=486)			
Leucopenia/neutropenia	Female	VAC	131	/	152	(86.2)	14	/	25	(56.0)	75	/	106	(70.8)
		VAI	124	/	155	(80.0)	12	/	25	(48.0)	67	/	105	(63.8)
	Male	VAC	181	/	234	(77.4)	16	/	40	(40.0)	90	/	142	(63.4)
		VAI	161	/	225	(71.6)	10	/	37	(27.0)	81	/	133	(60.9)
Infection	Female	VAC	90	/	161	(55.9)	13	/	25	(52.0)	73	/	106	(68.9)
		VAI	89	/	161	(55.3)	9	/	25	(36.0)	56	/	105	(53.3)
	Male	VAC	127	/	246	(51.6)	20	/	40	(50.0)	87	/	142	(61.3)
		VAI	88	/	240	(36.7)	15	/	37	(40.5)	75	/	133	(56.4)
Renal toxicity*	Female	VAC	8	/	160	(5.0)	5	/	24	(20.8)	5	/	106	(4.7)
		VAI	22	/	160	(13.8)	6	/	25	(24.0)	5	/	105	(4.8)
	Male	VAC	13	/	246	(5.3)	9	/	40	(22.5)	7	/	142	(4.9)
		VAI	12	/	239	(5.0)	5	/	38	(13.2)	13	/	133	(9.8)
Thrombocytopenia	Female	VAC	79	/	161	(49.1)	4	/	25	(16.0)	57	/	106	(53.8)
		VAI	72	/	161	(44.7)	0	/	25	(0.0)	38	/	105	(36.2)
	Male	VAC	102	/	245	(41.6)	4	/	40	(10.0)	70	/	142	(49.3)
		VAI	64	/	241	(26.6)	1	/	37	(2.7)	36	/	133	(27.1)
Mucositis	Female	VAC	6	/	160	(3.8)	3	/	25	(12.0)	59	/	106	(55.7)
		VAI	6	/	160	(3.8)	0	/	24	(0.0)	40	/	105	(38.1)
	Male	VAC	5	/	246	(2.0)	3	/	39	(7.7)	50	/	142	(35.2)
		VAI	5	/	240	(2.1)	2	/	37	(5.4)	55	/	133	(41.4)
Diarrhea	Female	VAC	1	/	160	(0.6)	1	/	12	(8.3)	18	/	106	(17.0)
		VAI	5	/	160	(3.1)	0	/	14	(0.0)	9	/	105	(8.6)
	Male	VAC	4	/	246	(1.6)	1	/	23	(4.3)	18	/	142	(12.7)
		VAI	1	/	240	(0.4)	0	/	26	(0.0)	12	/	133	(9.0)
Liver toxicity	Female	VAC	7	/	160	(4.4)	1	/	25	(4.0)	15	/	106	(14.2)
		VAI	11	/	159	(6.9)	2	/	24	(8.3)	9	/	105	(8.6)
	Male	VAC	15	/	245	(6.1)	3	/	38	(7.9)	23	/	142	(16.2)
		VAI	9	/	239	(3.8)	0	/	37	(0.0)	8	/	133	(6.0)
Central neurologic toxicity	Female	VAC	1	/	160	(0.6)	2	/	24	(8.3)	5	/	106	(4.7)
		VAI	4	/	160	(2.5)	0	/	24	(0.0)	7	/	105	(6.7)
	Male	VAC	2	/	244	(0.8)	0	/	39	(0.0)	7	/	142	(4.9)
		VAI	3	/	240	(1.3)	0	/	36	(0.0)	6	/	133	(4.5)
Peripheral neurologic toxicity	Female	VAC	11	/	159	(6.9)	3	/	25	(12.0)	26	/	106	(24.5)
		VAI	15	/	159	(9.4)	1	/	24	(4.2)	25	/	105	(23.8)
	Male	VAC	17	/	245	(6.9)	3	/	39	(7.7)	35	/	142	(24.6)
		VAI	8	/	240	(3.3)	2	/	37	(5.4)	34	/	133	(25.6)
Cardiac toxicity	Female	VAC	3	/	133	(2.3)	5	/	23	(21.7)	2	/	106	(1.9)
		VAI	9	/	143	(6.3)	4	/	22	(18.2)	2	/	105	(1.9)
	Male	VAC	6	/	210	(2.9)	8	/	36	(22.2)	3	/	142	(2.1)
		VAI	6	/	208	(2.9)	8	/	33	(24.2)	1	/	133	(0.8)

VAI: vincristine, dactinomycin, ifosfamide, VAC: vincristine, dactinomycin, cyclophosphamide

Adverse events were evaluated using the NCI CTCAE-v2 scale in the EE99-R1 and EICESS92 trials, and NCI CTCAE-v1 scale in the IRS-IV trial.

