Randomized phase 2 trial of the Vincristine-Irinotecan combination with or without Temozolomide, in children and adults with relapsed or refractory rhabdomyosarcoma: an EpSSG/ITCC trial.

Short title: VIT randomized trial in rhabdomyosarcoma

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Abstract

Purpose

The VIT-0910 trial was conducted to evaluate efficacy and safety of the vincristine-irinotecan combination with and without temozolomide (VIT and VI, respectively) in relapsed or refractory rhabdomyosarcoma.

Methods

In this randomized European phase-2 trial, patients aged 0.5-50 years received 21-day cycles combining vincristine (1.5mg/m2 d1, d8), irinotecan (50mg/m2 d1-d5) with/without temozolomide (125mg/m² d1-d5, 150mg/m² from cycle-2), until progression/unacceptable toxicity. The primary endpoint was objective response rate (ORR) after two cycles. Secondary endpoints included best response, progression-free survival (PFS), overall survival (OS) and adverse events. A Simon 2-stage design was initially planned to separately analyze 40 patients/arm. After amendment, the trial sample size was increased to 120 and a comparison between arms, adjusted for confounding factors, was added to the statistical plan. (ClinicalTrials.gov, NCT01355445)

Results

Overall, 120 patients (60 per arm) were recruited in 37 European centers. Median age was 11 years (range, 0.75-45); 89% patients had a relapsed rhabdomyosarcoma. ORR was 44% (24 of 55 evaluable patients) for VIT versus 31% (18/58) for VI (adjusted odds ratio, adj-OR=0.50, 95%CI, 0.22-1.12, p=0.09). The VIT-arm achieved significantly better OS (adjusted hazard ratio, adj-HR=0.55, 95%CI, 0.35-0.84, p=0.006) compared to VI, with consistent PFS results (adj-HR=0.68, 95%CI, 0.46-1.01, p=0.059). Overall, patients experienced adverse events ≥ grade 3 more frequently with

VIT than VI (98% versus 78%, respectively; p=0.009), including a significant excess of hematological toxicity (81% versus 61%; p=0.025).

Conclusion

The addition of temozolomide to VI improved chemotherapy efficacy for patients with relapsed rhabdomyosarcoma, with manageable increase in toxicity. VIT is considered the new standard treatment in these patients in the European Paediatric Soft Tissue Sarcoma Group (EpSSG) and will be the control arm in the next randomized trial.

Context summary

Key objective: This study, from the European paediatric Soft tissue Sarcoma study Group (EpSSG) and the Innovative Therapies for Children with Cancer consortium (ITCC), is the first European prospective randomized study testing chemotherapy combinations in relapsed and refractory rhabdomyosarcoma.

Knowledge generated: The study showed that the addition of the chemotherapy drug temozolomide to vincristine and irinotecan chemotherapy improved tumor response and survival of patients with relapsed or refractory rhabdomyosarcoma. The study has defined the combination of vincristine, irinotecan and temozolomide as a new standard chemotherapy treatment option for relapsed rhabdomyosarcoma.

Relevance: The combination of vincristine, irinotecan and temozolomide is the standard (control) treatment in the recently launched EpSSG Frontline and Relapse Rhabdomyosarcoma study which will test innovative combinations of new treatments combined with backbone chemotherapy in relapsed rhabdomyosarcoma.

Introduction

At the time of relapse, Rhabdomyosarcoma (RMS) is generally refractory to treatment leading to poor overall survival (OS) of less than 20%.[1] Main prognostic factors at relapse are the type of recurrence, prior radiotherapy treatment, initial tumor size and time of relapse from diagnosis.[2] New systemic therapies are urgently needed to improve outcome of relapsed RMS.

The combination of vincristine and irinotecan (VI) using a 2-week regimen for irinotecan was highly active in newly diagnosed metastatic RMS, with an objective response rate (ORR) of 70%.[3] Subsequently, this regimen was compared to a shorter schedule of irinotecan (1x 5 days every 21 days) in the ARST0121 randomized phase-2 trial in first relapse or progression of RMS. No significant difference was observed between the longer and shorter regimens (ORR=26 and 37%, respectively). The authors recommended the more convenient shorter regimen to be taken forward.[4].

Irinotecan, as a prodrug, is metabolized in vivo into its active metabolite SN-38 which acts as a topoisomerase I inhibitor which is active in S-Phase of the cell cycle leading to replication disruption. This mechanism of action supports its use in combination with alkylating agents such as temozolomide.[5,6] The dose limiting toxicities of irinotecan (diarrhea) and temozolomide (myelosuppression) are non-overlapping and schedule-dependent synergy between these two drugs has been demonstrated in RMS mouse xenograft models.[7] When we designed the trial, the combination of VI with temozolomide (VIT) had not been evaluated prospectively in RMS. This European open-label, multicenter, randomized phase-2 trial evaluated the efficacy and safety of the combination of VI with or without temozolomide in patients with relapsed or refractory RMS.

