Title: Antitumor Activity of Lurbinectedin, a Selective Inhibitor of Oncogene Transcription, in Patients with Relapsed Ewing Sarcoma: Results of a Basket Phase II Study

Authors: Vivek Subbiah,[1] Irene Braña,[2] Alessandra Longhi,[3] Valentina Boni,[4] * Jean-Pierre Delord,[5] Ahmad Awada,[6] Pascaline Boudou-Rouquette,[7] John Sarantopoulos,[8] Geoffrey I. Shapiro,[9] Anthony Elias,[10] Ravin Ratan,[1] Cristian Fernandez,[11] Carmen Kahatt,[11] Martin Cullell-Young,[11] Mariano Siguero,[11] Ali Zeaiter,[11] and Sant P. Chawla.[12]

Affiliations:

1. The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
2. Hospital Universitari Vall d'Hebron, Barcelona, Spain.
3. Istituti Ortopedici Rizzoli, Bologna, Italy.
4. START Madrid–Centro Integral Oncológico Clara Campal, Hospital Universitario Madrid Sanchinarro, Madrid, Spain.
5. Institut Claudius Regaud, Toulouse, France.
6. Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium.
7. Hôpital Cochin, Paris, France.
8. Cancer Therapy & Research Center, San Antonio, TX, USA.
9. Dana-Farber Cancer Institute, Boston, MA, USA.
10. University of Colorado Cancer Center, Aurora, CO, USA.
11. PharmaMar, Colmenar Viejo, Madrid, Spain.

12. Sarcoma Oncology Center, Santa Monica, CA, USA.

* V. Boni is currently working at NEXT Madrid, Universitary Hospital QuironSalud Madrid, Madrid, Spain.

Running title: Antitumor activity of lurbinectedin in relapsed ES.

Key words: Lurbinectedin, phase II study, Ewing sarcoma.

Corresponding author: Vivek Subbiah, M.D.

Department of Investigational Cancer Therapeutics, Division of Cancer Medicine,

The University of Texas MD Anderson Cancer Center,

1515 Holcombe Blvd., Houston, TX, 77030, USA

Phone: 713-563-1930

Fax: 713-792-0334

E-mail: vsubbiah@mdanderson.org

Financial support:

The study was funded by Pharma Mar S.A, including grants from the Centro para el Desarrollo Tecnológico Industrial (CDTI) during the conduct of the study. **V. Subbiah** is an Andrew Sabin Family Foundation Fellow at The University of Texas MD Anderson Cancer Center. V. Subbiah acknowledges support of The Jacquelyn A. Brady Fund. V. Subbiah is supported by NIH grant R01CA242845. MD Anderson Cancer Center Department of Investigational Cancer Therapeutics is supported by the Cancer Prevention and Research Institute of Texas (RP1100584), the Sheikh Khalifa Bin Zayed

Al Nahyan Institute for Personalized Cancer Therapy (1U01 CA180964), NCATS Grant UL1 TR000371 (Center for Clinical and Translational Sciences), and the MD Anderson Cancer Center Support Grant (P30 CA016672).

Potential conflicts of interest:

V. Subbiah reports grants from Eli Lilly/LOXO Oncology, Blueprint Medicines Corporation, Turning Point Therapeutics, Boston Pharmaceuticals; and grants from Helsinn Pharmaceuticals during the conduct of the study; in addition, V. Subbiah reports a grant and advisory board/consultant position with Eli Lilly/Loxo Oncology during the conduct of the study; research grants from Roche/Genentech, Bayer, GlaxoSmithKline, Nanocarrier, Vegenics, Celgene, Northwest Biotherapeutics, Berghealth, Incyte, Fujifilm, D3, Pfizer, Multivir, Amgen, Abbvie, Alfa-sigma, Agensys, Boston Biomedical, Idera Pharma, Inhibrx, Exelixis, Blueprint Medicines, Altum, Dragonfly Therapeutics, Takeda, National Comprehensive Cancer Network, NCI-CTEP, University of Texas MD Anderson Cancer Center, Turning Point Therapeutics, Boston Pharmaceuticals, Novartis, Pharma Mar, Medimmune; an advisory board/consultant position with Helsinn, Incyte, QED Pharma, Daiichi-Sankyo, Signant Health, Novartis, Relay therapeutics, Roche, Medimmune; travel funds from Pharma Mar, Incyte, ASCO, ESMO; other support from Medscape; all outside the submitted work. **V. Boni** reports employment as Director of Clinical Cancer Research, NEXT Madrid, Universitary Hospital QuirónSalud Pozuelo; institutional financial support for clinical trials from Abbvie, ACEO, Adaptaimmune, Amcure, AMGEN, AstraZeneca, BMS Cytomx, GSK, Genentech/Roche, H3, Incyte, Janssen, Kura, Lilly, Loxo, Nektar, Macrogenics, Menarini, Merck, Merus, Nanobiotix, Novartis, Pfizer, PharmaMar, Principia, PUMA, Sanofi, Taiho, Tesaro, BeiGene, Transgene, Takeda,

