The tyrosine kinase inhibitor crizotinib does not have clinically meaningful activity in heavily pre-treated patients with advanced alveolar rhabdomyosarcoma with *FOXO* rearrangement. European Organization for Research and Treatment of Cancer phase 2 trial 90101 "CREATE"

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Summary

***Background*:** Alveolar rhabdomyosarcomas (ARMS) can harbour MET/ALK alterations. We prospectively assessed crizotinib in patients with advanced/metastatic ARMS.

***Methods*:** Eligible patients with a central diagnosis of ARMS received oral crizotinib 250mg twice daily. Patients were attributed to *MET/ALK*+ or *MET/ALK*- sub-cohorts by assessing the presence or absence of the forkhead box O1 (*FOXO1;* a marker of MET upregulation) and/or anaplastic lymphoma kinase (*ALK*) gene rearrangement. The primary endpoint was the objective response rate (ORR). Secondary endpoints included duration of response (DOR), disease control rate (DCR), progression-free survival (PFS), progression-free rate (PFR), overall survival (OS), and safety.

***Findings*:** Nineteen of 20 consenting patients had centrally confirmed ARMS. Molecular assessment revealed rearrangement of *FOXO1* in 17 tumours, and *ALK* in none. Thirteen eligible patients were treated, but only 8 were evaluable for the primary endpoint due to the observed aggressiveness of the disease. Among 7 evaluable *MET*+/*ALK*- patients, only one achieved a confirmed partial response (ORR: 14.3%; 95% CI: 0.3–57.8) with a DOR of 52 days. Further *MET*+/*ALK*- efficacy endpoints were: DCR: 14.3% (95% CI: 0.3–57.8), median PFS: 1.3 months (95% CI: 0.5–1.5), median OS: 5.6 months (95% CI: 0.7–7.0). The remaining *MET*+/*ALK*- and *MET*-/*ALK*- patients had early progression as best response. Common treatment-related adverse events were fatigue (5/13 [38.5%]), nausea (4/13 [30.8%]), anorexia (4/13 [30.8%]), vomiting (2/13 [15.4%]) and constipation (2/13 [15.4%]). All 13 treated patients died early due to progressive disease.

***Interpretation*:** Crizotinib is well tolerated, but lacks clinically meaningful activity as a single agent in patients with advanced metastatic ARMS. Assessing single agents in aggressive, chemotherapy-refractory ARMS is challenging and future trials should explore established chemotherapy +/- investigational compounds in earlier lines of treatment.

Clinical trial number: EORTC 90101, ClinicalTrials.gov NCT01524926

Keywords: Alveolar rhabdomyosarcoma; ARMS; metastasis; *FOXO1*; *ALK*; crizotinib

Highlights:

- Chemotherapy-refractory ARMS is a clinically aggressive disease commonly associated with *FOXO1* rearrangement, but a low incidence of *ALK* alterations.
- Crizotinib is well-tolerated, but has limited single-agent activity in chemotherapy-refractory ARMS without *ALK* rearrangement.
- Future trials in this disease should test conventional chemotherapy +/- novel agent.

Introduction

Rhabdomyosarcoma (RMS) is a rare malignancy, however it is the most common sarcoma in children and adolescents, with an incidence of approximately 50% of all soft tissue sarcomas in this age group [1,2]. In adult patients RMS is an orphan disease, accounting for only 3% of all soft tissue sarcomas [1-3]. There are different subtypes of RMS: pleomorphic, embryonal, alveolar (ARMS), and the botryoid and spindle cell variants [1,2,4]. Microscopically, ARMS consists of small densely packed round cells that resemble pulmonary alveoli, although a more solid variant has also been identified [1,2,5].

In ARMS, specific chromosomal translocations occur in 70-80% of patients [2,6]. The disease is typically characterized by a fusion of the paired box 3 (*PAX3)* or *PAX7* gene with forkhead box O1 (*FOXO1)* [1,7]. In approximately 60% of ARMS, translocation t(2;13)(q35;q14) occurs; while in about 20% of ARMS translocation t(1;13)(p36;q14) is found [1,2]. The t(2;13)(q35;q14) translocation results in the expression of its chimeric transcription factors *PAX3-FOXO1*, while the t(1;13)(p36;q14) translocation results in the expression of *PAX7-FOXO1* [1,2]. Both fusion genes encode the subsequent chimeric proteins, which are more abundant and transcriptionally more potent than their wild type counterparts [8-11]. Studies suggest that the presence of the PAX3-FOXO1 and PAX7-FOXO1 fusion proteins downstream contribute towards tumourigenesis [8,11]. PAX-FOXO1 stimulates tumour cell proliferation, angiogenesis, activates the myogenic program and inhibits apoptosis [2,12]. PAX3 is a main regulator of myogenesis while PAX7 induces satellite cell specification [1,13,14].

PAX3 activates the transcription of a number of target genes, involved in myogenic cells lineage, including *MET*, *MYOD* (myogenic differentiation 1), and *LBX1* (ladybird homeobox 1), and was shown to cause ligand-independent activation of MET in preclinical models [1,15-17]. *MET* encodes for the MET tyrosine kinase cell surface receptor, which is activated by its ligand hepatocyte growth factor (HGF), and MET phosphorylation in turn stimulates multiple signal pathways that play an important role in cell survival, proliferation, angiogenesis, migration, invasiveness, and metastasis [19-21]. The ARMS-specific PAX3-FOXO1 fusion leads to MET overexpression, frequently observed in this entity [1]. Although, Rees *et al* assessed the role of a putative hepatocyte growth factor (HGF)–MET pathway in in a panel of 68 clinical primary RMS samples and found MET was surprisingly a consistent feature of embryonal and not alveolar RMS [18].