Methods

Trial design

The VIT-0910 trial (ClinicalTrials.gov, NCT01355445) was an international open-label, randomized two-parallel group phase-2 trial conducted by the European Paediatric Soft Tissue Sarcoma Group (EpSSG) and Innovative Therapies for Children with Cancer (ITCC), in 37 centers from five countries (On-line Table-S1). Study protocol was approved by an independent ethics committee and the appropriate institutional review boards.

Patients

Key eligibility criteria included histologically confirmed RMS; relapsed, progressive or refractory RMS in which standard treatments had failed; age 6 months to 50 years; Karnofsky or Lansky performance status $\geq$70%; life expectancy $\geq$3 months; adequate organ function (details in full protocol). Following the recommendation from the Independent Data Monitoring Committee (IDMC) based on data analysis of the first 80 patients, the protocol was amended in December-2015 to continue accrual in the trial in relapsed patients only.

Patients with prior exposure to irinotecan or temozolomide were not eligible. Written informed consent was obtained from all patients and/or their parents/guardians before enrolment.

Randomization and masking

The chemotherapy regimen VI or VIT was allocated by randomization at study entry. Centralized randomization software (TENALEA®) was used, ensuring the concealment of the next patient allocation. Balanced 1:1 randomization was based on a minimization algorithm taking into account disease status (relapsed or progressive

in patients who have already shown a response to chemotherapy, here termed "relapse"), versus refractory (defined as progression after receiving chemotherapy without prior response) and country for the first 80 patients; for the 40 additional patients, all recruited in the relapse stratum, the algorithm also included prior radiotherapy (yes-versus-no) and disease staging at study entry (metastases: yes-versus-no). Patients and investigators were not blinded to treatment assignment, but the centralized retrospective radiological review committee was blinded to group allocation.

Treatment

The study treatment consisted of 21-day cycles of VI or VIT. In the VIT-arm, the starting dose of temozolomide was 125 mg/m² d1-d5, escalating to 150 mg/m² at cycle-2 for patients without grade ≥3 toxicity, based on Kushner's published regimen in neuroblastoma.[8] Cefixime was recommended for prophylaxis of irinotecan gut toxicity. Treatment was continued until progression or unacceptable toxicity for up to 12 cycles. Further continuation of treatment was individually discussed for patients who did not experience disease progression after 12 cycles. Treatment schedule and chemotherapy details are in On-line Figure-S1.

Local therapy was allowed after two cycles; it was tailored to patient and tumor characteristics, and included complete surgical removal wherever feasible, radiotherapy, or a combination of both.

Outcomes and assessments

Tumor assessment based on computed tomography or magnetic resonance imaging was performed every two courses during study treatment. After completion of study

treatment, tumor evaluation was recommended every three months during the first two years, then every six months up to six years from study entry until disease progression. The primary endpoint was ORR, i.e. complete or partial response, after two cycles. Tumor response was evaluated using the three-dimensional WHO response criteria for the primary lesion and according to RECIST-1.1 criteria for metastatic sites.[9,10] Tumor evaluations until reported progression were reviewed by an independent response review committee. Clinical progression without radiological confirmation, but which shortly led to death, was counted as progression.

Secondary efficacy endpoints included centrally-reviewed best response over the whole study treatment duration (before local treatment if any), progression-free survival (PFS) and overall survival (OS). PFS was defined as the time interval from the start of treatment to the date of tumor progression, relapse, or death from any cause. OS was defined as the time interval from the start of treatment until death from any cause.

Adverse events (AE), evaluated by clinical and laboratory examinations at the beginning of each cycle of study treatment, and weekly for hematological tests, were graded according to NCI-CTCAE-v4.0. A grade≥3 AE was classified as a severe AE.

Data cut-off was set at April 1, 2019.

Statistical considerations

The trial was originally designed as a non-comparative randomized Phase-2 trial. An Optimum Simon two-stage design based on the objective response at two cycles was used to define the statistical rule and the sample size. Accounting for an 8% dropout rate, 40 patients in each arm were required to test the null hypothesis $p0 \leq 0.20$ at a 1-sided alpha of 10% and ensure a 90%-power under the alternative hypothesis $p1 \geq 0.40$. Following the IDMC recommendation to continue accrual in relapsed patients only, and

assuming better outcomes in this stratum, the design parameters were revised (p0=0.35 and p1=0.55), leading to an increased sample size up to a total of 120 patients including 108 relapsed patients. Based on IDMC recommendations, another amendment was submitted in July-2018 to allow formal comparison of all endpoints between the randomized groups.

