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Late toxicity comparison of alkylating-based maintenance regimen with cyclophosphamide (VAC) vs ifosfamide (VAI) in Ewing sarcoma survivors treated in the randomized clinical trial Euro-EWING99-R1 in France

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Abstract

In Euro-EWING99-R1 randomized trial, cyclophosphamide was shown to be noninferior to ifosfamide in the consolidation of standard-risk Ewing sarcoma (SR-EWS) after a common induction with VIDE (vincristine-ifosfamide-doxorubicin-etoposide). We present the results of the late effects analysis of VAC (vincristine-dactinomycin-cyclophosphamide) vs VAI (vincristine-dactinomycin-ifosfamide) conducted in Euro-EWING99-R1 French cohort. Of 267 French randomized patients, 204 were alive and free-of-relapse at 5-years including 172 with available long-term follow-up data concerning cardiac, renal and/or gonadal functions (sex-ratio M/F = 1.3, median age at diagnosis = 14 years): 84 randomized in VAC (median cumulative doses: cyclophosphamide = 9.7 g/m², ifosfamide = 59.4 g/m²) and 88 in VAI (ifosfamide = 97.1 g/m²). With a median follow-up of 10 years (range = 5-17), five

Abbreviations: AMH, anti-mullerian hormone; CTCAE, Common Terminology Criteria for Adverse Events; EFS, event-free survival; EORTC, European Organization for Research and Treatment of Cancer; EWS, Ewing sarcoma; FSH, follicle-stimulating hormone; GFR, glomerular filtration rate; GPOH, Gesellschaft für Pädiatrische Onkologie und Hämatologie; LH, luteinizing hormone; LTFU, long-term follow-up; LV, left ventricular; LVEF, left ventricular ejection fraction; LVSF, left ventricular fractional shortening; SMN, second malignant neoplasms; SR-EWS, standard-risk Ewing sarcoma; TRP, tubular reabsorption of phosphate; UKCCSG, United Kingdom Children's Cancer Study Group; VAC, vincristine-dactinomycin-cyclophosphamide; VAI, vincristine-dactinomycin-ifosfamide; VDC/IE, vincristine-doxorubicin-cyclophosphamide/ifosfamide-etoposide; VIDE, vincristine-ifosfamide-doxorubicin-etoposide.

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For affiliation refer to page 1665

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late relapses and five second malignancies were recorded. The 10-year event-free survival among 5-year free-of-relapse survivors was similar between VAC and VAI (93% vs 95%, $P = .63$). We estimated the 10-year cumulative probabilities of cardiac and kidney toxicities at 4.4% (95% confidence interval [95% CI] = 1.1%-7.6%) and 34.8% (95% CI = 26.8%-42.0%), respectively. Cardiac toxicity cumulative probability was similar in both arms, whereas kidney toxicity was higher in VAI (at 10 years, 43.0% vs 25.7%, $P = .02$), resulting from significant difference in glomerular toxicity (31.1% vs 13.1%, $P < .01$). At 10 years, gonadal toxicity was observed in 27% and 28% of pubertal men and women, respectively, without significant difference between VAC and VAI. Kidney and gonadal toxicities represent major issues in Euro-EWING99-R1, with significantly higher risk of kidney toxicities with VAI, without significant gonadal toxicity reduction. These results support the need to limit cumulative doses of both alkylating agents and to use mixed regimen as in VIDE-VAC or VDC/IE (vincristine-doxorubicin-cyclophosphamide/ifosfamide-etoposide).

KEYWORDS

antineoplastic combined chemotherapy protocols/adverse effects, child, cyclophosphamide/adverse effects, Ewing/drug therapy, Ifosfamide/adverse effects

What's new?

The Euro-EWING99-R1 trial compared the alkylating agents cyclophosphamide with ifosfamide in combination treatment for Ewing sarcoma. Here, the authors compare the late events between the two combination treatments using data from 172 patients enrolled in Euro-EWING99-R1. The combination containing ifosfamide carried a higher risk of kidney toxicity, but both regimens carried high rates of gonadal toxicity. Some of this toxicity could be avoided, they suggest, by using a mixed regimen to limit the dose of both alkylating agents.