Anaplastic lymphoma kinase (ALK) can also be overexpressed in RMS [22,23]. Studies have yielded conflicting results regarding the correlation between ALK positive staining and *PAX3/PAX7-FOXO1* fusion status, which are generally found to be independent events [22]. Aberrant ALK expression can result in phosphorylation of the ALK tyrosine kinase receptor and the subsequent abnormal activation of multiple downstream signalling cascades, including the Janus kinase (JAK)/signal transducer and activator of transcription (STAT), phosphatidylinositol-3 kinase/AKT (PI3-K/AKT), and RAS/extracellular signal-regulated kinase (ERK) pathways, which are associated with increased cell proliferation, prolonged tumour cell survival and enhanced cell migration [24-26]. ALK expression is an independent negative prognostic factor in ARMS [22].

Aberrant MET and ALK expression may promote resistance to chemotherapy resulting in poorer treatment outcome [1,3,22]. The presence of both MET and ALK pathway alterations in ARMS supports the assessment of crizotinib in this disease, as the drug inhibits both targets.

Crizotinib (Xalkori®, PF-02341066, Pfizer Inc.) is a small molecule tyrosine kinase inhibitor (TKI) targeting MET, ALK, ROS proto-oncogene 1 receptor tyrosine kinase (ROS1) and RON (Recepteur d'Origine Nantais) [27-31]. Crizotinib interferes with the ALK/MET pathways by competitively

preventing adenosine triphosphate (ATP) from binding to the ALK and MET receptors, therefore abrogating their phosphorylation [27-31]. This blocks the downstream cascade of events, thereby inhibiting the growth and survival of ALK or MET dependent cells [27-34]. Crizotinib is approved for the treatment of patients with advanced non-small cell lung cancer (NSCLC) whose tumours are either *ALK* or *ROS1* positive, and the recommended oral dose in adult patients is 250mg twice daily [29,30].

The European Organization for Research and Treatment of Cancer (EORTC) initiated a multinational, multi-tumour, prospective phase 2 clinical trial (EORTC 90101 "CREATE") to evaluate the efficacy and safety of crizotinib in patients with advanced tumours driven by MET and/or ALK alterations. CREATE included 6 disease-specific cohorts, and we report here the results of the independent ARMS cohort.

Materials and methods

Study design

This was a multicentre, single agent, non-randomized, open-label, two-stage phase 2 trial, assessing crizotinib in patients with locally advanced/ metastatic ARMS. The patient population was divided by protocol into *MET/ALK* altered and *MET/ALK* non-altered sub-cohorts, which were analysed separately.

Ethics approval was obtained for this study(ClinicalTrials.gov identifier NCT01524926), which was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonisation-Good Clinical Practice, and participating country and institution regulations.

Patient enrolment

Patient enrolment was based on a multi-step registration procedure. Step 1 prerequisites for registration were a local diagnosis of advanced ARMS deemed incurable by conventional surgery, radiotherapy or systemic therapy. Prior treatment with chemotherapy, the availability of a formalin-fixed paraffin embedded (FFPE) tumour-containing tissue block from primary tumour and/or metastatic site for trial purposes, and written informed consent of the patient for central collection of the tissue and all other trial-specific procedures were mandatory inclusion criteria for ARMS patients.

Criteria for step 2 included receipt of the tissue by a central biorepository (BioRep, Milan, Italy) with presence of tumour in the shipped material and confirmation of the correct diagnosis of ARMS by central reference pathology.

Screened patients were treated after completion of both steps, provided all other eligibility criteria were met. Details on the patient selection are described in the study protocol: (http://www.eortc.be/services/doc/protocols/90101v10.0.pdf)

Documentation of the presence of a specific rearrangement leading to *MET* and/or *ALK* alteration was not required for a patient to enter the treatment phase (step 3). FISH analysis was done while patients were already receiving therapy, to avoid delaying the start of treatment for patients in need for an experimental treatment, considering the aggressiveness of typical chemotherapy-refractory ARMS.

Treatment, safety and efficacy assessment

Eligible patients with centrally confirmed diagnosis of ARMS were treated with oral crizotinib at a starting dose of 250 mg twice daily. One treatment cycle was defined as 21 days in duration. Treatment was continued until documented disease progression, unacceptable toxicity, or patient refusal. Treatment dose and schedule modifications were defined in the protocol.

Safety information was collected at baseline, day 15 of cycle 1 and 2, and at the end of every cycle applying the Common Terminology Criteria for Adverse Events [CTCAE] version 4.0. Tumour assessments were performed every other cycle by the local investigator or radiologist according to RECIST 1.1 using computed tomography or magnetic resonance imaging. Objective responses were centrally reviewed.

Assessment of MET/ALK alterations in archival tumour tissue

Patients were attributed to *MET/ALK* altered or *MET/ALK* non-altered sub-cohorts on the basis of the presence or absence of a rearrangement of either *FOXO1* (synonym: *FHKR)* upregulation (as a surrogate of *MET*) and/or *ALK* in archival tumour tissue, using commercial fluorescence break apart probe sets (Vysis® LSI® *FKHR* (13q14) Dual Colour Break Apart Rearrangement Probe; and Vysis® LSI® *ALK* Dual Colour Break Apart Rearrangement Probe, both from Abbott Molecular). The archival tissue originated from either the primary tumour or a metastatic lesion. The FISH analysis was done at the University Hospitals Leuven, Leuven (Belgium) using unstained 4µm slides. The protocol required at least 15% of cells with re-arrangement for a positive test results. At least *FOXO1* or *ALK* had to be rearranged according to these criteria to define the tumour of a patient as being *MET/ALK* altered.

Outcomes

The main objective was to study the activity of crizotinib in ARMS patients with activated MET/ALK signalling. The primary endpoint was the ORR per RECIST 1.1 with response confirmation, assessed by the local investigator. This endpoint was chosen based on response pattern seen with crizotinib in the labelled indication of NSCLC and due to the relative absence of reliable reference data on PFS or PFR in adult patients with chemotherapy-refractory ARMS. Secondary endpoints included DOR, DCR, PFS, PFR, OS, safety, and correlative/translational research endpoints. DCR was defined as the percentage of patients achieving a complete (CR) or partial response (PR) or stable disease (SD), as best response.