Comparison of treatment arms was controlled for predefined covariates: disease status (relapse-versus-refractory disease), disease staging at study entry (metastases: yes-versus-no) and histological subtype (alveolar-versus-non-alveolar), using multivariate logistic regressions for the ORR at 2 cycles and the best response, and using Cox models for the PFS and OS. Treatment effect estimates (Odds ratio of failure, OR, and hazard ratio, HR, respectively) were estimated with their 95%-Confidence Intervals (95%CI) and tested at a two-sided 5%-alpha level.

In addition to the Kaplan-Meier estimates of PFS and OS curves, we provided the adjusted survival curves estimated in the multivariate models.

The efficacy analysis was performed both on the entire study population, and on the main subset of patients at relapse (study population after amendment). Heterogeneity of treatment effect across the main subgroups (based on predefined covariates) was tested using interaction tests and illustrated by forest plots.

AEs were described by system organ class (SOC). Maximum grade observed over the whole treatment duration was tabulated per type of AE and illustrated using a butterfly plot. We estimated relative risk of severe AE in VIT compared to VI, overall and for each SOC.

The analysis of response after two cycles included all patients who started study treatment except those with no imaging after two cycles (and no clinical progression). All patients with at least one tumor evaluation during the study treatment were included

in the analysis of the best response. The primary analysis of survival outcomes (OS and PFS) was performed in the intention-to-treat population (ITT), including the entire follow-up duration regardless of possible non-study maintenance treatment. We performed a post-hoc sensitivity analysis of PFS and OS by censoring the observations at the date of start of a systemic treatment other than planned study drugs, if a systemic treatment was administered before progression.

A 2-sided p-value<0.05 was considered as significant for all VIT-versus-VI comparison tests.

All statistical analyses were performed using Stata® software, version 15.0 (StataCorp LLC College Station, USA).

Results

Patient characteristics

Overall, 120 patients were enrolled between March-2012 and April-2018: 60 in the VI-arm and 60 in the VIT-arm. All but two patients in the VI-arm started study treatment (Figure-1). As detailed in Table-1, we observed a non-significant excess of patients with unfavorable site of primary tumor, large tumor at diagnosis, refractory disease and metastatic disease at study entry in the VIT-arm compared to the VI-arm. Additionally, there were slightly fewer patients with progression or early relapse (occurring in the 18 months from diagnosis) in the VIT-arm than in the VI-arm.

Efficacy results on the whole population by treatment group

In the whole population, ORR after two cycles was 44% in the VIT-arm (24/55 evaluable patients) and 31% in the VI-arm (18/58), significantly higher than the pre-specified minimum efficacy threshold p0=20% in both arms (Table-2). Controlling for

the pre-specified covariates, the adjusted OR was 0.50 (95%CI, 0.22-1.12) for the VIT-arm compared to the VI-arm, with a 2-sided p=0.09.

Considering the best response over the whole treatment duration, we observed significantly more objective responses in the VIT-arm than in the VI-arm (33/58, 57% versus 22/58, 38%, adjusted-OR=0.40; 95%CI, 0.18-0.88; 2-sided p=0.023).

Overall, with a median follow-up of 57 months, 104 disease progressions or relapses were reported and 91 patients died, all but one from disease (Table-2, Figure-2). In the multivariate Cox model adjusted for possible predefined confounding factors, the VIT-arm was found to be associated with a reduction in the risk of progression or relapse compared to the VI-arm, with an adjusted-HR_{PFS}=0.68, 95%CI, 0.46-1.01, which was nearly statistically significant (p=0.059).

The VIT-arm was associated with a significant reduction in the risk of death compared to the VI-arm with an adjusted-HR_{OS}=0.55, 95%CI 0.35-0.84, and p=0.006.

In the sensitivity analysis censoring observations at the start date of other anti-cancer treatment, the benefit associated with VIT compared to VI appeared larger and significant in terms of PFS (adjusted-HR_{PFS}=0.64, 95%CI, 0.42-0.98, p=0.039), and stable and still significant in terms of OS (adjusted-HR_{OS}=0.59, 95%CI 0.37-0.93, p=0.02).

