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Impact of gender on efficacy and acute toxicity of alkylating agent -based chemotherapy in Ewing sarcoma: Secondary analysis of the Euro-Ewing99-R1 trial

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Abstract ***Background:*** Based on the randomised Euro-EWING99-R1 trial, vincristine, adriamycin, cyclophosphamide (VAC) may be able to replace vincristine, adriamycin, ifosfamide (VAI) in the treatment of standard-risk Ewing sarcoma. However some heterogeneity of treatment effect by gender was observed. The current exploratory study aimed at investigating the influence of gender on treatment efficacy and acute toxicity.

Patients and methods: Impact of gender on event-free survival (EFS), acute toxicity by course, switches between treatment arms and cumulative dose of alkylating agents was evaluated in multivariable models adjusted for age including terms to test for heterogeneity of treatment effect by gender. The analysis of the EFS was performed on the intention-to-treat population.

Results: EFS did not significantly differ between the 509 males and 347 females ($p = 0.33$), but an interaction in terms of efficacy was suspected between treatment and gender ($p = 0.058$): VAC was associated with poorer EFS than VAI in males, hazard ratio (HR) (VAC/VAI) = 1.37 [95% confidence interval (CI), 0.98–1.90], contrasting with HR = 0.81 [95%CI, 0.53–1.24] in females. Severe toxicity was more frequent in females, whatever the toxicity type. Thirty patients switched from VAI to VAC (9/251 males, 4%, and 21/174 females, 12%) mostly due to renal toxicity, and three from VAC to VAI (2/258 males, 0.8%, and 1/173 females, 0.6%). A reduction of alkylating agent cumulative dose >20% was more frequent in females (15% versus 9%, $p = 0.005$), with no major difference between VAC and VAI (10% versus 13%, $p = 0.15$).

Conclusion: Differences of acute toxicity rate and cumulative doses of alkylating agents could not explain the marginal interaction observed in the Euro-EWING99-R1 trial data. Effects of gender-dependent polymorphism/activity of metabolic enzymes (e.g. known for CYP2B6) of ifosfamide versus cyclophosphamide should be explored. External data are required to further evaluate whether there is heterogeneity of alkylating agent effect by gender.

Trial numbers: NCT00987636 and EudraCT 2008-003658-13.

1. Introduction

Alkylating agents ifosfamide and/or cyclophosphamide are part of the chemotherapeutic regimens in all studies of Ewing sarcoma treatment. A beneficial synergistic effect of ifosfamide with other chemotherapeutic agents has been published [1–3]. However, data on the value of ifosfamide as compared to cyclophosphamide are not consistent. We previously reported on the randomised Euro-E.W.I.N.G.99-R1 trial, an international trial comparing non-inferiority of cyclophosphamide versus ifosfamide-based consolidation regimens given after an intense induction chemotherapy, in standard-risk localised Ewing sarcoma (NCT00020566) [4]. Overall, it was concluded that cyclophosphamide might be able to replace ifosfamide in consolidation treatment of standard-risk Ewing sarcoma. However, some uncertainty surrounding non-inferiority of vincristine (V), actinomycin D (A), cyclophosphamide (C) compared to VA-Ifosfamide (I) remained at this stage, in particular because of a trend towards a benefit in favour of ifosfamide for male patients [4]. With a *p*-value equal to 0.083 in the main analysis, the interaction between treatment and gender was considered to be a marginal interaction. However, interaction tests are known to be underpowered and it is common practice to use a threshold of 0.10 for interaction tests. If alkylating treatment effect truly varies according to gender, such differences in efficacy, if proven, would have a major impact on future clinical practice. The current manuscript reports an exploratory analysis performed to investigate the influence of gender on treatment efficacy and acute toxicity and to determine whether age was a possible confounder.

2. Methods

2.1. Study design

The Euro-E.W.I.N.G.99-R1 trial was a two-parallel group non-inferiority randomised trial of two different consolidation regimens, conducted in 202 European paediatric and adult oncology centres in 13 countries, via four national or international cooperative groups; i.e. GPOH (German Paediatric Oncology and Hematology), EORTC (European Organization for Research and Treatment of Cancer), CCLG (Children's Cancer and Leukaemia Group, United Kingdom (UK)) and the French groups (i.e. SFCE, GSF-GETO (Groupe Sarcomes Français), part of French EORTC centres).