Statistical analysis

A Simon's optimal two-stage design was implemented with the aim of excluding an ORR ≤10% under the alternative assumption that 30% ORR can be achieved with crizotinib in *MET/ALK* rearranged disease. The type I error and power were set at 10%. The study was conceptually focused on this genetically defined sub-cohort, while patients with *MET/ALK* non-altered tumours were supposed to serve as a non-randomized, treated internal control. The entry of "all comers" independent of their *MET/ALK* status avoided delaying treatment for patients in need of an active intervention, given the progression dynamics of chemotherapy-resistant ARMS, and to provide reference data for both subsets for future clinical trials. The entry of *MET/ALK* negative cases was considered ethical due to the lack of validated treatment alternatives for this disease after failure of chemotherapy. We expected the vast majority of cases to have a rearrangement of *FOXO1* and thus, using the *FOXO1* rearrangement as a surrogate, these patients were categorised as *MET* positive.

In stage 1, if at least two out of the first 12 eligible and evaluable *MET/ALK* altered ARMS patients achieved a confirmed RECIST PR or CR, a maximum of 35 patients were to be enrolled. In stage 2, if <6 out of the 35 eligible and evaluable patients responded, the treatment was declared ineffective. If ≥6 out of the 35 patients responded, further study of crizotinib was warranted.

Stopping rules and activity endpoints details are provided in the protocol. Analyses were performed using the SAS version 9.4(SAS Institute, Cary, United States).

Results

Patient disposition, reference pathology, clinical screening and enrolment

Between April 12, 2013 and November 4, 2016, 9 sites in 5 European countries recruited 20 patients with the local diagnosis of ARMS. Nineteen (95.0%) of these 20 patients had centrally confirmed ARMS. The non-confirmed, non-eligible case had no tissue available for reference pathology and could not be included, and did not enter the screening or treatment phase of the trial.

Thirteen of the 19 patients with centrally confirmed ARMS started treatment with crizotinib. Reasons for not entering the treatment phase in the 6 remaining patients were ineligibility (n=2), patient withdrawal (n=2) and rapid progression prior to study entry (n=2). Only 8 eligible patients with confirmed ARMS were evaluable for the primary and secondary endpoints of this trial due to early progression of many of the treated cancers. The trial profile is shown in figure 1.

Molecular epidemiology

FISH analysis was completed within a median time of 5 days (range: 1-13 days) after receipt of technically useful slides from the central biorepository. Among the 19 patients with centrally confirmed diagnosis, 18 (94.7%) had a *FOXO1* gene rearrangement, and none of them had an *ALK* gene rearrangement detected using the validated FISH probes. These patients were grouped together according to protocol as the *FOXO/ALK* rearranged subset (*MET+/ALK-*). Only one patient (5.3%) had no detectable *FOXO1* or *ALK* rearrangement (*MET-/ALK-*). An overview on all relevant genetic findings and treatment outcome per patient is shown in table 1.

Recruitment to both the *MET+/ALK-* and *MET-/ALK-* sub-cohorts was suspended early on November 4, 2016 due a high rate of early radiological and/or clinical progression on the experimental treatment, a decision endorsed by the trial steering committee and the EORTC Protocol Review Committee.

Patient characteristics

Among the total group of 19 patients with confirmed ARMS, 12 patients with *MET+/ALK-* disease and one patient with *MET-/ALK-* disease entered the treatment phase. Characteristics of the 13 treated patients are shown in table 2. One paediatric patient was included. The median age was 30.0 years (range: 6.0-48.0), 38.5% (5/13) had an ECOG PS of 1, all patients (13/13) had received prior chemotherapy and 38.5% (5/13) had undergone prior major surgery. The majority of patients had received at least three prior lines of therapy and 15% had previously undergone high-dose chemotherapy and bone marrow/stem cell support.

Crizotinib treatment

As of September 11, 2017, with a median follow-up of 154 days (range: 21-212), all patients have stopped receiving treatment. The mean relative dose intensity was 97.7%. None of the patients had dose reductions or dose modifications, but one patient interrupted crizotinib due to haematological adverse events. Total treatment duration with crizotinib ranged from 7-103 days, with a very short median duration of 22 days and a median number of treatment cycles of only 1 (range: 1-5) in the *MET+/ALK-* sub-cohort. All 13 patients came off study due to disease progression. This included 5 patients with symptomatic deterioration who according to local investigator did not qualify for radiological confirmation of disease progression due the aggressiveness of the underlying malignancy.

Activity of crizotinib

Among the 13 eligible and treated patients, 8 were evaluable with at least one RECIST assessment after treatment start. A single, short lasting, confirmed partial response was observed in one of 7 evaluable *MET+/ALK-* patients (14.3% ORR; 95% confidence interval (CI): 0.3-57.8%; DOR: 52 days). The single evaluable *MET-/ALK-* patient did not achieve an objective response. Key efficacy data are summarized in table 3.

None of the remaining eligible and evaluable patients in this trial achieved RECIST SD. Disease progression was the best response in 6 out of 7 *MET+/ALK-* patients (85.7%). The *MET-/ALK-* patient had disease progression at the first on treatment assessment.

Median PFS was 1.3 months (95% CI: 0.5-1.5) in *MET+/ALK-* cases. All patients progressed within 4 months after treatment start. The Kaplan-Meier estimates for PFS are shown in figure 2A. Median OS was 5.6 months (95% CI: 0.7-7.0) in *MET+/ALK-* cases and the OSR at 6 months was only 28.6% (95% CI: 4.1-61.2%). The Kaplan-Meier estimates for OS are shown in figure 2B. All patients entering the treatment phase of this trial died early due to progression of ARMS.

Figure 2C illustrates the maximum target lesion shrinkage, figure 2D summarizes the poor clinical course of the treated patients.

Safety and toxicity

No new or unexpected safety signals for crizotinib were detected in the ARMS patients. The most common treatment-related adverse events were fatigue (38.5% of patients), nausea (30.8%), anorexia (30.8%), vomiting (15.4%) and constipation (15.4%). The reported treatment-related grade 3 adverse events were fatigue (2 patients), no grade 4 events were observed. Adverse events details are shown in Tables 4A and 4B.

Serious adverse events included a systemic inflammatory response syndrome (1 patient), respiratory infection (1 patient), dehydration (1 patient) and chest pain (1 patient). Only the first event was considered possibly related to study treatment.