Efficacy results in patients at relapse

As detailed in Table-2, results were comparable when focusing on relapsed patients only. In this subgroup, the ORR after 2 cycles was 47% in the VIT-arm (22/52), significantly higher than pre-specified minimum efficacy threshold p0=35% (1-sided p=0.045), whereas the ORR of 33% in the VI-arm (18/55) was insufficient, leading to an adjusted OR of 0.53 (95%CI, 0.23-1.22; p=0.14). The adjusted-HR_{PFS} was 0.68

(95%CI, 0.45-1.03; p=0.069) and the adjusted-HR_{OS} was 0.57 (95%CI, 0.36-0.90; p=0.016).

Subgroup analyses

As illustrated by the forest-plots (Supplementary Figures-S2, S3, S4), we did not observe any significant heterogeneity of treatment effect across subgroups, neither for the objective response at 2 cycles, nor for the PFS or the OS.

Treatments

The median number of cycles was 6 (range 1-18) for the VIT-arm and 4 (range 1-26) for the VI-arm (Table-3). The proportion of patients with relative dose intensity <0.8 was significantly higher in the VIT-arm (47% versus 22%, p=0.006).

Overall, 55 patients discontinued treatment early due to progressive disease and 13 due to toxicity, with a non-significant trend for fewer early terminations due to progression and more due to toxicity in the VIT-arm (p=0.30). Sixteen patients received 12 or more cycles of VIT/VI.

In addition, 17 patients had additional systemic therapy (Table-3) after stopping VI/T and before progression: 13/57 (23%) in the VIT-arm and 4/55 (7%) in the VI-arm (p=0.02).

Among the 46 patients with local/loco-regional disease at study entry, 20 had a local treatment (5 surgery alone, 7 radiotherapy alone, and 8 both) with no significant difference between treatment groups (p=0.65).

Safety

A significantly higher proportion of patients experienced a grade ≥3 AE in VIT compared to VI, both for all AE (98% versus 78%, respectively, p=0.009) and also for AEs classified as related to study treatment (93% versus 69%, p=0.002).

There was also a significant excess of serious adverse events classified as related to the study treatment in VIT-arm (38% versus 19%, p=0.023).

We observed a significant excess of severe hematological toxicity in VIT (81% versus 61%, p=0.025, Figure-3). Focusing on gastrointestinal events, we did not observe any significant difference in terms of grade ≥3 diarrhea (24% versus 17%, p=0.33) as well as grade ≥3 nausea and/or vomiting of (26% versus 17%, p=0.24). There were no study treatment-related deaths.

Discussion

This randomized European phase-2 trial suggests that, in patients with relapsed or refractory or relapsed rhabdomyosarcoma, the addition of temozolomide to vincristine and irinotecan improves chemotherapy efficacy. The ORR after two cycles in the VIT-arm was 47% in patients at relapse, significantly higher than the predefined p0=35%, whereas the ORR rate was insufficient in the VI-arm. Considering the best response over the whole treatment duration in the entire population, we observed significantly more objective responses in the VIT-arm than in the VI-arm. We also observed a nearly significant PFS benefit and a large and significant OS benefit for the VIT-arm. The better outcomes with VIT were observed despite having a significant decrease in planned dose-intensity, mainly due to toxicity. Overall, the significant excess of acute toxicity of the VIT combination, mostly hematological toxicity, was manageable.

This is the first randomized controlled trial evaluating VIT in the setting of progressive/relapsed RMS, other published studies evaluating VIT in this setting were retrospective studies.[11,12] The VIT-combination has also been prospectively evaluated in the ARST08P1-trial by the Children's Oncology Group (COG), in a non-randomized study evaluating VIT with lower doses of temozolomide as first line treatment in metastatic RMS.[13] The authors concluded that the addition of temozolomide to intensive multiagent chemotherapy did not improve outcome for patients with metastatic RMS.

This study was the first EpSSG trial for patients with relapsed or refractory RMS, with the goal of defining the standard chemotherapy at relapse to which novel agents could be added or other innovative therapies compared. The control arm of the trial was based on the results of ARST0121-trial where the shorter schedule of irinotecan was found to be no different in efficacy from the protracted schedule.[4]

Although the study populations were not entirely comparable as our study also included patients with second or subsequent relapse (23 and 25.4% of the relapsed patients in the VIT- and VI-arm, respectively), results of the VIT-combination still compare favorably with the ARST0121-study.[1] In this risk-based therapy, 6-month failure-free survival was 50% in patients with unfavorable features receiving multi-agent chemotherapy (with or without tirapazamine), similar to the results in the VIT-arm presented here (6-month PFS=45% overall, 55% at first relapse/progression). When looking specifically at the comparable patient population (first relapse/progression), our results in the VIT-arm (6-month and 24-month PFS 55% and 23%, respectively) are also quite similar to the results of ARST0921-trial comparing temsirolimus and bevacizumab in combination with vinorelbine-cyclophosphamide

(temsirolimus-arm: 6- and 24-month PFS=65% and 19%, respectively; bevacizumab-arm: 50% and 7%).[14]

Although toxicity was deemed manageable in the VIT-arm, the increased VIT toxicity raises the question of whether it is possible to add new targeted therapy or immunotherapy to this chemotherapy backbone. Such combinations should be tested in experienced early Phase centers.