Eligible patients were less than 50 years of age, with a localised, biopsy-proven Ewing sarcoma, classified as standard-risk disease, i.e. either good histological response to pre-operative treatment (<10% viable tumour cells), and/or small tumour (<200 mL) resected at diagnosis or with radiotherapy alone as local treatment. Appropriate ethics committees approved the trial in accordance with legislation in each country.

Induction chemotherapy was similar in all patients and consisted of six courses of VI, doxorubicin (D), etoposide (E) (VIDE) [5,6]. After local treatment (surgery, radiotherapy or both), if carried out at this time, the first consolidation course was common to both arms and consisted of VAI (vincristine, adriamycin, ifosfamide), after which treatment was allocated by randomisation either to seven courses of VAI with ifosfamide (3 g/m^2/day) on Day 1 and Day 2, or to seven courses of VAC (vincristine, adriamycin, cyclophosphamide) with cyclophosphamide (1.5 g/m^2/day) on Day −1. The total dose of alkylating agents over the consolidation treatment was 48 g/m^2 ifosfamide in the VAI-arm versus 6 g/m^2 ifosfamide plus 10.5 g/m^2 cyclophosphamide in the VAC arm, which were presumed to be equivalent. Randomisation was balanced and stratified according to age, gender, cooperative group and local treatment. The sample size of the EE99-R1 trial (N = 856) was calculated to test the non-inferiority of VAC compared to VAI.

2.2. Statistical analyses

In the main analysis the primary end-point to evaluate the impact of treatment was event-free survival (EFS), defined as the time from randomisation to first event (relapse, second malignancy or death whatever the cause). At the cut-off date, 1st June 2012, the median follow-up of the study population was 6.0 years. The hazard ratio (HR) of failure associated with treatment effect (VAC versus VAI) was estimated with its 95% confidence interval in a multivariable Cox regression model controlling for age, gender, cooperative group and local treatment. In the previously published analysis, age was included in the model as a binary variable corresponding to the randomisation strata (< versus ⩾25 years).

In the current exploratory analysis, the multivariable Cox models included treatment, gender and age as main effects, and were stratified by cooperative group and local treatment (surgery after VIDE induction with or without postoperative radiotherapy/initial surgery of primary/definitive radiotherapy). As age could be a confounding factor and misspecification of a continuous co-variable may impact the validity and accuracy of model-based estimates; we examined different codings of the age, i.e. binary </⩾25, three strata centred on puberty, or as a continuous variable (see Appendix for

more details) [7,8], (see Appendix for more details). We then estimated the HR of treatment effect by gender, by adding an interaction term to the model selected at the previous step. In order to evaluate whether the treatment by gender interaction estimate varied with age, we also tested a third order interaction, treatment × gender × age. The analysis of the EFS was performed on the intention-to-treat population including all patients by allocated treatment group (N = 856).

The impact of gender on acute toxicity following consolidation chemotherapy was investigated as well. VIDE induction chemotherapy courses were not considered in the analysis as they were administered before randomisation, and similarly in both randomised groups acute toxicity was assessed after each course, using a list of 21 selected items from the NCI-CTC-v2.0. [9]. Grade 4 haematological, grade >2 infection, renal, cardiac or neurological and grade >3 of all other extra-haematological toxicities were considered as severe. Toxicity items were also pooled by body system. For each toxicity item, a logistic regression was used to model the risk of severe toxicity by treatment (VAC versus VAI), gender (males versus females), age (< versus ⩾25 years) and cooperative group, including an interaction term between treatment and gender. Initially we analysed toxicity data at the patient level, considering the maximum grade for each patient over the whole consolidation treatment, and then at the level of the chemotherapy courses, using a Generalised Estimated Equation approach to take into account repeated observations per patient [10]. In the case of insufficient data, we used Firth's approach to reduce the small sample size bias [11]. To account for multiple comparisons, we set the p-value threshold at 0.05 for significant interaction tests. In this toxicity analysis, we censored courses administered after a switch from one treatment arm to the other.