1 | INTRODUCTION

Cooperative clinical trials have resulted in a substantial improvement in cure rates of children and young adults treated for Ewing sarcoma (EWS), especially in standard-risk (SR) EWS. SR-EWS are defined as localized tumors with good histologic response to chemotherapy with <10% residual viable cells, or small tumors, <200 mL, resected at diagnosis or receiving radiotherapy alone as local treatment. The 3-year overall survival of SR-EWS exceeds now 85%.¹

The international Euro-EWING99-R1 randomized trial for SR-EWS questioned the alkylating agent type to use in consolidation treatment. The conclusion was that cyclophosphamide-based consolidation treatment (vincristine-dactinomycin-cyclophosphamide = VAC) may be able to replace ifosfamide-based treatment (vincristine-dactinomycin-ifosfamide = VAI) (hazard ratio of event = 1.12 [91.4% CI = 0.89-1.41]), but with higher rate of thrombocytopenia in VAC-arm and more acute tubular toxicity in VAI-arm.¹ However, the question of differences between both arms in terms of late toxicities has not yet been answered.

Individually, the toxicity of both alkylating agents is well-known, with ifosfamide known to induce nephrotoxicity in the long-term²⁻⁴ contrary to cyclophosphamide,⁵ and cyclophosphamide known to induce gonadal toxicity,⁶⁻¹⁰ whereas ifosfamide is

considered safer.^{11,12} However, the toxicity of combination of high cumulative doses of both agents as in VAC-arm or of very high cumulative doses of ifosfamide as in VAI-arm has not been previously studied. Moreover, epipodophyllotoxin and anthracyclines used in induction are potentially associated with other late side-effects including second malignant neoplasms (SMN)¹³⁻¹⁵ and cardiomyopathy.¹⁵⁻¹⁷

The objective of our study was to describe and compare late mortality, SMN, heart, kidney and gonadal functions between VAC and VAI-arms in the Euro-EWING99-R1 French randomized cohort.

2 | METHODS

2.1 | Study population and data collection

Euro-EWING99-R1 trial compared seven VAC courses (experimental arm) to seven VAI (control arm) for consolidation treatment after a common induction chemotherapy of six VIDE courses (vincristine-ifosfamide-doxorubicin-etoposide) (Figure S1).¹ Theoretical cumulative doses of ifosfamide and cyclophosphamide were, respectively, 60 and 10.5 g/m² in VAC-arm, and 102 g/m² and no cyclophosphamide in VAI-arm. Randomization was stratified on age, gender, center

and type of local treatment. Overall, 856 patients were included in 13 European countries between 1999 and 2010.

The current analysis included the 5-year free-of-relapse SR-EWS survivors randomized in Euro-EWING99-R1 in France for whom long-term follow-up (LTFU) data were available.

2.2 | Health condition outcomes

LTFU evaluations with cardiac, kidney and gonadal function assessments were scheduled at 2, 4 and 6 years after diagnosis, and additional evaluations were recorded.

Cardiac left ventricular (LV) dysfunction was defined on echocardiography as LV ejection fraction (LVEF) <50% and/or LV fractional shortening (LVFS) <30%.

Kidney tubular injury was defined as decreased fractional tubular reabsorption of phosphate ($TRP = [1 - (Ph_u \times Cr_{sg}) / (Ph_{sg} \times Cr_u)] \times 100$) <80% and/or elevated beta-2-microglobulinuria (>370 $\mu\text{g}/24$ hour or urinary beta-2-microglobulin/creatinine ratio >300 $\mu\text{g}/\text{g}$). Glomerular damage was defined as an estimated GFR <90 mL/min/1.73 m^2 (CKD-EPI equation).¹⁸ Proteinuria (>300 mg/24 hour or urinary protein/creatinine ratio >300 mg/g creatinuria) was analyzed separately. Global kidney toxicity was defined as a composite criterion combining tubular dysfunction, glomerular dysfunction and proteinuria.