The planned dose of temozolomide was higher in the current trial than in the ARST08P1-COG trial, which concluded that adding temozolomide to multi-agent chemotherapy did not improve outcome compared to historical controls.[13] In our trial, the better outcomes on VIT were observed despite having a significant decrease in planned dose-intensity, mainly due to toxicity. Whether a similar outcome would be observed with lower planned dose remains unknown.

We acknowledge several limitations of our study. First, the study was not initially designed to compare efficacy outcomes between treatment groups, leading, after amendment, to underpowered comparisons, both overall and even more in subgroups. Based on current knowledge, PFS would have been a more appropriate primary endpoint than ORR.[15] However, when the study was designed in 2012, assessment of objective response was still current practice in rhabdomyosarcoma. In addition, the study was based on the COG study published by Mascarenhas et al. in JCO in 2010, evaluating two different schedules of VI combination, using objective response as primary endpoint.[4] Lastly, we did evaluate progression-free and overall survival as secondary endpoints. We also acknowledge that the use of several types of imaging and response criteria hampers optimal response assessment. Differences in treatment effect estimates between unadjusted and adjusted analyses also complicate the interpretation of the results; this is explained by slight imbalances in patient

characteristics between treatment groups, which would have been avoided if the randomization had been controlled for these prognostic factors. Another issue is the higher proportion of patients who received further chemotherapy after end of study treatment and before progression in the VIT-arm compared to the VI-arm, which may confuse the interpretation of survival outcomes. Reported OS results should be interpreted with care. However, OS remained significantly better for VIT and the improvement in PFS were became statistically significant in the sensitivity analysis when observations were censored at the date of start of another anti-cancer treatment. Lastly, we have no clear explanation for the larger effect on OS than on PFS of the VIT-arm compared to the VI-arm, as there was no significant difference of treatment modalities at progression/relapse. A similar finding was reported in the trial evaluating maintenance treatment in high-risk localized RMS.[16]

Based on our study results, the VIT-combination is considered the new EpSSG standard treatment in patients with relapsed RMS who have previously received alkylating agent. We discounted the option of adding temozolomide to the first-line chemotherapy regimen in RMS because active cytotoxic drugs in RMS have reached a plateau in their capacity to prevent relapse, and temozolomide would add an additional alkylating agent to cyclophosphamide and ifosfamide already used in front-line. It was thus decided to pursue its evaluation in patients with relapsed or refractory disease.

The EpSSG has recently launched its new multi-arm multi-stage frontline and relapse rhabdomyosarcoma study (FaR-RMS) and VIT will be the new standard control arm in relapsed patients. Depending on expected combination toxicity, experimental arms will include VI or VIT backbone, combined with innovative agents.

Data Sharing Statement

The dataset used and analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1: Patient and tumor characteristics

Characteristics	VIT N=60		VI N=60		Total N=120		p VIT vs VI
Age at inclusion							
Median (Range)	12 Y	(9.1 M ; 45 Y)	10.5 Y	(3 Y; 45 Y)	11 Y	(9.1 M ; 45 Y)	0.94
Age group							0.66
< 18 years, N and %	46	77%	48	80%	94	78%	
≥ 18 years, N and %	14	23%	12	20%	26	22%	
Primary site (at initial diagnosis)							0.23
Favorable(1), N and %	8	13%	13	22%	21	18%	
Unfavorable, N and %	52	87%	47	78%	99	83%	
Histology							1
Alveolar, N and %	34	57%	34	57%	68	57%	
Non alveolar N and %	26	43%	26	43%	52	43%	
Tumor size at initial diagnosis (MD=1)							0.15
≤ 5 cm, N and %	18	31%	26	43%	44	37%	
> 5 cm, N and %	41	69%	34	57%	75	63%	
Prior chemotherapy with doxorubicin (MD=1)							0.39
Yes, N and %	46	77%	49	83%	95	80%	
No(2), N and %	14	23%	10	17%	24	20%	
Prior radiotherapy (MD=3)							0.43
Yes, N and %	47	81%	51	86%	98	84%	
No, N and %	11	19%	8	14%	19	16%	
Disease status at inclusion							0.38
Relapse, N and %	52	87%	55	92%	107	89%	
Including first relapse	40		41		81		
Refractory, N and %	8	13%	5	8%	13	11%	
Disease staging at inclusion							0.28
Local or loco-regional progression, N and %	19	32%	27	45%	46	38%	
Metastatic only, N and %	21	35%	19	32%	40	33%	
Both, N and %	20	33%	14	23%	34	28%	
Time interval between diagnosis and first relapse/progression							
Median time interval (months)	15.0	(2.1-76.6)	14.3	(0.3-67.8)	14.5	(0.3-76.6)	0.34
Categories							0.26
<1.5 year, N and %	35	58%	41	68%	76	63%	
≥1.5 year, N and %	25	42%	19	32%	44	37%	