Incyte, Innovio, MSD, PsiOxus, Seattle Genetics, Mersana, Daiichi, Nektar, Astellas, ORCA, Boston Therapeutics, Dynavax, DebioPharm, Boehringer Ingelheim, Regeneron, Millennium, Synthon, Spectrum, Rigontec, and Zenith; advisory board/consultant position with Puma Biotechnology, Ideaya Biosciences, Loxo Therapeutics, CytomX Therapeutics, Guidepoint, and Oncoart; and honoraria (for speaking) from Eli Lilly; all outside the submitted work. **J-P. Delord** reports research funding from Genentech, Bristol-Myers Squibb, MSD Oncology, Astra Zeneca, and Transgene; and an advisory board/consultant position with Novartis, Roche/Genentech, Bristol-Myers Squibb, MSD Oncology and Pierre Fabre; all outside the submitted work. **P. Boudou-Rouquette** reports travel and accommodation fees from Pharma Mar; and company honoraria from Ipsen; all outside the submitted work. **C. Kahatt**, and **A. Zeaiter** report personal fees for salary as full time employee and stock ownership from Pharma Mar, outside the submitted work. **C. Fernandez**, **M. Cullell-Young**, and **M. Siguero** report personal fees for salary as full time employee from Pharma Mar, outside the submitted work. All remaining authors have declared no conflicts of interest.

Word count for text: 2,625.

Word count for abstract: 245.

Figure/Table count: 2/4.

Reference count: 34.

STATEMENT OF TRANSLATIONAL RELEVANCE

Novel therapeutic agents are needed for patients with relapsed/refractory Ewing sarcoma (ES) who have a dismal prognosis. Lurbinectedin blocks transcription and induces DNA double-strand breaks, leading to apoptosis. In pre-clinical models it was shown that lurbinectedin is effective in suppressing the activity of the oncogenic transcription factor EWS-FLI1 through relocalization to the nucleolus and delayed tumor growth in mice bearing Ewing sarcoma xenografts. This Basket clinical trial demonstrated clinical antitumor activity of lurbinectedin with an objective response rate of 14.3%, clinical benefit rate (response or disease stabilization for ≥4 months) of 39.3% and disease control rate (response or disease stabilization of any duration) of 57.1% in a cohort of patients with relapsed ES. Lurbinectedin could represent a valuable addition to relapsed ES, which constitutes a highly unmet medical need.

ABSTRACT

Purpose: Lurbinectedin suppresses the oncogenic transcription factor EWS-FLI1 through relocalization to the nucleolus, and delays tumor growth in mice bearing Ewing sarcoma xenografts. Based on this rationale, lurbinectedin was evaluated in patients with relapsed Ewing sarcoma (ES).

Patients and methods: This open-label, single-arm, Basket phase II trial included a cohort of 28 treated adult patients with confirmed ES, measurable disease as per Response Evaluation Criteria In Solid Tumors (RECIST) v.1.1, Eastern Cooperative Oncology Group performance status ≤2, adequate organ function, no central nervous system metastasis, and pre-treated with ≤2 chemotherapy lines for metastatic/recurrent disease. Patients received lurbinectedin 3.2 mg/m^2 as a 1-hour infusion every 3 weeks. Primary endpoint was overall response rate (ORR) as per RECIST v.1.1. Secondary endpoints included time-to-event parameters and safety profile.