Testicular exocrine insufficiency was defined as elevated gonadotropin levels (FSH ≥ 20 IU/L) with preservation of spontaneous puberty, inhibin B concentration decrease (according to reference values¹⁹) and/or oligo/azoospermia. Testicular endocrine dysfunction was defined as basal LH concentration increase (>12 IU/L) and testosterone concentration decrease (<3.5 ng/mL). Premature ovarian insufficiency was defined as primary amenorrhea, or cessation of menses or use of hormone replacement therapy before age 40 years with evidence of hypergonadotropic hypogonadism (FSH ≥ 30 IU/L, estradiol <12 pg/mL). Moreover, we considered diminished ovarian reserve as defined by AMH concentration decrease (<1.3 ng/mL), low antral follicle count and/or use of medically assisted reproduction.

The most recent evaluation was considered for each toxicity. Transient toxicities (reversible at last evaluation) were also reported. Follow-up time was defined as the time since diagnosis until the most recent function evaluation, and time to onset of toxicity as the time since diagnosis until the first occurrence of an abnormal measurement. Each toxicity was graded at last evaluation according to the Common Terminology Criteria for Adverse Events (CTCAE, v5.0).

2.3 | Statistical analyses

Descriptive statistics were used to characterize the study population. Comparisons between VAC and VAI-arms were performed using Fisher's exact test for categorical variables and nonparametric Wilcoxon-Mann-Whitney test or Student test for continuous variables. Event-free survival (EFS) was defined as the time interval between diagnosis

and relapse, SMN or death from any cause, whichever occurred first. Survival curves were estimated using the Kaplan-Meier method with 95% confidence interval (95% CI). Cumulative probabilities of persistent toxicities at the last evaluation were estimated using the Kaplan-Meier method, with censoring observations at time of relapse, SMN, death and loss of follow-up, assuming that relapse, SMN and death were independent competing events of cardiac, kidney and gonadal toxicities. To minimize a potential detection bias, which could result in an over-estimation of toxicity incidence when considering only evaluated patients, we decided to present cumulative probabilities of toxicities on the whole population. The log-rank test was used for comparisons between the VAC and VAI-arms. All tests were two-sided with alpha set to 0.05. Statistical analyses were performed with SAS 9.4 (SAS Institute, Cary, North Carolina) and R 3.6.2 (<http://www.R-project.org>).