A total number of 5 deaths occurred on treatment or within 4 weeks of treatment discontinuation, but none of them was treatment related.

Discussion

Alveolar rhabdomyosarcoma belongs to the expanding group of sarcomas characterized by fusions of the *PAX3* or *PAX7* gene with *FOXO1* [1,2,6,7]. PAX3 activates the transcription of a number of target genes, including *MET* [1,15-17]. The MET receptor is significantly overexpressed in ARMS

[1]. In addition to MET and likely unrelated to the *PAX3/PAX3-FOXO1* fusion status, ALK can be overexpressed in RMS [22].

The theoretical presence of both MET and ALK pathway alterations in ARMS provided a strong rationale to test the MET, ALK and ROS1 TKI crizotinib in this disease. Preclinical studies have shown that crizotinib can block the downstream cascade of events as described above [26,29], thereby inhibiting the growth and survival of ALK or MET dependent cells, which translates into impressive anti-tumour effects of the compound in the labelled indication of NSCLC [29].

We were not able to demonstrate clinically meaningful activity of crizotinib in adult patients with chemotherapy-refractory ARMS. Only one patient had a short objective response. A striking finding was that none of our non-responding patients achieved disease stabilization according to RECIST, which underscores the clinical aggressiveness of the disease. To this end, some patients could not even enter the study due to rapid progression during trial screening; many others had early disease progression on crizotinib preventing further imaging assessments, and all died within less than 6 months after study entry due to progressive ARMS. This made a significant proportion of our study population non-evaluable for the primary endpoint, which led to the ethical decision by the Steering Committee to discontinue recruitment of further patients to this arm of EORTC 90101 "CREATE", before having reached the critical number 12 eligible ARMS patients with gene alterations evaluable for response. Only 7 *MET*+/*ALK*- patients were evaluable for response, after recruitment of 19 patients with documented diagnosis of ARMS.

The poor outcome of crizotinib treatment in rhabdomyosarcoma observed in our study is similar to findings in other recent clinical trial, e.g. a phase 2 study by Schuetze *et al* with dasatinib where only 1/13 patients achieved stable disease with a median PFS of 0.9 months [35], and a phase 2 trial by Pappo *et al* with R1507 (a monoclonal antibody to the insulin-like growth factor-1 receptor) that achieved 1 PR, 3 unconfirmed PR and median PFS of 5.6 weeks in 36 enrolled patients [36]. The results of these trials support the conclusion that previously treated RMS is a rapidly progressive, aggressive cancer and new trial designs are needed to test novel agents in this disease.

While *FOXO1* alterations were present in all but one centrally confirmed ARMS case in our trial, no patient had an *ALK* gene rearrangement based on the use of a validated commercial FISH probe. We did not perform additional immunohistochemistry for ALK or other tests in the available tissue samples, since we believe that ALK may not be a relevant target in our series in the absence of a genetic event and the reported overexpression of ALK in ARMS is rather a passenger effect [37]. While expression of MET was not tested in this cohort of patients we assume that based on the lack of clinical benefit in this patient cohort, any MET activation would play a minor role in driving the disease. *PAX3* had been shown to activate MET during muscle development thus it may be more relevant for lineage-specific differentiation than as pro-survival pathway [18]. Pandey *et al* recently published that upon recurrence, tumor cells gain increasing independence from the PAX3-FOXO1 mechanism [38]. This supports the need for fresh biopsies in clinical trials with targeted agents.

A recent integrated genetic and epigenetic analysis on RMS revealed that 32% of patients had genomic dysregulation of signalling intermediates activating the RAS/RAF and the PI3-K pathway [39]. While these genes have not been sequenced in our study they are known to confer resistance to kinase inhibitors.

Based on our observations, heavily pre-treated ARMS represent a subgroup of patients with a particularly aggressive disease and poor prognosis. The high risk of losing patients quickly while on a single, targeted drug suggests it would be better to test novel therapies as an earlier treatment line [40], preferably in combination with chemotherapy – unless the genomic marker is more predictive. Chemotherapy can also influence expression levels of RTKs, which possibly provides another

reason to test targeted therapy in earlier lines of treatment. In theory it could be more accurate to test post-chemotherapy resection specimens for target expression levels, although repetitive sampling in such patients may be challenging, especially in symptomatic patients with rapidly progressive disease. An alternative approach would be to test novel targeted agents in the context of ongoing or planned RMS trials, randomizing patients to conventional chemotherapy +/- novel agent. This concept has a long tradition mainly in paediatric sarcoma trials, and may also apply here.

The investigators of EORTC 90101 only entered one paediatric patient with ARMS. Our efficacy findings cannot be extrapolated to younger patients with other subtypes of rhabdomyosarcoma, nevertheless our data are consistent with those from a clinical trial of ceritinib in paediatric patients including ARMS [41].

In summary, crizotinib is well tolerated but does not have clinically relevant activity as a single agent in adult patients with chemotherapy-refractory ARMS.

Contributors

PS developed and designed the trial, proposed it to EORTC and Pfizer, and searched the published reports. Protocol writing was a collaboration effort between PS, EORTC Headquarters staff and AW. RS was responsible for reference pathology and MDR performed the FISH analysis. PS, AW, ML, SA, PR, SB, GF, VG, RS and SS contributed to patient accrual and data collection. SC did the data analysis and PS, SM, AN and SC oversaw the management of the clinical trial and data collection. AN maintained the trial database. PS coordinated reference pathology, FISH testing and the central review of radiological images, supported by AW. BG was member of the trial steering committee and provided paediatric expertise.

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Role of funding source

This work was an investigator-initiated trial. EORTC was the legal sponsor. Pfizer Inc. provided the investigational agent and funding, but had no role in the study design, data collection, analysis, interpretation, writing of the report, or decision to publish this report. The database is held by EORTC, and EORTC statisticians performed the analysis.

The lead investigator had access to all data in the study, was responsible for providing regular information to the relevant committees monitoring this trial and had final responsibility for the decision to submit for publication. All authors were responsible for data interpretation and final approval of the manuscript for submission.