Results: ORR was 14.3% (95% confidence interval [CI], 4.0-32.7%), with median duration of response of 4.2 months (95%CI, 2.9-5.5 months). Median progression-free survival was 2.7 months (95%CI, 1.4-4.3 months), clinical benefit rate was 39.3% and disease control rate was 57.1%. With 39% censoring, median overall survival was 12.0 months (95%CI, 8.5-18.5 months). Most common grade 3/4 adverse events were neutropenia (57%), anemia, thrombocytopenia, and treatment-related febrile neutropenia (14% each). No deaths or discontinuations were due to toxicity.

Conclusions: Lurbinectedin was active in the treatment of relapsed ES and had a manageable safety profile. Lurbinectedin could represent a valuable addition to therapies for ES, and is currently being evaluated in combination with irinotecan in advanced ES in a phase Ib/II trial.

Trial code: ClinicalTrials.gov identifier: NCT02454972.

INTRODUCTION

Ewing sarcoma (ES), formerly referred to as the Ewing family of tumors (EFTs), is an aggressive form of sarcoma that comprises malignancies such as classic ES, peripheral neuroectodermic tumors (PNET) and Askin tumor. ES is the second most common malignant bone tumor among children, adolescents and young adults, striking them in the prime of their lives (1). ES may also appear in soft tissue, with the most common sites being trunk and limbs (2). The average incidence of ES is 2.93 cases per million per year (3), and most patients are under the age of 20 years.

The prognosis of ES varies depending on primary tumor site, presence of metastases and tumor size. First-line treatment with surgery, radiotherapy and multi-agent chemotherapy has resulted in 5-year disease-free survival rates of 60-70% in patients with localized sarcoma, but less than 20% if metastases are present at diagnosis (4), with an inferior outcome being observed in patients younger than 18 years (5, 6). There is no established treatment for relapsed ES. Management of relapsed disease mostly consists of different combinations of the same agents used as prior therapy (e.g., alkylators or topoisomerase inhibitors), radiotherapy of lung and bone, and surgical removal of metastases. However, failure on second-line therapy is very common, and the agents used are associated with short- and long-term toxicity (7, 8). Patients with relapsed ES have a dismal prognosis, with a 5-year survival rate of 13% (9). Development of therapeutics in ES is challenging due to its rarity and the absence of classic kinase targets (10). Therefore, there is an urgent need for new therapeutic agents with different mechanisms of action to manage this patient population.

Lurbinectedin (Zepzelca®) is a synthetic tetrahydroisoquinoline alkaloid structurally related to trabectedin that inhibits oncogenic transcription primarily through binding to guanine-rich DNA sequences around gene promoters, thereby altering the 3D

DNA structure and evicting oncogenic transcription factors from their binding sites (11-13). Lurbinectedin adducts may also inhibit mRNA synthesis and induce the ubiquitination and degradation of RNA polymerase II (14), and favor the production of DNA double-strand breaks and trigger apoptotic cell death (15). Lurbinectedin has received US Food and Drug Administration (FDA) accelerated approval for treatment of patients with metastatic small cell lung cancer (SCLC) with disease progression on or after platinum-based chemotherapy (16). A previous phase II study had shown efficacy for trabectedin in pretreated patients with advanced ES, including 3 partial responses (PRs) and 7 disease stabilizations in a cohort of 20 patients; progression-free survival (PFS) rate at 6 months was 25% (17). Pre-clinical studies showed that lurbinectedin is more effective than trabectedin in suppressing the activity of the oncogenic transcription factor EWS-FLI1 in mice through relocalization to the nucleolus (18, 19). *In vivo*, administration of lurbinectedin delayed tumor growth in mice bearing ES xenografts (19). Lurbinectedin also showed an improved therapeutic index relative to trabectedin, with suppression of EWS-FLI1 activity observed in mice at clinically achievable concentrations (19). Compared with trabectedin, lurbinectedin had a more favorable pharmacokinetic (PK) profile, as suggested by a higher recommended dose (RD) and greater exposure values at the RD when administered as single-agent every three weeks (q3wk) (20, 21).