3 | RESULTS

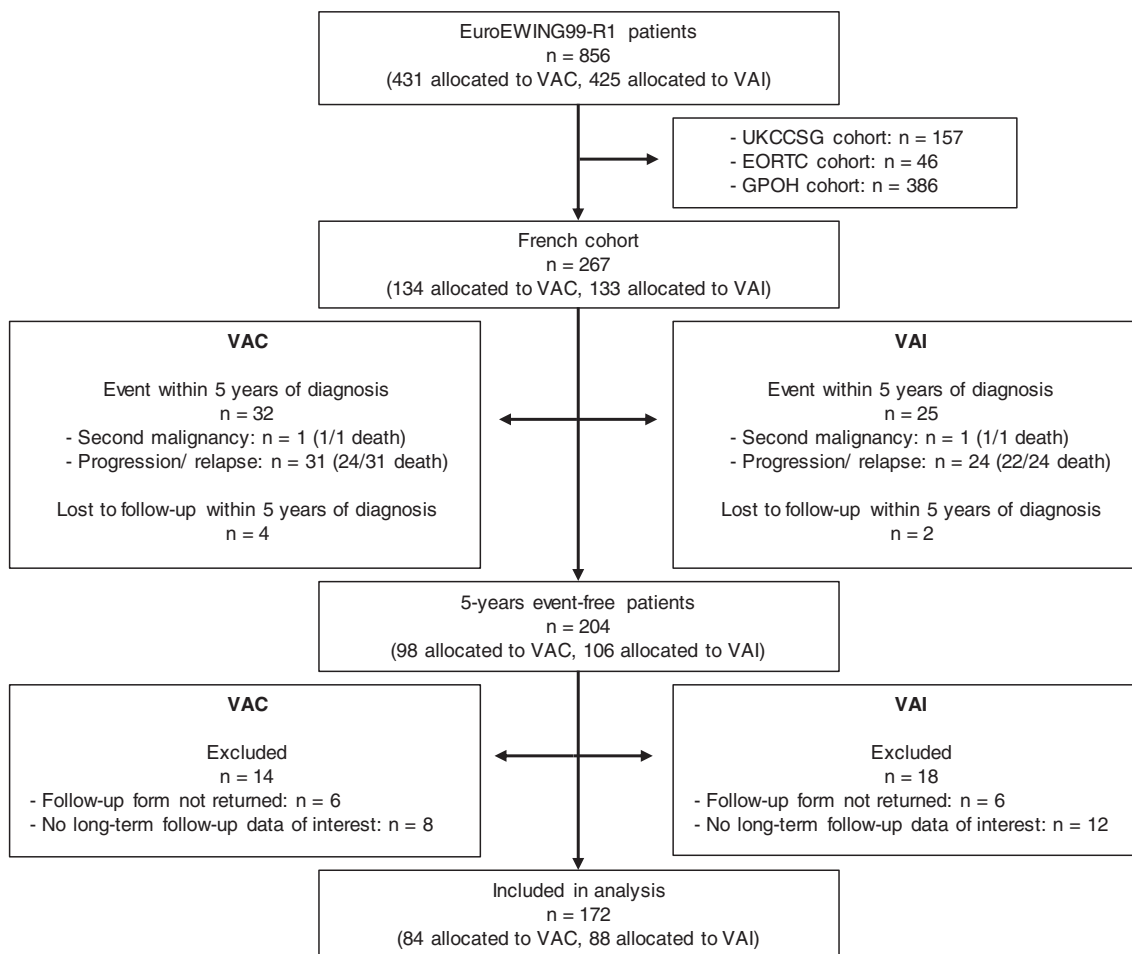
Among 267 French SR-EWS patients randomized in Euro-EWING99-R1, 204 were alive and free-of-relapse at 5 years. LTFU data were available for 172 of them, treated from 1999 to 2008 in 32 French centers (Figure 1, Table 1). Median age at diagnosis was 14 years (range = 1-43). Primary tumor site was mainly lower limb (35%). Ninety-seven percent of the patients had surgery as local treatment, alone (65%) or in combination with radiation therapy (32%).

3.1 | Survival data

With a median follow-up of 10 years from diagnosis (range = 5-17), 10 events were observed: five late relapses (three in VAC-arm and two in VAI-arm, occurring 5 to 13 years after diagnosis, Table S1) and five SMN (two in VAC-arm and three in VAI-arm, occurring 6 to 9 years after diagnosis, Table S2). Two patients died, both due to late EWS relapses (one in each arm, at 10 and 12 years after EWS diagnosis). In this subset of 5-years free-of-relapse survivors, the estimated 10-year EFS was 94% (95% CI = 90-98), similar between VAC and VAI-arms (93% vs 95%, $P = .63$, Figure S2).

3.2 | Cardiac toxicity

Cardiac function evaluations at least 5 years after diagnosis were available in 111 patients (65%, 52 in VAC-arm, 59 in VAI-arm) and identified persistent cardiac dysfunction at the last evaluation in eight (five in VAC-arm, three in VAI-arm, Table S3). One of them was diagnosed during EWS treatment (VAI-arm), whereas the seven others were diagnosed between 3.8 and 12.5 years postdiagnosis. Cardiac toxicity was moderate (63% grade-1, 37% grade-2). Overall, the estimated 10-year cumulative probability of persistent cardiac toxicity in the whole population was 4.4% (95% CI = 1.1-7.6), without a significant difference between VAC and VAI-arms ($P = .21$, Figure 2A).



UKCCSG: United Kingdom Children's Cancer Study Group
EORTC: European Organisation for Research and Treatment of Cancer
GPOH: Gesellschaft für Pädiatrische Onkologie und Hämatologie

FIGURE 1 Participant flow diagram

Additionally, transient myocardial dysfunction with isolated slight LVSF decrease (28%-29%) and spontaneous recovery was observed in 9/111 other patients (three in VAC-arm, six in VAI-arm) from 1 to 6 years after diagnosis. None of these patients with persistent ($n = 8$) or transient ($n = 9$) LV dysfunction had received chest radiation.