Conflict of interest

PS: no competing interest
AW: no competing interest
ML: no competing interest
SA: no competing interest
PR: received honoraria from Pfizer outside the scope of this study
SB: received honoraria from Pfizer for consulting and educational activities
SR: no competing interest
VG: received honoraria from Bristol-Myers Squibb, Novartis, Pfizer; advisory board for Bristol-Myers Squibb, Novartis, Pfizer, Roche; received travel grant from Bristol-Myers Squibb, MSD, Novartis, Pfizer
MDR: no competing interest
RS: no competing interest
BG: no competing interest

SM: no competing interest
SC: no competing interest
AN: no competing interest
SS: received honoraria from Lilly for educational activities

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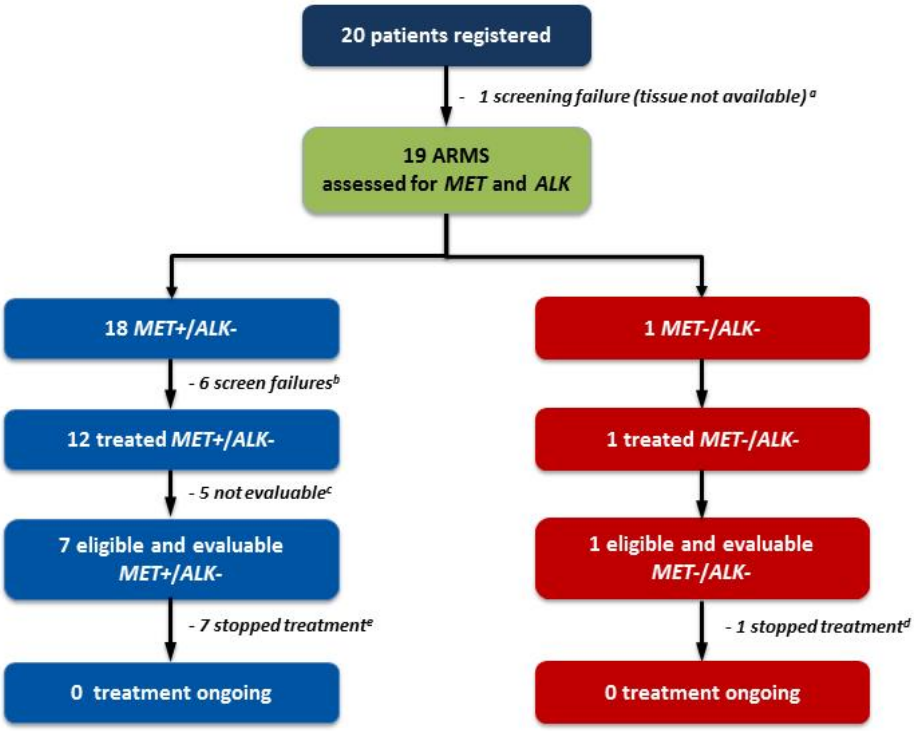
Figure 1. Recruitment of alveolar rhabdomyosarcoma patients in EORTC 90101.



[a]:1 tissue not available; [b]: 2 patient decision, 2 ineligible (1 no measurable lesion, 1 elevated alanine transaminase), 2 progressive disease (1 spinal cord compression, 1 general deterioraton of health status); [c]: symptomatic progressive disease without radiololgical documentation ; [d,e]: progressive disease

Table 1. Molecular characteristics of centrally confirmed alveolar rhabdomyosarcomas in this trial and their response to crizotinib.

Patient number	Origin of tested archival tumour material	*FOXO1* (MET pathway) gene rearrangement by FISH (% of positive tumour cells)	*ALK* gene rearrangement by FISH (% of positive tumour cells)	Treatment status	Duration of treatment		Best RECIST response	Survival status	Overall survival (days)
					Cycles	Days			
MET+/*ALK*- patients									
47	Metastatic	87	0	Stopped	5	91	PR	Death	173
50	Metastatic	15	0	Stopped	2	42	PD	Death	260
83	Primary	75	2	Stopped	1	22	Missing	Death	34
100	Primary	91	0	Not treated	-	-	-	-	-
102	Metastatic	81	0	Stopped	1	13	Missing	Death	16
104	Primary	77	0	Stopped	1	22	PD	Death	41
111	Primary	93	0	Stopped	1	21	Missing	Death	62
119	Primary	73	0	Not treated	-	-	-	-	-
130	Primary	76	0	Stopped	2	44	PD	Death	170
136	Metastatic	74	0	Stopped	2	29	PD	Death	101
152	-	48	0	Not treated	-	-	-	-	-
162	Primary	27	0	Stopped	1	13	Missing	Death	44
174	Primary	79	0	Stopped	2	41	PD	Death	212
179	Primary	95	0	Stopped	1	8	Missing	Death	14
181	Primary	84	4	Stopped	1	PD	PD	Death	21
183	Primary	95	0	Not treated	-	-	-	-	-
184	Primary	85	0	Not treated	-	-	-	-	-
187	Metastatic	95	0	Not treated	-	-	-	-	-
MET-/*ALK*- patient									
11	Primary	0	0	Stopped	2	38	PD	Death	138

Legend: Missing, clinical progression without radiological confirmation; PD, progressive disease; RECIST, Response Evaluation Criteria in Solid Tumours

Table 2. Key patient characteristics.