As the dose of alkylating agent was collected for each course, we computed the cumulative dose of alkylating agents per metre-square over the whole consolidation treatment. We calculated the weighted sum of both agents, assuming equivalence of 4 g/m^2 ifosfamide and 1 g/m^2 cyclophosphamide. The dose reduction compared to the expected cumulative dose of ifosfamide, 48 g/m^2, was then modelled using an analysis of variance. A dose reduction greater than 20% was also analysed using a logistic regression model. These analyses were adjusted for age and included patients who switched from one treatment arm to the other in the group they had initially been allocated to. Patients who prematurely stopped treatment because of progression or death were excluded from this analysis.

All estimates are given with their 95% confidence intervals [95% CI] and tested with two-sided tests. Statistical analyses were performed using SAS Software 9.3.

3. Results

Overall, 856 patients (509 males, 59%; 347 females, 41%) were recruited between 2000 and 2010: 425 VAI (251 males, 174 females) and 431 VAC (258 males, 173 females). Three years EFSs were 75.4% and 78.2% for VAC and VAI treated patients, respectively [4]. As illustrated in the figure in the Appendix (Figure 5), males were significantly older than females in the study population (mean age, 16.1 years [95%CI, 15.4–16.9] versus 14.7 [13.9–15.7] respectively, $p = 0.02$). Age could thus be a confounding factor in the relationship between gender and outcome.

3.1. Efficacy analysis

An event occurred in 231 patients. EFS curves by gender and treatment arm are given in Fig. 1. Comparison of the models including treatment, age and gender as main effects with different typing of variables for age led us to consider age as a continuous variable (details in Appendix). In the model including these three main effects with no interaction term, age was the only factor associated with a significant impact on the risk of failure, with a poorer outcome with increasing age, HR (/10 years) = 1.25 [1.09–1.43], p-value = 0.0017, whereas we observed a trend for a poorer outcome in males compared to females, HR (males/females) = 1.13 [0.87–1.48], p-value = 0.36. When the treatment by gender interaction term was added to this model, results were consistent with the initial analysis, with a difference of treatment effect between genders being slightly larger (HR (VAC/VAI) = 1.37 [0.98–1.90] in males; HR (VAC/VAI) = 0.81 [0.53–1.24] in females) than in the initial analysis, with a p-value for the interaction test equal to 0.058 (Table 1). The difference of EFS between genders was restricted to the VAC arm: males had worse EFS than females with VAC. This marginal interaction between treatment

and gender did not vary across age as the third order interaction treatment × gender × age was not significant, p-value = 0.79. This result is illustrated by the forest-plot (Fig. 2).

3.2. Toxicity analyses

As detailed in Table 2 for the toxicity observed per patient, more females than males experienced severe toxicities, with Odds Ratio (males/females) lower than one for all but one toxicity item. This higher risk of severe toxicity in females was significant for many toxicity types, such as haematological, infectious, renal toxicity. We observed an increased risk of haematological toxicity and a decreased risk of renal toxicity with VAC compared to VAI. As illustrated by the forest plot (Fig. 3), the effect of treatment VAC versus VAI on the risk of toxicity did not significantly differ between genders; none of the treatment by gender interaction tests was significant. Overall conclusions were similar in the analysis of toxicity at the level of courses (details in Appendix).

3.3. Cumulative dose of alkylating agents

Thirty patients switched from VAI to VAC (9/251 males, 4%, and 21/174 females, 12%), mostly due to renal toxicity, and three from VAC to VAI (2/258 males, 0.8%, and 1/173 females, 0.6%).

The variation of cumulative dose of alkylating agents compared to the theoretical dose was not significantly different between treatment arms (mean = −6.9% [95%CI, −8.1; −5.6] in the VAC arm versus −7.9% [−9.2; −6.7] in VAI-arm, $p = 0.23$), while females received significantly lower cumulative doses than males (−8.8% [−10.2; −7.4] versus −6.1% [−7.2; −4.9], $p = 0.003$) (Fig. 4). The difference between genders was similar in VAC and VAI arms (interaction test, $p = 0.96$). A dose reduction greater than 20% was more frequent in females than in males (15% versus 9%, OR (males/females) = 0.53 [0.34–0.82], $p = 0.005$), with no major difference between VAC and VAI (10% versus 13%, OR(VAC/VAI) = 0.73 [0.47–1.13], $p = 0.15$), and no significant interaction between gender and treatment effect, $p = 0.37$.