3.3 | Kidney toxicity

Results of tubular toxicity evaluations performed ≥ 5 years postdiagnosis were available for 76 patients (44%, 32 in VAC-arm, 44 in VAI-arm) with a median time interval from diagnosis of 9 years in both arms. Signs of persistent tubular damage at the last evaluation were observed in 27 patients (11 in VAC-arm, 16 in VAI-arm). Overall, the estimated 10-year cumulative probability of persistent tubular toxicity in the whole population was 17.5% (95% CI = 11.1-23.5), without a significant difference between VAC and VAI-arms ($P = .54$). Moreover, transient TRP alterations were observed in five patients (one in VAC-arm,

four in VAI-arm), with early TRP decline between 1 and 5 years after diagnosis and spontaneous normalization 2 to 12 years after diagnosis.

Results of glomerular function evaluations performed ≥ 5 years postdiagnosis were available for 130 patients (76%, 59 in VAC-arm, 71 in VAI-arm), with comparable evaluation delays between both arms (median = 9.1 and 8.6 years in VAI and VAC-arms, respectively). Persistent glomerular dysfunction at the last evaluation was observed in 38 patients (11 in VAC-arm, 27 in VAI-arm). Toxicity was mild in 79% of cases (60-89 mL/min/1.73 m²) and moderate in 21% (30-59 mL/min/1.73 m²). The lowest estimated GFR was 35 mL/min/1.73 m² (two patients, one in each arm). Median time to onset of glomerular damage was 5 years postdiagnosis (range = 1-14), similar between both arms. Overall, the estimated 10-year cumulative probability of persistent glomerular toxicity in the whole population was 22.5% (95% CI = 15.6-28.8), significantly higher in VAI-arm compared to VAC (31.1% vs 13.1%, $P < 0.01$). Additionally, transient slight GFR decrease (74-89 mL/min/1.73 m²) was observed in six patients (three in each arm) within 3 to 7 years postdiagnosis.

TABLE 1 Baseline characteristics of the overall, included and excluded patients

	Total (n = 204)	Included (n = 172)	Excluded (n = 32)	P
Sex				
Male	110 (53.9)	96 (55.8)	14 (43.8)	.29
Female	94 (46.1)	76 (44.2)	18 (56.2)	
Age at diagnosis (years): mean ± SD				
Median (min-max)	14.7 ± 8.2	14.5 ± 8.0	15.8 ± 9.5	.81
<25 years	14.0 (1.2-43.4)	14.0 (1.2-43.4)	13.0 (2.3-43.4)	
≥25 years	183 (89.7)	156 (90.7)	27 (84.4)	.34
	21 (10.3)	16 (9.3)	5 (15.6)	
Follow-up duration (years): mean ± SD				
Median (min-max)	10.4 ± 2.8	10.9 ± 2.7	8.0 ± 2.5	<.01
	10.3 (5.1-17.3)	10.8 (5.2-17.3)	7.5 (5.1-14.2)	
Age at last follow-up (years): mean ± SD				
Median (min-max)	25.4 ± 8.5	25.6 ± 8.3	23.9 ± 9.1	.12
	23.7 (8.6-60.4)	24.1 (8.6-60.4)	22.4 (11.4-51.2)	
Tumor site				
Head and neck	17 (8.3)	16 (9.3)	1 (3.1)	.79
Thoracic level	42 (20.6)	36 (20.9)	6 (18.8)	
Abdominal and pelvic level	48 (23.5)	39 (22.7)	9 (28.1)	
Lower limb	71 (34.8)	60 (34.9)	11 (34.4)	
Upper limb	26 (12.7)	21 (12.2)	5 (15.6)	
Tumor type				
Osseous lesion ± soft tissue component	173 (84.8)	145 (84.3)	28 (87.5)	.79
Soft tissue only	31 (15.2)	27 (15.7)	4 (12.5)	
Local treatment				
Surgery only	132 (64.7)	114 (66.3)	18 (56.2)	.23
Radiotherapy only	7 (3.4)	7 (4.1)	0 (0)	
Surgery + radiotherapy	65 (31.9)	51 (29.7)	14 (43.8)	
Cumulative doses of chemotherapy				
Vincristine, mg/m ²	17.7 ± 3.0	17.6 ± 3.0	18.2 ± 3.2	.22
Ifosfamide, g/m ²	79.2 ± 19.9	78.8 ± 19.8	81.2 ± 20.9	.22
Doxorubicin, mg/m ²	353.8 ± 17.9	352.8 ± 18.5	359.1 ± 13.2	.02
Etoposide, g/m ²	2.5 ± 0.4	2.5 ± 0.4	2.7 ± 0.2	.02
Actinomycin D, mg/m ²	10.7 ± 1.7	10.6 ± 1.8	10.9 ± 1.4	.60
Cyclophosphamide, g/m ²	4.9 ± 4.9	4.9 ± 4.9	4.7 ± 4.9	.81
Events				
Relapse	6 (2.9)	5 (2.9)	1 (3.1)	1
Secondary malignancy	5 (2.5)	5 (2.9)	0 (0)	1
Death	2 (1.0)	1 (0.6)	1 (3.1)	.29
Events within a period of 10 years				
Relapse	5 (2.5)	4 (2.3)	1 (3.1)	.58
Secondary malignancy	5 (2.5)	5 (2.9)	0 (0)	1
Death	2 (1.0)	1 (0.6)	1 (3.1)	.29
10-year EFS	94.2 (90.7-97.8)	94.1 (90.4-98.0)	96.6 (90.1-100)	.97