	MET/ALK status		
	MET+/ALK- (N=12)	*MET-/ALK-* (N=1)	Total (N=13)
Age (years)			
Median	28.5	32.0	30.0
Range	16.0 - 48.0	32.0 - 32.0	16.0 - 48.0
Eastern Cooperative Oncology Group performance status			
0	1 (8.3%)	1 (100.0%)	2 (15.4%)
1	5 (41.7%)	0 (0.0%)	5 (38.5%)
2	6 (50.0%)	0 (0.0%)	6 (46.2%)
Sex			
Male	11 (91.7%)	0 (0.0%)	11 (84.6%)
Female	1 (8.3%)	1 (100.0%)	2 (15.4%)
Any previous major surgery	4 (33.3%)	1 (100.0%)	5 (38.5%)
Any prior systemic anticancer therapy	12 (100.0%)	1 (100.0%)	13 (100.0%)
Chemotherapy	12 (100.0%)	1 (100.0%)	13 (100.0%)
Autologous or allogenic stemcell or bone marrow transplant	2 (16.7%)	0 (0.0%)	2 (15.4%)
Prior systemic treatments			
Neo-adjuvant	1 (8.3%)	1 (100.0%)	2 (15.4%)
Adjuvant	3 (25.0%)	1 (100.0%)	4 (30.8%)
Maintenance	4 (33.3%)	0 (0.0%)	4 (30.8%)
1st line	12 (100.0%)	0 (0.0%)	12 (92.3%)
2nd line	11 (91.7%)	0 (0.0%)	11 (84.6%)
3rd line	9 (75.0%)	0 (0.0%)	9 (69.2%)
4th line	4 (33.3%)	0 (0.0%)	4 (30.8%)
5th line	1 (8.3%)	0 (0.0%)	1 (7.7%)
More than 5th line	1 (8.3%)	0 (0.0%)	1 (7.7%)

Table 3. Response assessment and efficacy summary, according to investigator assessment.

	MET/ALK status		
	MET+/ALK- (N=7) N (%)	*MET-/ALK-* (N=1) N (%)	Total (N=8) N (%)
Best RECIST 1.1 response			
Partial response	1 (14.3%)	0 (0.0%)	1 (12.5%)
Progressive disease	6 (85.7%)	1 (100.0%)	7 (87.5%)
Objective Response rate	14.3%	0%	12.5%
(95% CI)	(0.3 -57.8)	(-)	(0.3-52.6)
Disease control rate	14.3%	0%	12.5%
(95% CI)	(0.3 -57.8)	(-)	(0.3-52.6)
Progression-free survival			
Progression of ARMS or died	7 (100.0%)	1 (100.0%)	8 (100.0%)
6-months progression-free survival rate	0.0%	0.0%	0.0%
(95% CI)	(-)	(-)	(-)
Survival status			
Dead	7 (100.0)	1 (100.0%)	8 (100.0%)
Reason of death			
Progression of ARMS	7 (100.0)	1 (100.0%)	8 (100.0%)
6-months survival rate	28.6%	0.0%	25.0%
(95% CI)	(4.1, 61.2)	(-)	(3.7, 55.8)

Legend: CI, confidence interval

Figure 2A. Kaplan-Meier estimates for progression-free survival for the *MET+/ALK-* and *MET-/ALK-* sub-cohorts per protocol.