*Severe renal toxicity (grade 2 or more): at least one episode of increased plasmatic creatinine > 1.5 baseline, or a glomerular filtration rate decrease <60ml/min/1.73m² or a tubular phosphate reabsorption decrease <80%.

Supplemental Figure S5: Forest plot of the odd ratios (OR) of leucopenia/neutropenia in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.

Trial	Cyclophosphamide no. of events	total	Ifosfamide no. of events	total	Odds Ratio	Adjusted OR	95%-CI	Interaction OR [95%CI]
EE99								
Male	181	246	161	241		1.49	[0.95; 2.36]	0.67 [0.30; 1.47]
Female	131	164	124	163		2.25	[1.17; 4.31]	
EICESS92								
Male	16	41	10	38		1.69	[0.62; 4.57]	1.18 [0.26; 5.44]
Female	14	25	12	25		1.43	[0.45; 4.55]	
IRS-IV								
Male	90	142	81	133		1.07	[0.65; 1.76]	0.82 [0.38; 1.76]
Female	75	106	67	105		1.31	[0.73; 2.36]	
Overall								
Male	287	429	252	412		1.36	[0.99; 1.86]	0.82 [0.49; 1.36]
Female	220	295	203	293		1.66	[1.11; 2.49]	

0.3 0.5   1   2   5

Ifo more toxic   Cyclo more toxic

Test for heterogeneity for the interaction term: p= 0.81

Overall treatment-by-gender interaction : p=0.43

The Odd Ratios (ORs) given on the right side represent the OR of the treatment-by-sex interaction ($OR_{Cyclo/Ifo \ in \ males}$/ $OR_{Cyclo/Ifo \ in \ females}$) estimated independently for each trial and in the pooled dataset, using the logistic regression 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. Heterogeneity of the interaction (treatment x sex) across trials was assessed using the 3-order interaction term.

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Supplemental Figure S6: Forest plot of the odd ratios (OR) of infection in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.

Trial	Cyclophophamide no. of events	total	Ifosfamide no. of events	total	Odds Ratio	Adjusted OR	95%-CI	Interaction OR [95%CI]
EE99								
Male	127	246	88	241		1.95	[1.34; 2.84]	1.65 [0.92; 2.96]
Female	90	164	89	163		1.19	[0.75; 1.87]	
EICESS92								
Male	20	41	15	38		1.47	[0.59; 3.66]	0.83 [0.19; 3.59]
Female	13	25	9	25		1.78	[0.57; 5.60]	
IRS-IV								
Male	87	142	75	133		1.23	[0.75; 2.00]	0.65 [0.31; 1.37]
Female	73	106	56	105		1.89	[1.07; 3.34]	
Overall								
Male	234	429	178	412		1.62	[1.22; 2.14]	1.11 [0.71; 1.71]
Female	176	295	154	293		1.46	[1.04; 2.05]	

0.3 0.5 1 2 7

Ifo more toxic   Cyclo more toxic

Test for heterogeneity for the interaction term: p= 0.12

Overall treatment-by-gender interaction : p=0.65

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Supplemental Figure S7: Forest plot of the odd ratios (OR) of renal toxicity in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.