4. Discussion

As the randomisation was balanced and stratified according to gender, we were able to evaluate differences in outcome and toxicity between cyclophosphamide and ifosfamide with respect to gender. EFS analysis showed a marginal interaction between treatment effect and gender ($p = 0.058$): Males had a poorer outcome with VAC as compared to VAI, HR (VAC/VAI) = 1.37 [0.98–1.90], contrasting with HR = 0.81 [0.53–1.24] in females.
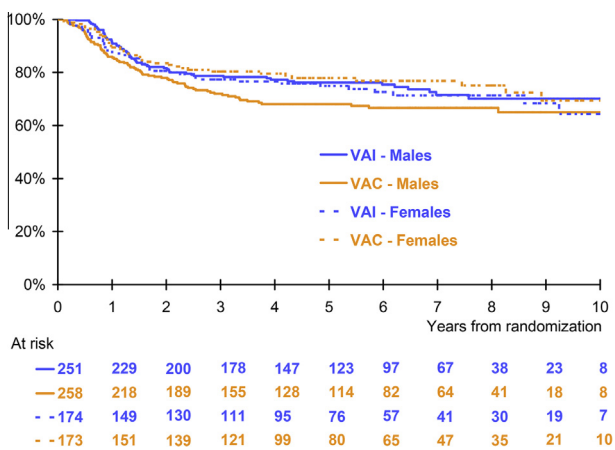

Fig. 1. Unadjusted event-free survival (EFS) curves by gender and treatment group.

Table 1
Cox multivariable model of event-free survival (EFS).

Factors	Hazard ratio (HR)	[95% Confidence interval (CI)]	P-value
Age			
10 year	1.26	[1.10–1.44]	0.001
Gender			
Males versus Females	0.86	[0.59–1.27]	0.45
Treatment VAC versus VAI			
In males	1.37	[0.98–1.90]	0.06
In females	0.81	[0.53–1.24]	0.33

The Hazard Ratios, HR, 95% confidence intervals [95%CI] and p-values were estimated in a Cox multivariable model including age, gender, treatment and treatment by gender interaction, and stratified on cooperative group and local treatment. The HR associated with age (/10 years) corresponds to the relative increase of failure rate associated with a 10-year difference of age. AIC = 2129.
Gender by treatment interaction test, p value = 0.058.
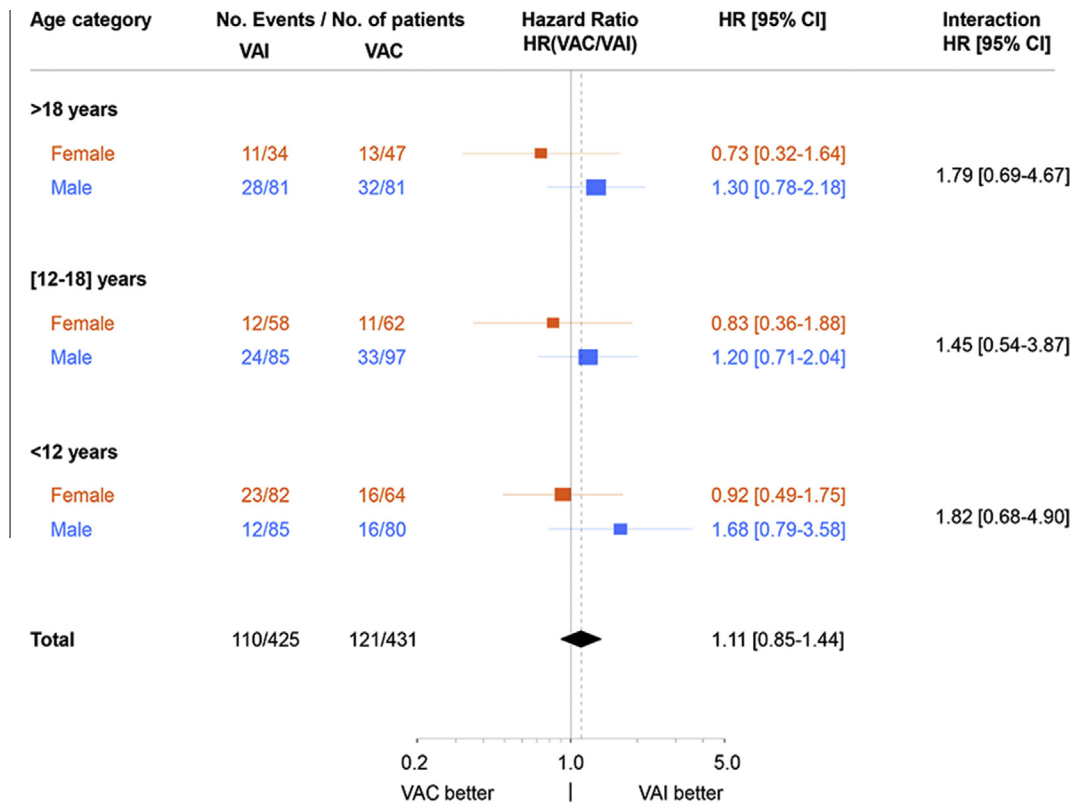
Fig. 2. Event-free survival (EFS) analysis – Effect of treatment by gender, across age categories. Age × Gender × Treatment interaction, p-value = 0.91. *HR(VAC/VAI) = Hazard Ratio of failures associated with treatment effect, estimated in model adjusted on age, in three categories, and gender, and stratified on local treatment and cooperative group. For this analysis, age was categorised in three categories in order to illustrate the results by a forest plot. Interaction HR = Ratio between HR(VAC/VAI) in males and HR(VAC/VAI) in females. 95% CI: 95% Confidence Intervals. The vertical dotted line represents the HR estimated on the whole population in multivariable analysis.