This study evaluated the monotherapy activity of lurbinectedin in terms of response rate, progression free survival, clinical benefit rate and disease control rate in a cohort of patients with relapsed ES.

MATERIALS AND METHODS

This single-arm, open-label, Basket phase II trial evaluated the efficacy and safety of lurbinectedin in nine cohorts of patients with difficult-to-treat tumors. This report is focused on the cohort of patients with ES (labelled as EFTs in the study protocol) treated at 11 sites in Belgium, France, Italy, Spain and the U.S. The trial was conducted in compliance with ICH Good Clinical Practice guidelines. The protocol was approved by the centers' Research Ethics Committees. Signed written informed consent was obtained for each patient before study-specific procedures. The trial is registered at https://www.clinicaltrials.gov as NCT02454972.

Eligibility Criteria

Eligible patients were aged ≥18 years; with ES previously treated with ≤2 chemotherapy lines in the metastatic/recurrent setting; measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) v.1.1 (22) and documented disease progression; Eastern Cooperative Oncology Group performance status score ≤2; and adequate bone marrow, hepatic, renal and metabolic function who had recovered from any previous toxicities.

Patients were excluded if they had been pretreated with lurbinectedin or trabectedin; had prior/concurrent malignant disease (unless in complete remission for >5 years); had impending need for radiotherapy; were pregnant or lactating women, or women of childbearing potential who were not using effective contraceptives; or had central venous system (CNS) involvement, relevant cardiac disease, severe dyspnea or daily intermittent oxygen requirement, active infection, unhealed wounds, external drainages, immunocompromise (including human immunodeficiency virus [HIV] infection), or limited ability to comply with treatment or follow-up.

Study Treatment

All patients were given lurbinectedin 3.2 mg/m^2 as a 1-hour intravenous (i.v.) infusion once every 3 weeks (q3wk). Treatment delays and dose reductions were allowed to manage toxicity at the investigator's discretion. Treatment was administered until disease progression, unacceptable toxicity, treatment delay >3 weeks (except if clear clinical benefit), requirement of >2 dose reductions, intercurrent illness precluding study continuation, and patient refusal and/or non-compliance with study requirements. Standard antiemetic prophylaxis was administered before each lurbinectedin infusion. Only secondary prophylaxis with granulocyte colony-stimulating factor (G-CSF) was allowed.

Study Assessments

Antitumor activity was evaluated in patients who had at least one complete infusion of lurbinectedin, and who either had at least one tumor assessment (as per RECIST v.1.1) or were considered treatment failures (i.e., discontinued treatment due to toxicity/clinical disease progression or died due to the disease before the first tumor assessment). Radiological tumor assessments (computed tomography [CT] scans or magnetic resonance imaging [MRI]) were conducted every 6 weeks until Cycle 6, and every 9 weeks thereafter. Any patients showing a response had to have a confirmatory assessment using the same technique at least 4 weeks later.

Safety was evaluated in all patients who received at least one lurbinectedin infusion through the assessment of adverse events (AEs), laboratory tests, physical examination and vital signs. Laboratory tests were conducted weekly during Cycles 1 and 2, and on Day 1 of subsequent cycles. Safety was monitored throughout treatment and up to 30 days after the last lurbinectedin infusion, start of a new antitumor therapy

or death, whichever occurred first. Any lurbinectedin-related AE was followed until recovery. AEs and laboratory abnormalities were graded with the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.4 (23), and coded using the Medical Dictionary for Regulatory Activities (MedDRA) v.21.0.