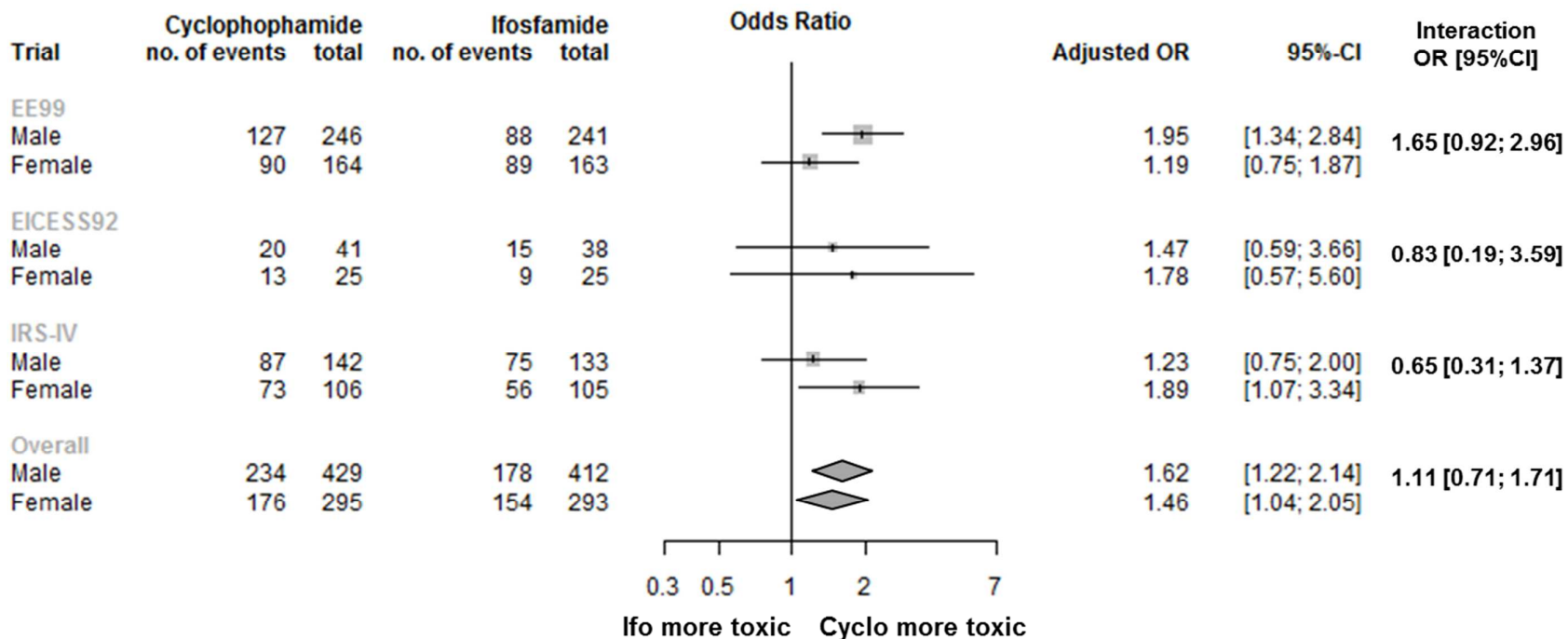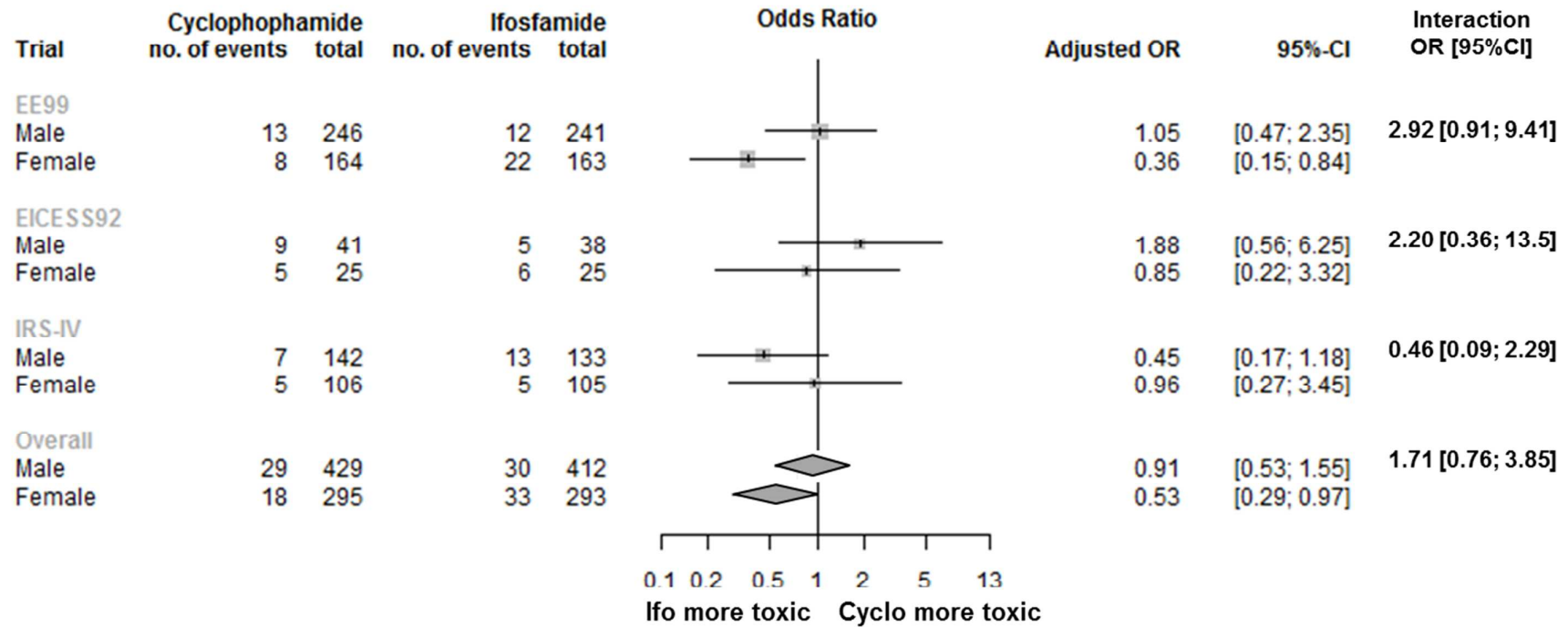| Trial	Cyclophophamide no. of events	total	Ifosfamide no. of events	total	Odds Ratio	Adjusted OR	95%-CI	Interaction OR [95%CI]
EE99								
Male	13	246	12	241		1.05	[0.47; 2.35]	2.92 [0.91; 9.41]
Female	8	164	22	163		0.36	[0.15; 0.84]	
EICESS92								
Male	9	41	5	38		1.88	[0.56; 6.25]	2.20 [0.36; 13.5]
Female	5	25	6	25		0.85	[0.22; 3.32]	
IRS-IV								
Male	7	142	13	133		0.45	[0.17; 1.18]	0.46 [0.09; 2.29]
Female	5	106	5	105		0.96	[0.27; 3.45]	
Overall								
Male	29	429	30	412		0.91	[0.53; 1.55]	1.71 [0.76; 3.85]
Female	18	295	33	293		0.53	[0.29; 0.97]	