Table 2
Proportion of patients who experienced a severe toxicity, by gender and treatment group, and Odds-Ratio estimated in multivariable models.

Toxicity item	N	Observed percentages				Multivariable model						Inter°
		Males		Females		Gender effect		Treatment effect in males		Treatment effect in females		
		VAI	VAC	VAI	VAC	Odds ratio (OR) M/F [confidence interval (CI)]	P	OR VAC/VAI [CI]	P	OR VAC/VAI [CI]	P	P
		%	%	%	%							
Haematology grade 4	808	73	80	86	90	0.44 [0.25–0.75]	0.003	1.53 [0.99–2.36]	0.05	1.49 [0.74–3.00]	0.26	0.95
Anaemia	808	5	8	20	17	0.20 [0.10–0.41]	<.0001	1.69 [0.80–3.58]	0.17	0.82 [0.46–1.45]	0.49	0.13
Leucopaenia	808	54	62	74	80	0.42 [0.27–0.65]	0.0001	1.43 [0.99–2.06]	0.06	1.39 [0.82–2.35]	0.22	0.93
Neutropenia	764	72	77	80	87	0.69 [0.42–1.15]	0.15	1.39 [0.89–2.16]	0.15	1.85 [0.97–3.52]	0.06	0.47
Thrombocytopenia	808	27	42	45	49	0.46 [0.30–0.70]	0.0003	1.98 [1.34–2.91]	0.001	1.21 [0.78–1.89]	0.40	0.10
Infection grade ⩾2	807	34	41	47	47	0.58 [0.38–0.89]	0.01	1.37 [0.94–2.00]	0.11	1.00 [0.64–1.57]	1.00	0.30
General condition grade ⩾3	789	7	6	10	5	0.71 [0.34–1.48]	0.36	0.84 [0.40–1.76]	0.64	0.51 [0.21–1.23]	0.13	0.40
Gut toxicity grade ⩾3	807	7	9	13	9	0.50 [0.25–0.99]	0.05	1.31 [0.67–2.59]	0.43	0.67 [0.33–1.38]	0.28	0.18
Stomatitis	806	2	2	4	4	0.53 [0.16–1.78]	0.31	0.97 [0.28–3.40]	0.96	1.00 [0.32–3.19]	0.99	0.97
Vomiting	806	5	5	8	4	0.54 [0.24–1.25]	0.15	1.08 [0.47–2.49]	0.86	0.52 [0.20–1.34]	0.18	0.26
Diarrhoea	806	0	2	3	1	0.18 [0.03–0.98]	0.05	2.98 [0.53–16.8]	0.21	0.27 [0.05–1.48]	0.13	0.05
Skin toxicity grade ⩾3	805	3	1	4	2	0.68 [0.23–1.98]	0.48	0.52 [0.15–1.84]	0.31	0.53 [0.15–1.90]	0.33	0.99
Renal toxicity grade ⩾2	806	16	12	29	15	0.49 [0.30–0.80]	0.004	0.69 [0.41–1.16]	0.16	0.44 [0.25–0.77]	0.004	0.25
Serum creatinine	805	1	2	3	3	0.30 [0.07–1.26]	0.10	1.74 [0.40–7.62]	0.46	0.81 [0.24–2.71]	0.73	0.43
Proteinuria	691	1	1	4	2	0.16 [0.03–0.86]	0.03	1.48 [0.22–9.98]	0.69	0.53 [0.15–1.87]	0.33	0.38
Haematuria	711	0	2	4	3	0.11 [0.01–0.94]	0.04	3.87 [0.43–34.9]	0.23	0.66 [0.18–2.42]	0.53	0.18
Glomerular filtration	611	3	1	6	3	0.53 [0.18–1.57]	0.25	0.25 [0.04–1.40]	0.12	0.48 [0.14–1.68]	0.25	0.54
Tubular function	432	25	16	39	16	0.54 [0.30–0.98]	0.04	0.54 [0.28–1.02]	0.06	0.28 [0.13–0.60]	0.001	0.20
Hepatic toxicity grade ⩾3	802	4	6	7	4	0.54 [0.22–1.32]	0.18	1.66 [0.73–3.80]	0.23	0.64 [0.25–1.66]	0.36	0.14
Hyperbilirubinemia	769	1	2	5	1	0.21 [0.05–0.85]	0.03	1.82 [0.41–8.05]	0.43	0.32 [0.08–1.30]	0.11	0.10
Transaminase elevation	798	3	5	4	3	0.80 [0.28–2.28]	0.67	1.55 [0.62–3.89]	0.35	0.87 [0.28–2.73]	0.82	0.44
Cardiac toxicity grade ⩾2	657	3	3	7	2	0.45 [0.15–1.31]	0.14	1.06 [0.33–3.39]	0.92	0.35 [0.09–1.32]	0.12	0.22
Cardiac function	628	2	2	1	0	1.83 [0.31–11.0]	0.51	0.98 [0.25–3.91]	0.98	0.35 [0.02–6.73]	0.49	0.54
LV-SF impairment	512	3	3	8	3	0.32 [0.10–1.11]	0.07	1.09 [0.26–4.49]	0.91	0.44 [0.11–1.74]	0.24	0.37
Neurotoxicity grade ⩾2	805	5	7	11	8	0.34 [0.15–0.75]	0.01	1.62 [0.73–3.57]	0.23	0.62 [0.28–1.34]	0.22	0.09
Central neurotoxicity	804	1	1	3	1	0.47 [0.12–1.81]	0.28	0.71 [0.16–3.26]	0.66	0.32 [0.06–1.87]	0.21	0.50
Peripheral neurotoxicity	803	3	7	9	7	0.30 [0.12–0.73]	0.01	2.26 [0.95–5.40]	0.07	0.69 [0.30–1.57]	0.37	0.05