(1): Favorable sites included orbit (N=7), head and neck non para-meningeal sites (N=12) and genitourinary sites apart from bladder and prostate (N=5).

(2): 24 patients had not received doxorubicin prior to study entry; they had all received IVA courses (ifosfamide-vincristine-dactinomycin), followed by vinorelbine-cyclophosphamide in 5 patients.

Table 2: Efficacy results in both treatment groups, on the whole population and only in patients enrolled at relapse

Outcome	Whole population		Patients at relapse	
	VIT	VI	VIT	VI
	N=60	N=60	N=52	N=55
Response at two cycles				
Distribution of the response				
- Complete response	5 (9%)	2 (3%)	5 (11%)	2 (4%)
- Partial response	19 (35%)	16 (28%)	17 (36%)	16 (30%)
- Stable disease	21 (38%)	21 (36%)	17 (36%)	18 (33%)
- Progressive disease	10 (18%)	19 (33%)	8 (17%)	18 (33%)
- Missing data	5	2	5	1
Objective response rate at 2 cycles (95%CI)	44% (30-58%)	31% (20-45%)	47% (32-62%)	33% (21-47%)
1-sided p-value (test versus p0) (1)	p<0.0001	p=0.018	p=0.045	p=1.00
Odds ratio of failure (2)				
- Unadjusted OR (95%CI)	0.58 (0.27-1.26)	1	0.57 (0.25-1.27)	1
2-sided p-value	p=0.17		p=0.17	
- Adjusted OR (95%CI) (3)	0.50 (0.22-1.12)	1	0.53 (0.23-1.22)	1
2-sided p-value	p=0.09		p=0.14	
Best Response over the whole treatment (4)				
Distribution of the response				
- Complete response	9 (16%)	4 (7%)	9 (18%)	4 (7%)
- Partial response	24 (41%)	18 (31%)	22 (44%)	18 (33%)
- Stable disease	16 (27%)	17 (30%)	12 (24%)	14 (26%)
- Progressive disease	9 (16%)	19 (33%)	7 (14%)	18 (33%)
- Missing data	2	2	2	1
Best objective response rate (95%CI)	57% (43-70-%)	38% (26-52%)	62% (47-75%)	40% (28-55%)
Odds ratio of failure (2)				
- Unadjusted OR (95%CI)	0.46 (0.22-0.97)	1	0.43 (0.19-0.96)	1
2-sided p-value	p=0.042		p=0.040	
- Adjusted OR (95%CI) (3)	0.40 (0.18-0.88)	1	0.42 (0.19-0.93)	1
2-sided p-value	p=0.023		p=0.032	
Progression-free survival (PFS)				
Number and type of events				
- Disease progression or relapse	52	52	44	48
- Death as first event (5)	0	1	0	1
Median PFS (95%CI) in months	4.7 (4.1-8.5)	3.2 (2.4-7.3)	5.0 (4.2-10.0)	3.5 (2.4-7.4)