Note: Qualitative data are expressed as the number (percentage) of patients, quantitative data as the mean ± SD or the median (minimum-maximum) and survival as a Kaplan-Meier estimate (95% CI).

Abbreviation: EFS, event-free survival.

Proteinuria was assessed in 65 patients (38%, 30 in VAC-arm, 35 in VAI-arm), with a median delay postdiagnosis of 9 years (range = 5-15, similar in both arms). Fourteen of them (four in

VAC-arm, 10 in VAI-arm) had significant proteinuria (range = 31-63 mg/mmol).

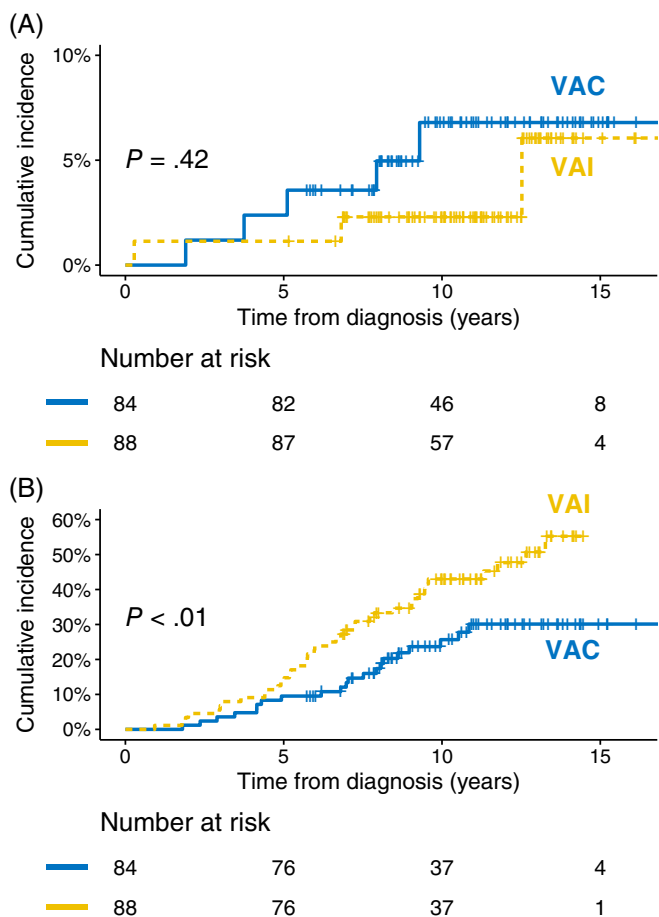


FIGURE 2 Cumulative probabilities of persistent cardiac dysfunction (A) and kidney toxicity (glomerular and/or tubular and/or elevated proteinuria) (B) for the whole analysis population according to randomly assigned group