The Odds Ratios, OR, 95% confidence intervals [CI] and p-values (Wald test), P, were estimated in multivariable logistic regressions including age (<, ⩾25 years), gender, cooperative group, treatment and treatment by gender interaction. N: number of patients included in the toxicity analysis. As we excluded courses administered after a switch from one treatment arm to the other, patients who did not receive any course allocated by randomisation are excluded. The denominator N varies across the table due to missing information for some specific toxicity items.
OR(M/F): Odds Ratio of males compared to females. Inter°: treatment by gender interaction.
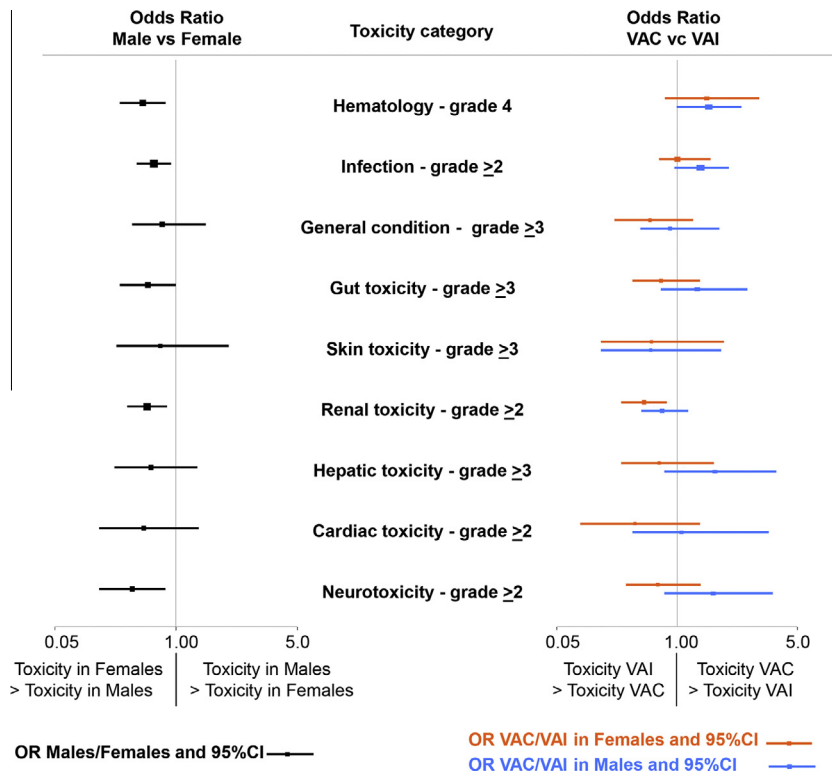
Fig. 3. Toxicity analysis – Effect of treatment by gender on the different toxicity items. Left and right panels represent gender effect and treatment effect according to gender, respectively. Odds ratios were estimated in the multivariable models including treatment, gender, treatment-by-gender interaction, age, cooperative group. Each effect was estimated for a toxicity category, separately. 95%CI: 95% Confidence Intervals. The vertical lines represent the null hypothesis (no gender effect for the left panel, no treatment effect for the right panel).
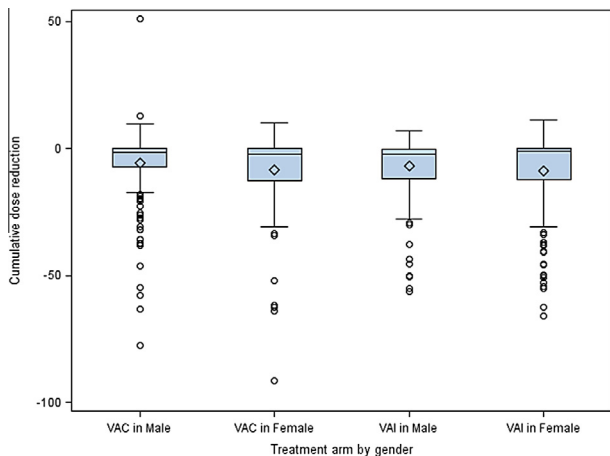
Fig. 4. Box plot of the cumulative alkylating doses compared to protocol dose, by treatment group and by gender. The bottom and top edges of the box indicate the first and third quartiles. The diamond and line inside the box indicate the mean and the median, respectively. The whiskers indicate the minimum value (respectively, maximum) higher (respectively, lower) than Q1-1.5*IQR (respectively, Q3+1.5*IQR) with IQR denoting the interquartile range.

This finding was very stable when adjusted for age (also with different typing of this variable) in the multivariable model, as well as across age. Severe toxicity was more frequent in females than in males, whatever the toxicity type, with no significant interaction between treatment and gender effect.