Endpoints

All study endpoints were assessed by the investigators. The primary endpoint was the antitumor activity of lurbinectedin in terms of overall response rate (ORR, percentage of patients with complete [CR] or partial response [PR]) as per RECIST v.1.1). Secondary endpoints were duration of response (DoR; time from the date of first response to the date of first disease progression or death from any cause in patients with response); clinical benefit rate (CBR, percentage of patients with response or disease stabilization for ≥4 months); disease control rate (DCR, percentage of patients with response or disease stabilization of any duration); progression-free survival (PFS, time from the date of first infusion to the date of disease progression, death from any cause or last tumor evaluation); PFS at 4 and 6 months; overall survival (OS, time from the date of first infusion to the date of death or loss to follow-up); OS at 6 and 12 months; and pharmacogenomics and safety profile of lurbinectedin.

Statistical Analysis

Up to 25 evaluable patients were to be enrolled to test the null hypothesis that 1% or fewer patients would achieve a response to lurbinectedin ($p \leq 0.01$) *vs.* the alternative hypothesis that 10% or more patients would achieve a response to lurbinectedin ($p \geq 0.10$). The variance of the standardized test was based on the null hypothesis. The type I error (alpha) associated with this one-sided test was 0.025 and the type II error

(beta) was 0.2; thus, statistical power was 80%. With these assumptions, the null hypothesis could be rejected if the number of patients who achieved a confirmed response was ≥2.

Frequency tables were prepared for categorical variables. Continuous variables were described using summary tables with the median, mean, standard deviation, minimum and maximum for each variable. Non-continuous variables were described using frequency tables with counts and percentages. Binomial exact estimates and 95% confidence intervals (CIs) were used to evaluate the primary endpoint (ORR), CBR and DCR. The Kaplan-Meier method was used to evaluate time-to-event endpoints. For DoR and PFS, patients who did not progress or die by data cutoff were censored at the date of their final tumor evaluation. For OS, patients who were still alive were censored at data cutoff. SAS v.9.4 was used for all statistical analyses.

Data-sharing Statement

Individual participant data are not publicly available since this requirement was not anticipated in the study protocol considering that this trial started patient enrolment in 2015. Clinical trial summary results were placed at ClinicalTrials.gov (https://www.clinicaltrials.gov).

RESULTS

Characteristics of Patients and Treatment

A total of 29 patients with ES were enrolled into the study between 25 August 2015 and 16 November 2020. Of these, 28 patients were treated with lurbinectedin and were evaluable for both safety and efficacy.

Baseline characteristics of these 28 treated patients are summarized in Table 1. Most patients (57%) were male, with median age 33 years (range, 18-74 years). ES were mostly extraosseous (58%; PNET in 50%); the other 42% were osseous. Ten patients (36%) had ≥3 metastatic sites, with the most common sites being lung, bone and pleura. All patients had received previous systemic therapy, with a median of 2 lines (range, 1-5 lines) each. The most common prior anticancer agents were vincristine (93%), doxorubicin, ifosfamide (89% each), cyclophosphamide (79%), etoposide (71%), irinotecan (61%), and temozolomide (50%).

A total of 135 treatment cycles were administered, for a median of 4 cycles (range, 1-14 cycles) per patient. Eleven patients (39.3%) received ≥6 cycles each. Median relative dose intensity was 97.7% (range, 69.7-104.5%). Treatment-related adverse events resulted in dose administration delays in 7 patients (29%) and dose reduction in 6 patients (25%); all delays and reductions were due to hematological toxicity (mostly afebrile neutropenia).

Efficacy

Median follow-up was 8.3 months (95% CI, 4.0 months - upper limit not reached). Partial response was observed in 4 patients with extraosseous (n=2) and osseous (n=2) Ewing sarcoma (ORR=14.3%; [95%CI, 4.0-32.7%]) (Table 2 and Table 3). Median DoR was 4.2 months (95%CI, 2.9-5.5 months). Disease stabilization was observed in 12 patients (43%), which lasted ≥4 months in 7 of them (25%) (Table 3). Hence, CBR was 39.3% (95%CI, 21.5-59.4%) and DCR was 57.1% (95%CI, 37.2-75.5%). Median PFS was 2.7 months (95%CI, 1.4-4.3 months) (Figure 1). With a censoring of 39% (11 of 28 patients alive), median OS was 12.0 months (95%CI, 8.5-18.5 months) (Table 2).