Outcome	Whole population		Patients at relapse	
	VIT	VI	VIT	VI
	N=60	N=60	N=52	N=55
PFS rates (95% CI)				
- at 6 months	45% (32-57)	42% (29-54)	50% (36-63)	44% (30-56)
- at 1 year	33% (21-45)	28% (17-40)	36% (23-49)	29% (30-56)
- at 2 years	18% (9-29)	15% (8-26)	19% (10-31)	16% (8-27)
Hazard ratio (HR)				
- Unadjusted HR (95%CI)	0.81 (0.55-1.19)	1	0.77 (0.51-1.16)	1
2-sided p-value	p=0.28		p=0.22	
- Adjusted HR (95%CI) [3]	0.68 (0.46-1.01)	1	0.68 (0.45-1.03)	1
2-sided p-value	p=0.059		p=0.069	
Progression-free survival (PFS) – censored at first other chemotherapy before progression				
Number and type of events				
- Disease progression or relapse	42	50	35	46
- Death as first event [5]	0	1	0	1
Median PFS (95%CI) in months	4.8 (4.1-8.5)	3.2 (2.4-6.7)	7.6 (4.2-10)	3.5 (2.4-7.4)
PFS rates (95% CI)				
- at 6 months	47% (34-60)	41% (29-53)	53% (38-66)	43% (30-56)
- at 1 year	31% (18-44)	26% (15-38)	35% (21-49)	27% (15-39)
- at 2 years	19% (9-32)	13% (5-24)	21% (10-36)	13% (6-25)
Hazard ratio (HR)				
- Unadjusted HR (95%CI)	0.74 (0.49-1.11)	1	0.68 (0.44-1.06)	1
2-sided p-value	p=0.14		p=0.09	
- Adjusted HR (95%CI) [3]	0.64 (0.42-0.98)	1	0.62 (0.39-0.96)	1
2-sided p-value	p=0.039		p=0.03	
Overall survival (OS)				
Number and cause of deaths				
- Death due to disease progression	43	47	36	43
- Death from another cause [5]	0	1	0	1
Median OS (95%CI) in months	15.0 (10.0-21.2)	10.3 (7.1-12.6)	17.3 (11.7-22.9)	10.8 (7.4-14.9)
OS rates (95% CI)				
- at 6 months	80% (67-88)	70% (57-80)	81% (67-89)	75% (61-84)
- at 1 year	56% (42-67)	43% (30-55)	61% (46-73)	45% (32-58)
- at 2 years	33% (21-45)	22% (12-34)	36% (22-49)	24% (13-36)
Hazard ratio (HR)				
- Unadjusted HR (95%CI)	0.71 (0.48-1.09)	1	0.69 (0.44-1.08)	1
2-sided p-value	p=0.12		p=0.10	
- Adjusted HR (95%CI) [3]	0.55 (0.35-0.84)	1	0.57 (0.36-0.90)	1
2-sided p-value	p=0.006		p=0.016	

Outcome	Whole population		Patients at relapse	
	VIT	VI	VIT	VI
	N=60	N=60	N=52	N=55
Overall survival (OS) – censored at first other chemotherapy before event				
Number and cause of deaths				
- Death due to disease progression	35	45	28	41
- Death from another cause (5)	0	1		1
Median OS (95%CI) in months	12.4 (9.8-17.3)	10.3 (7.1-12.6)	15 (9.8-22.3)	10.4 (7.4-12.6)
OS rates (95% CI)				
- at 6 months	79% (66-88)	70% (56-80)	80% (65-88)	74% (60-84)
- at 1 year	51% (36-64)	40% (27-53)	57% (40-70)	42% (29-55)
- at 2 years	27% (15-41)	20% (10-31)	32% (17-47)	21% (11-33)
Hazard ratio (HR)				
- Unadjusted HR (95%CI)	0.73 (0.47-1.13)	1	0.67 (0.41-1.08)	1
2-sided p-value	p=0.15		p=0.10	
- Adjusted HR (95%CI) (3)	0.59 (0.37-0.93)	1	0.59 (0.36-0.96)	1
2-sided p-value	p=0.02		p=0.03	

(1) The observed objective response rate after two cycles was tested against p0=20% when considering the whole study population and against p0=35% when focusing on patients at relapse, using 1-sided test.

(2) Failure is defined as stable disease or progressive disease.

(3) All adjusted estimates of treatment effect (VIT compared to VI) are based on multivariable models including treatment and predefined covariates: histological subtype (alveolar versus non-alveolar), disease staging at study entry (metastases: yes versus no) and disease status (relapse versus refractory disease)

(4) Best response was based on tumor evaluations performed during study treatment or at the end of study treatment, before any local treatment as well as before start of another systemic treatment if any.

(5) One patient died from surgical complications (hemorrhage) after hepatic transplant for a recurrent biliary duct rhabdomyosarcoma transplanted after seven VI-courses