Our study has a number of strengths. It is based on data from a large cohort, with outcomes collected prospectively. The treatments tested were allocated by randomisation, which was stratified by gender and age. Our data allowed us to disentangle the impact of gender from the possible confounding effect of age.

We acknowledge several limitations of our study. The current analysis is a secondary analysis of the EE99-R1 trial which was initially designed to test the main treatment effect and not the interaction. Consequently, with a total of 231 events, the study was not sufficiently powered to detect with a high level of confidence, a treatment by gender interaction for the efficacy or safety end-points. Approximately 450 events would be required to demonstrate with 80%-power a significant interaction with a two-sided alpha of 5% if this interaction is associated with a HR of 1.7, similar to what we have observed. With a borderline p-value of 0.058 for the effect of treatment by gender interaction on EFS, this exploratory analysis unfortunately neither convincingly confirms, nor clearly refutes the hypothesis of a treatment by gender interaction. Although, several authors apply a threshold of 0.10 for interaction tests [12]. In addition, the analysis of treatment by gender interaction on the risk of severe toxicity was limited by

the rarity of some specific toxicities leading to underpowered analyses. Sexual maturation may be more important on the different outcomes than age. This could unfortunately not be studied accurately as pubertal status was not fully recorded. However, results were stable when we included in the models the recorded pubertal status, or the age in three categories with different cut-offs by gender for the pubertal category (11–15 years in females and 13–17 in males) (data available on request).