Overall, persistent kidney toxicity was observed in 62 patients (21 in VAC-arm, 41 in VAI-arm; median delay postdiagnosis = 6 years; 85% grade-1, 15% grade-2; Table S4). The estimated 10-year cumulative probability of kidney toxicity in the whole population was 34.8% (95% CI = 26.8-42.0), significantly higher in VAI-arm compared to VAC (43% vs 26%, $P = .02$, Figure 2B).

3.4 | Gonadal toxicity

Among 66 pubertal or postpubertal men at last evaluation (28 in VAC-arm, 38 in VAI-arm), hormonal blood assessments were performed in 42 patients (64%, 18 in VAC-arm, 24 in VAI-arm, Table S5). Testosterone, FSH, LH and inhibin B concentrations were measured in 38, 40, 40 and 10 patients, respectively, and 14 men had performed semen analysis. An exocrine testicular dysfunction was observed in 17 patients (41%, 12/23 in VAI vs 5/19 in VAC, $P = .12$) and an endocrine testicular dysfunction in 4 (10%, 4/23 in VAI vs 0/18 in VAC, $P = .12$). Overall, male gonadal toxicity was identified in 18/43

evaluated patients (42%), with higher but nonsignificant rate in VAI-arm compared to VAC-arm (54.2% vs 26.3%, $P = .12$).

Among 67 pubertal or postpubertal women at last evaluation (34 in VAC-arm, 33 in VAI-arm), 4/27 girls without menstruations at EWS diagnosis did not develop spontaneous menarche (15%, 3/14 in VAC-arm, 1/13 in VAI) and 7/58 women used hormonal replacement therapy (12%, 5/30 in VAC-arm, 2/28 in VAI-arm; data not available in nine cases) (Table S6). Hormonal blood assessments were performed in 43 patients (64%, 22 in VAC-arm, 21 in VAI-arm), with FSH, estradiol and AMH concentrations measured in 42, 36 and 15 patients, respectively, and 10 women had performed an antral follicle count. A premature ovarian insufficiency was observed in 7 women (11%, 5/34 in VAC vs 2/33 in VAI, $P = .43$), whereas a diminished ovarian reserve was documented in 8 (12%, 5/34 in VAC vs 3/33 in VAI, $P = .71$). Among the 13 patients with at least one spontaneous pregnancy (six in VAC-arm, seven in VAI-arm), 14 live births were observed in 10 patients. Additionally, one medically assisted pregnancy led to one live birth. Overall, female gonadal toxicity was observed in 19/67 patients (28%) with higher but nonsignificant rate in VAC-arm compared to VAI (38.2% vs 18.3%, $P = .10$).

4 | DISCUSSION

We reported LTFU data of 172 SR-EWS survivors free-of-relapse at 5 years, randomized in EuroEWING99-R1 trial in France. The estimated conditional 10-year EFS in this population was 94% (95% CI = 90-98), similar between VAC and VAI-arms. First late relapses were rare, but could occur in this series until 13 years postdiagnosis. In the same time, SMN could occur as early as 6 years postdiagnosis. These two types of malignant events, though rare, justify to pursue oncological follow-up after 5 years.