0.1 0.2   0.5 1   2   5   13

Ifo more toxic   Cyclo more toxic

Test for heterogeneity for the interaction term: p= 0.19

Overall treatment-by-gender interaction : p=0.19

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3. **Description of the randomized controlled trials comparing alkylating agents, not included in the meta-analysis**

Supplemental Table S2: Information extracted from the 3 randomized trials conducted in women and not included in the meta-analysis

Author	Pathology	Treatment arms	Number of patients	Response rate (CR or PR)	Progression-free survival (PFS)[‡]			Overall survival (OS)
Buzdar [28]	Breast carcinoma	FAC+BCG+levamisole	117	72.6%	Median time to progression: 17 months			Median OS: 21.4 months
		FAI+BCG+levamisole	49*	65.3%	Median time to progression: 17.8 months			Median OS: 23.5 months
Nishida [29]	Ovarian epithelial cancer	PAC	53	NA	3y-PFS: 84.9%	5y-PFS: 79.0%	10y-PFS: 67.8%	NA
		PAI	52	NA	3y-PFS: 88.5%	5y-PFS: 88.5%	10y-PFS: 81.1%	NA
Pawinski [30]	Adenocarcinoma of uterine corpus	Cyclo	29	6.9%	Median time to progression: 7 weeks			NA
		Ifo	32	12.5%	Median time to progression: 8 weeks			NA

FAC: 5-fluorouracil, adriamycin, cyclophosphamide, FAI: 5-fluorouracil, adriamycin, cyclophosphamide, PAC: cisplatin, epirubicin, cyclophosphamide, PAI: cisplatin, epirubicin, ifosfamide, Cyclo: cyclophosphamide, Ifo: ifosfamide, CR: complete response, PR: partial response, NA: not available
* The FAI arm was closed because of increased bladder toxicity observed with ifosfamide resulting in a greater number of patients in the FAC arm.
‡: no information on the precision of the estimate (standard error, confidence interval or number of at-risk patients) was reported.

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