In the published literature a better outcome for females versus males in various malignancies has been suggested [13]. Even in children and adolescents, gender differences in efficacy and toxicity have been reported [14,15]. In a report on 352 children treated for anaplastic large cell lymphoma, Wrobel et al. report significantly higher rates of toxicity in females, including grade 4 haematologic toxicity and grade 3–4 stomatitis. In the meta-analysis performed by Collins et al. including 4838 patients with osteosarcoma using a multivariable model adjusted for age, females had a significantly better overall survival, a higher rate of good tumour necrosis and more frequent thrombocytopenia and mucositis [15]. However, these studies are not directly informative of an alkylating treatment by gender interaction. In Wrobel's study, all patients received both cyclophosphamide and ifosfamide. In 'Collins' study, approximately half of the patients received an alkylating agent which was always ifosfamide.

It is possible that gender differences in the metabolism of both ifosfamide and cyclophosphamide might contribute to a potential interaction between alkylating treatment, gender and outcome and to the gender differences in terms of acute toxicity.

Both ifosfamide and cyclophosphamide are inactive prodrugs, with hydroxylation as a primary activating step, mainly under CYP3A4 and CYP2B6 control, respectively [16–19]. Increased activity of CYP2B6 is observed in females [20–25], which could be explained by the male-specific inhibition of the constitutive androstane receptor (CAR) pathway by androstenol and androstanol, resulting in a decreased expression of CYP2B6. CAR activity does not influence CYP3A4 activity [26–34]. As a result a gender related effect of vincristine, mainly metabolised through CYP3A4 cannot be expected. Of actinomycin-D no metabolites have been disclosed and excretion itself is through urine and bile, making a gender related effect unlikely, as well. The differences in CYP2B6 expression between genders might have resulted in lower levels of cyclophosphamide-derived active hydroxylated compounds in males. Since we have no quantitative CYP-RNA measurements and no pharmacokinetic data, such substantiated proposition is speculative. Additionally to this gender related expression a puberty/gender based pharmacokinetic factor can be

of importance. For example, it has been shown that doxorubicin clearance is influenced by body fat, which changes markedly, and in different directions at puberty in males and females [35]. Both the metabolic and pharmacokinetic hypothesis challenges the equivalency of 4 g/m^2 of ifosfamide per 1 g/m^2 cyclophosphamide in males.

With regard to side effects being more frequent in females than in males, the higher CYP3A4 activity, as reported in females, might lead to higher peak levels of the neurotoxic metabolite of ifosfamide, the N-dechlorethylated ifosfamide [36,37] and to gender differences in neurotoxicity reported in the literature. But the higher frequency in haematological and gastrointestinal toxicity as well as renal toxicity in females in our study cannot be fully explained.

The marginal interaction between gender and type of alkylating agent on EFS persisted in the different analyses controlling for age. For basic biological confirmation of our hypothesis, determination of RNA levels and measurements of enzyme activities are needed. For clinical proof, the differences of acute toxicity rate or cumulative doses of alkylating agents between genders, as found, are as yet insufficient to explain the findings. Additional data from other sources are required to further evaluate the hypothesis of heterogeneity of alkylating agent effect by gender.

The observations made here, even if statistically not conclusive, warrant further research into gender-related differences in large drug efficacy studies. If major potential differences in efficacy and tolerability between genders are not considered, future trials might produce misleading results because of such an interaction. The results reported here should be considered as hypotheses-generating. However, if there was to be a true interaction between the alkylating agent used and gender, this would have a very important implication for the treatment of future patients. Further studies, such as another randomised trial, are therefore needed to confirm or refute the presence of an interaction. A meta-analysis of trials evaluating both alkylating agents is currently on-going.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

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