After a planned cumulative dose of 360 mg/m² of doxorubicin, we observed a 10-year cumulative probability of persistent LV dysfunction of 4.4%, without significant difference between VAI and VAC-arms (2.3 vs 6.8%, respectively). However, with a median follow-up to last cardiac evaluation of 8 years, our study needs to be pursued in the very long-term. Cardiac events are usually very late and even if previous studies did not identify late cardiac toxicity of cyclophosphamide or ifosfamide,^{20,21} a higher risk of cardiac dysfunction was reported in case of combination of anthracycline with high-cumulative doses of ifosfamide.¹⁶

Concerning kidney toxicity, we reported a high probability of persistent kidney damage with an estimated 10-year cumulative probability of 35%, significantly higher in VAI-arm compared to VAC (43% vs 26%, $P = .02$). This difference resulted mainly from glomerular damage, whereas tubular toxicity was not significantly different in both arms. As patients were exposed to ifosfamide in both arms, a persistent tubular toxicity was expected in VAI as well as in VAC-arm, as previously published.² However, if the glomerular toxicity rate observed in our series in VAC-arm (19% of studied patients) was very similar to that previously published,² it was much lower than that observed in VAI-arm (38% of studied patients). These results confirmed the glomerular toxicity of high cumulative doses of ifosfamide,⁵ but with a potential dose-effect relationship which

was not reported for glomerular toxicity²² contrary to tubular toxicity.³ Moreover, glomerular damage could be delayed after end of treatment,²² until 14 years postdiagnosis in our series. Consequently, with very high ifosfamide cumulative doses as used in EuroEWING99-R1 VAI-arm (102 g/m²), kidney toxicity appears to be a major concern, not only for tubular but also for delayed and persistent glomerular damage, justifying very long-term kidney follow-up.

Concerning gonadal toxicity, cyclophosphamide was reported as more toxic than ifosfamide in men in previous studies, but with a median ifosfamide cumulative dose of 54 g/m².²³ In our series, we reported higher gonadal toxicity rate in patients treated with VAI-arm (54%) compared to VAC-arm (26%). This result is in favor of a cumulative toxic dose-effect of ifosfamide on exocrine testicular function, especially for high cumulative doses (>60 g/m²) which comforts previous preliminary reports.²⁴ In women, gonadal toxicity was identified in 28% of the patients with higher rate in VAC-arm (38%) compared to VAI (18%, $P = .10$). This confirms a previous preliminary report in which males appear to be more susceptible to ifosfamide gonadal toxicity than females²⁴ resulting in a potential higher rate of male gonadal toxicity in VAI-arm compared to VAC, and a potential higher rate of female gonadal toxicity in VAC-arm compared to VAI. Interestingly, the largest previous study concerning the effect of alkylating agents on ovarian function did not identify the total cumulative dose of cyclophosphamide or ifosfamide as predictive factors of decreased ovarian reserve, but with lower cumulative doses of cyclophosphamide and ifosfamide (median = 4.6 and 48 g/m², respectively).²⁵

This analysis was limited by the inclusion of data from only one country resulting in a quite low number of patients analyzed compared to the number of patients included in this European trial. However, this choice was justified by the high rate of missing data at the international level concerning late effects. Moreover, the number of evaluated patients varied widely according to the type of toxicity and to time intervals.

In conclusion, persistent kidney damage appears to be a major concern in VAI-arm whereas gonadal toxicity is a frequent issue in both arms. Consequently, we could recommend to limit cumulative doses of both alkylating agents, and to prefer a mixed regimen with moderate doses of cyclophosphamide and ifosfamide like VIDE-VAC or VDC/IE (vincristine-doxorubicin- cyclophosphamide/ifosfamide-etoposide) instead of a regimen with very high cumulative doses of only one alkylating agent like VIDE-VAI. However, an interaction between gender and type of alkylating agents concerning gonadal toxicity is not excluded based on these results. Further analyses are necessary to explore this hypothesis.

AUTHOR CONTRIBUTIONS

Victoria Corvest: Conceptualization and methodology; Investigation, resources and data curation; Validation and formal analysis; Writing - original draft; Writing - review & editing; Visualization, supervision and project administration. **Perrine Marec-Bérard:** Conceptualization and methodology; Investigation, resources and data curation; Writing - review & editing. **Cyril Lervat:** Investigation, resources and data curation; Writing - review & editing. **Hélène Pacquement:** Investigation, resources and data curation; Writing - review & editing. **Maud**

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of our study are available from the corresponding author upon request.

ETHICS STATEMENT

Written informed consent from patient/parent was mandatory. Appropriate ethics committees approved the trial in each country. Clinical trial registration number: NCT00020566.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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