Table 3: Treatment characteristics

Treatment characteristics	VI N=58		VIT N=60		p-value[5]
Total number of VI/VIT cycles before progression (N=118)					
Median - (Range)	4	(1-26)	6	(1-18)	0.44
Number of cycles <12, N and %	50	86%	52	87%	
Number of cycles ≥12, N and %	8	14%	8	13%	
Reasons for early termination of study treatment (<12 cycles) (N=101, MD=1)					0.30
Progression, N and %	30	60%	25	49%	
Toxicity, N and %	4	7%	9	15%	
Other, N and %	16	32%	17	33%	
Investigator decision	13		14		
Patient decision	3		3		
Reduced Dose Intensity for at least one study drug (Relative Dose Intensity, RDI <0.8) (N=118)					0.006
No, N and %	45	78%	32	53%	
Yes, N and %	13	22%	28	47%	
If yes (drugs with RDI<0.8, potentially combined)					
Vincristine	8		15		
Irinotecan	9		16		
Temozolomide [1]	0		20		
Type of non systemic treatment performed before progression, overall (N=112, MD=6)					0.88
None, N and %	33	60%	37	65%	
Radiation therapy alone, N and %	10	18%	11	19%	
Surgery alone, N and %	4	7%	3	5%	
Surgery & radiation therapy, N and %	8	15%	6	11%	
Timing of non systemic treatment (N=42)					0.38
During VI/VIT chemotherapy[2], N and %	15	68%	11	55%	
After the end of VI/VIT chemotherapy[3], N and %	7	32%	9	45%	
Type of local treatment performed before progression, in patients with local/loco-regional disease (N=45, MD=1)					0.65
None, N and %	14	54%	11	58%	
Radiation therapy alone, N and %	3	12%	4	21%	
Surgery alone, N and %	3	12%	2	11%	
Surgery & radiation therapy, N and %	6	23%	2	11%	

Treatment characteristics	VI N=58		VIT N=60		p-value[5]
Other systemic anti-cancer treatment administered before progression (N=112, MD=6)					0.02
No, N and %	51	93%	44	77%	
Yes, N and %	4	7%	13	23%	
Vinorelbine-cyclophosphamide, N and %	4	7%	6	10%	
Other[4], N and %	0	0%	7	12%	
Anti-cancer treatment administered after progression/relapse (N=94, MD=10)					0.17
None, N and %	11	25%	10	20%	
Systemic treatment, N and %	24	55%	19	38%	
Surgery and/or radiation therapy, N and %	2	4%	4	8%	
Systemic treatment + Surgery and/or radiation therapy, N and %	7	16%	17	34%	

(1) Relative dose intensity for Temozolomide was calculated considering 125mg/m²/day for the first cycle and then 150m g/m²/day from the second cycle
(2) Including 3 patients (2 in the VI-arm and 1 in the VIT-arm) who had surgery during VI/VIT courses and completed local treatment with radiation therapy delivered after VI/VIT courses.
(3) For these patients who had local treatment after VI/VIT courses, the median number of VI/VIT courses administered before local treatment was 5 (range 2-18).
(4) Seven patients allocated to VIT received after the end of VIT courses systemic anti-cancer treatment other than navelbine-cyclophosphamide before progression: 2 high dose chemotherapy with busulfan-melphalan followed by stem cell transplantation; 1 carboplatin etoposide, 1 pazopanib, 2 vincristine-dactinomycin-cyclophosphamide and 1 oral etoposide
(5) Chi 2 test for qualitative variables and Student test for quantitative variables

Figure Legends

Figure 1: CONSORT flow diagram

(1) 2 patients in the VI-arm did not receive the study treatment: 1 due to patient's decision, 1 because he was reviewed as ineligible for the study before start of treatment.

(2) The primary outcome (ORR after 2 cycles) was not evaluable for five in the VIT-arm with incomplete tumor evaluation, as well as for 2 patients in the VI-arm who did not start treatment.

(3) One hundred twelve patients (58 in the VIT-arm and 54 in the VI-arm) were evaluable for safety. Eight patients were not evaluable for safety: 2 patients in the VI-arm who did not receive the study treatment and 6 patients with missing safety data (2 in the VIT-arm and 4 in the VI-arm).

Figure 2: Progression-free and overall survival curves, by treatment group

Kaplan-Meier estimate of the progression-free survival (A) and the overall survival (B) from start of study treatment.

Adjusted curves of progression-free survival (C) and overall survival (D), estimated from the multivariable Cox models including treatment and predefined covariates: histological subtype (alveolar versus non-alveolar), disease staging at study entry (metastatic relapse/progression versus loco-regional disease) and disease status (relapse versus refractory disease).

Figure 3: Safety analysis considering all reported Adverse Events

The panel on the left is a butterfly plot showing the proportion of patients experiencing an adverse event, classified or not as related to study treatment, whatever the grade

(light blue for VI and yellow for VIT-arm), and a severe adverse event, grade ≤3 (dark blue for VI and orange for VIT-arm) according to the randomization group. The panel on the right displays the relative risk of a severe adverse event in patients with VIT relative to patients with VI, with 95% confidence intervals. The toxicity items are regrouped by main categories (system organ class). Details of adverse events are given as supplementary material (on-line Table S2). For each adverse event type, the analysis is based on the maximum grade observed over the whole maintenance treatment duration. The categories of adverse event are ordered by decreasing value of the relative risk of severe adverse event.

The supplemental Figure S4 illustrates the safety analysis focused on adverse events classified as related to study treatment.