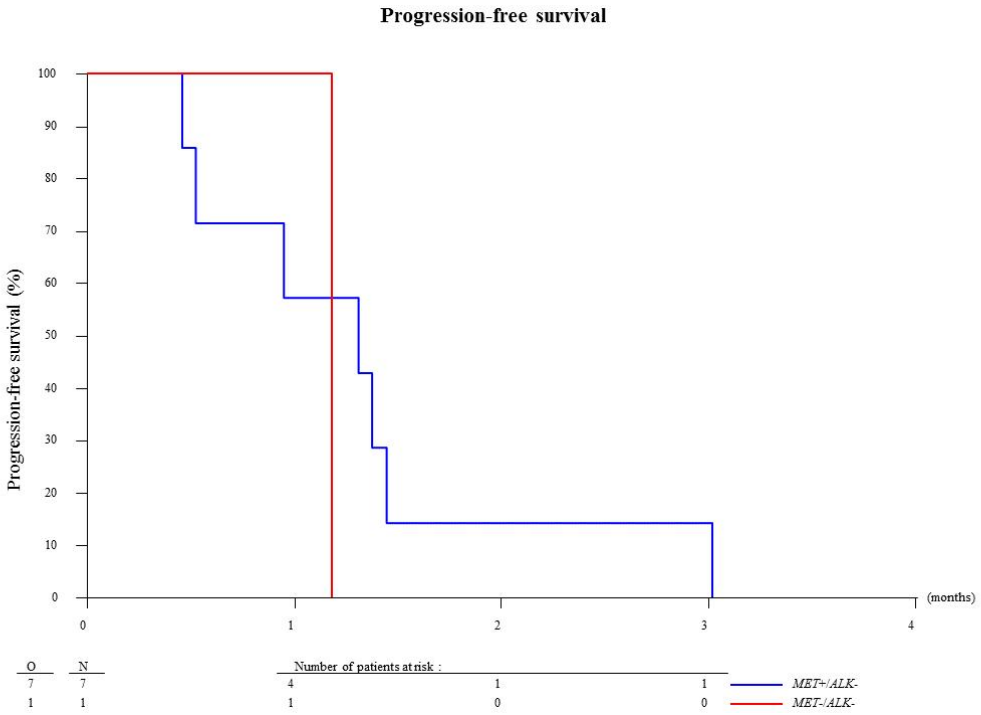
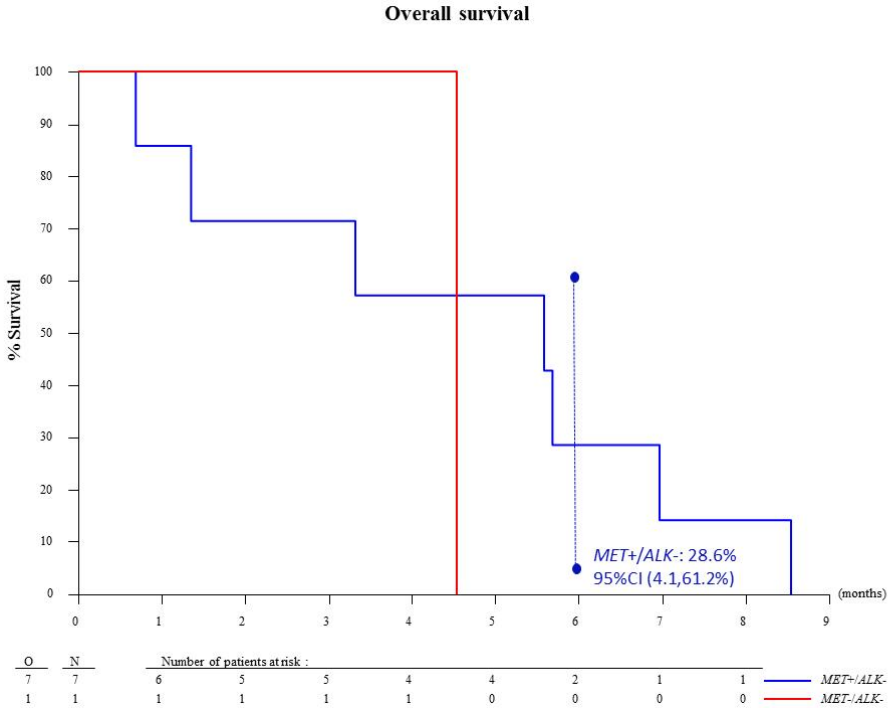
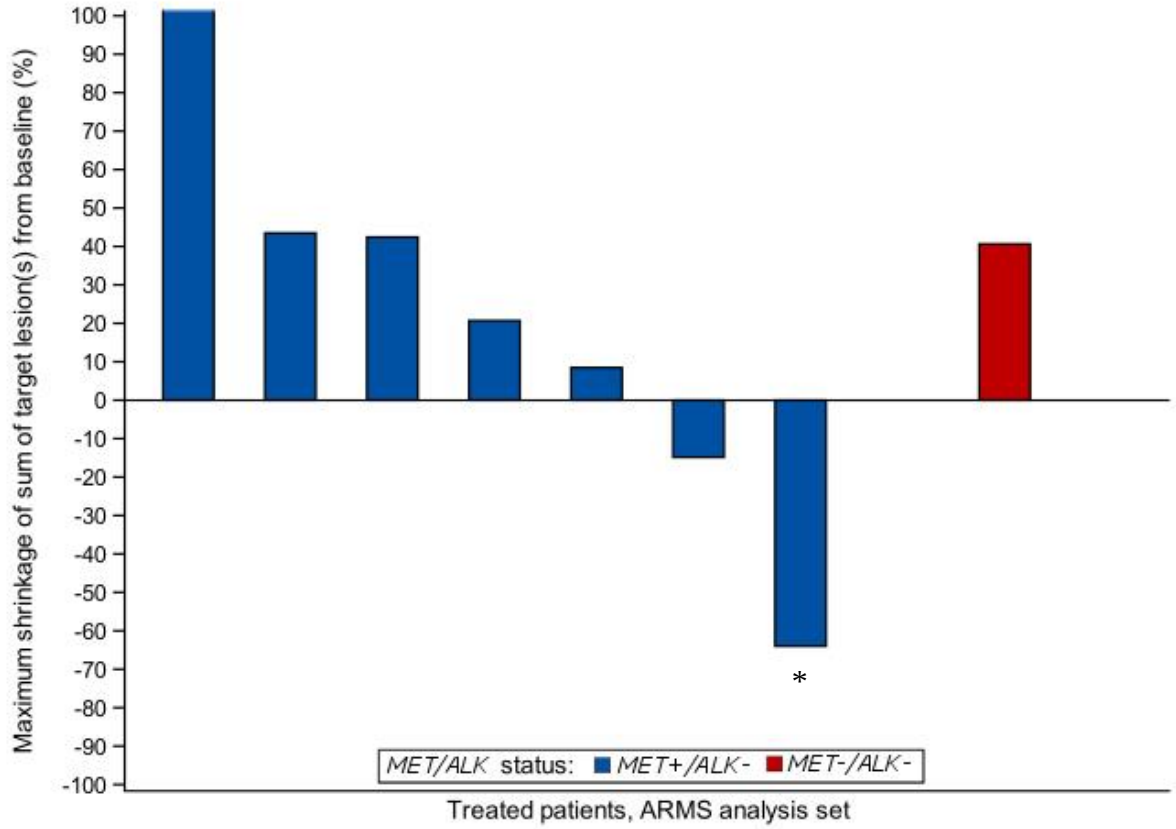


Figure 2B. Kaplan-Meier estimates for overall survival for the *MET+/ALK-* and *MET-/ALK-* sub-cohorts per protocol.



Legend: The vertical bar represent the 95% confidence interval (CI), for the 6 month estimate of the overall survival rate.

Figure 2C. Maximum shrinkage of target lesions (per protocol) in the *MET+/ALK-* and *MET-/ALK-* sub-cohorts, according to local investigator's assessment.



Legend: *Short-lasting objective and confirmed RECIST 1.1 partial response.

Figure 2D. Clinical course of patients in the alveolar rhabdomyosarcoma *MET+/ALK-* and *MET-/ALK-* sub-cohorts.

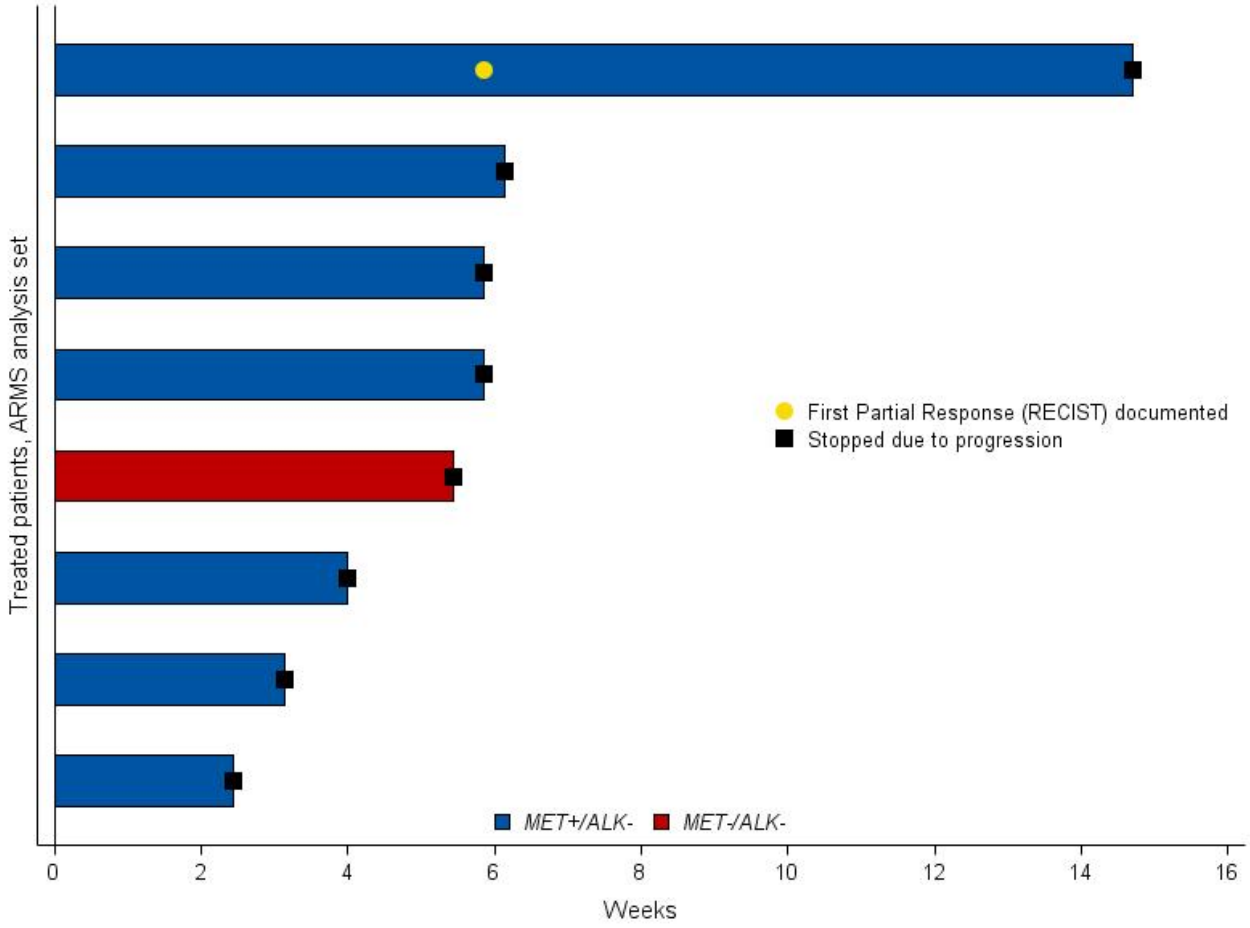


Table 4. Non-haematological adverse events that occurred in ≥ 10% of patients.

	All adverse events Present in ≥10% of patients (Safety population, N=13)						Treatment-related adverse events Present in ≥10% of patients (Safety population, N=13)					
CTC + MedDRA Term	Gr 1 N (%)	Gr 2 N (%)	Gr 3 N (%)	Gr 4 N (%)	Gr ≥3 N (%)	All grades N (%)	Gr 1 N (%)	Gr 2 N (%)	Gr 3 N (%)	Gr 4 N (%)	Gr ≥3 N (%)	All grades N (%)
NUMBER OF PATIENTS WITH AE's	2 (15.4)	5 (38.5)	4 (30.8)	2(15.4)	6 (46.2)	13 (100.0)	2 (15.4)	2 (15.4)	3 (23.1)		3 (23.1)	7 (53.8)
GASTROINTESTINAL DISORDERS												
Constipation	3 (23.1)	3 (23.1)				6 (46.2)	1 (7.7)	1 (7.7)				2 (15.4)
Diarrhoea	3 (23.1)					3 (23.1)						
Nausea	4 (30.8)	4 (30.8)				8 (61.5)	1 (7.7)	3 (23.1)				4 (30.8)
Vomiting	4 (30.8)					4 (30.8)	2 (15.4)					2 (15.4)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS												
Fatigue	1 (7.7)	3 (23.1)	3 (23.1)		3 (23.1)	7 (53.8)	1 (7.7)	2 (15.4)	2 (15.4)		2 (15.4)	5 (38.5)
Fever	2 (15.4)					2 (15.4)						
Pain	1 (7.7)	1 (7.7)				2 (15.4)						
Other AE	1 (7.7)		3 (23.1)		3 (23.1)	4 (30.8)						
INVESTIGATIONS												
Weight loss	2 (15.4)					2 (15.4)						
METABOLISM AND NUTRITION DISORDERS												
Anorexia	2 (15.4)	3 (23.1)				5 (38.5)	2 (15.4)	2 (15.4)				4 (30.8)
Dehydration	1 (7.7)		1 (7.7)		1 (7.7)	2 (15.4)						
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL. CYSTS AND POLYPS)												
Tumour Pain		2 (15.4)				2 (15.4)						
RENAL AND URINARY DISORDERS												
Other AE	1 (7.7)		1 (7.7)		1 (7.7)	2 (15.4)						
RESPIRATORY THORACIC AND MEDIASTINAL DISORDERS												
Dyspnoea	1 (7.7)	4 (30.8)				5 (38.5)						
Pleural Effusion		1 (7.7)	1 (7.7)		1 (7.7)	2 (15.4)						
SK SUBCUTANEOUS TISSUE DISORDERS												
Alopecia	1 (7.7)	1 (7.7)				2 (15.4)						

Legend: AE, Adverse event; Gr: Grade; CTC, Common Terminology Criteria

Table 5. Haematological and biochemical adverse events that occurred in ≥ 10% of patients.

	Grade1	Grade2	Grade3	Grade4	N (%)
Alkaline phosphatase	3	1			4 (30.8)
Anaemia		2	1		3 (23.1)
SGPT	2		1		3 (23.1)
Hypocalcaemia	1	1	1		3 (23.1)
Serum creatinine	4	1			5 (38.5)
Hyperglycaemia	2	1			3 (23.1)
Hyperkalaemia	2				2 (15.4)
Hyponatremia	2		3		5 (38.5)

Legend: Treatment emergent effects. Relationship not collected for these laboratory events; SGPT, serum glutamic-pyruvic transaminase.