Ten patients (40%) showed objective tumor shrinkage in target lesions: 7 patients with extraosseous ES, and 3 with osseous ES (Figure 1 and Figure 2).

After discontinuing lurbinectedin, 19 patients (67.9%) received further antitumor therapy (the most common drugs received were cyclophosphamide, gemcitabine and ifosfamide). Response to first subsequent therapy was observed in two patients (10.5%), neither of whom had shown response to lurbinectedin.

Safety

All 28 treated patients were evaluable for safety (Table 4). Most treatment-related AEs and laboratory abnormalities regardless of relationship were grade 1 or 2. The most common grade 3/4 AEs and abnormalities were hematological disorders, including neutropenia (57% of patients; grade 4 in 43%), leukopenia (46%; grade 4 in 11%), thrombocytopenia (14%; grade 4 in 4%), grade 3 anemia (14%), and treatment-related febrile neutropenia (14%; grade 4 in 4). Eleven patients (39.3%) required G-CSF support as secondary prophylaxis or treatment for neutropenia, three patients (10.7%) received red blood cell transfusions, and one patient (3.6%) received platelet transfusions. No treatment discontinuations or deaths were due to toxicity. Twenty-three patients (82%) discontinued lurbinectedin treatment due to disease progression as per RECIST v.1.1; the other five treatment discontinuations were due to the patient's decision to start another therapy (n=2), multiple cycle delays caused by the study disease or other illnesses (n=1), clinical decline unrelated to treatment (n=1), or death caused by disease progression (n=1).

DISCUSSION

A total of 28 patients with ES pretreated with a median of 2 lines of systemic therapy

each were treated with lurbinectedin in this Basket phase II trial. Confirmed response assessed by Investigators was observed in 4 patients (ORR=14.3%), with a median DoR of 4.2 months. Furthermore, 39.3% of patients had clinical benefit (response or disease stabilization for ≥4 months) and 57.1% showed disease control (response or disease stabilization of any duration). Of note, 5 patients (18%) had disease stabilization for ≥6 months, including 2 patients with ongoing stabilization at 8.2+ and 8.3+ months at the time of study termination. The number of patients with confirmed response assessed by Investigators was higher than the statistical boundary of ≥2 responses defined per protocol. Therefore, lurbinectedin at a dose of 3.2 mg/m^2 given as a 1-hour i.v. infusion q3wk was active in relapsed ES.

Management of patients with metastatic or treatment refractory ES is far from established, since robust evidence is lacking. The different polychemotherapy regimens currently used are based on small studies, and are largely dependent on institutional preferences. Patients with relapsed ES are usually treated with high-dose chemotherapy combinations such as cyclophosphamide and topotecan, or irinotecan and temozolomide with or without vincristine (24-29). Agents used in these regimens have shown little activity against relapsed ES when given as monotherapies, but synergistic activity when given as combination therapies. For instance, the response rate reported for topotecan in recurrent and refractory ES increased from 7% as single-agent (24, 30) to 32-35% when combined with cyclophosphamide (24, 26). The present study had the limitation of not including patients aged <18 years, a population with a high incidence of ES. Nevertheless, the response rate of 14.3% observed herein for single-agent lurbinectedin warrants further development of the drug in the treatment of relapsed ES. Combination of lurbinectedin with irinotecan or temozolomide might improve the antitumor activity of single-agent lurbinectedin in relapsed ES. An ongoing phase Ib/II trial

(NCT02611024) is currently evaluating lurbinectedin in combination with irinotecan in advanced solid tumors, including ES (31).

The safety profile of single-agent lurbinectedin was manageable. Reversible myelosuppression was the most common toxicity, and was managed with cycle delays, dose reductions, G-CSF support and transfusions. Severe hematological abnormalities were more frequent in this cohort of patients with ES than in a cohort of patients with second-line SCLC in this same Basket study (32), and among patients with platinum-resistant ovarian cancer in a randomized phase III trial (33). Thus, higher incidences were observed in patients with ES for grade 3/4 neutropenia (57% *vs.* 46% and 32% respectively), grade 3/4 thrombocytopenia (14% *vs.* 7% and 9%), and treatment-related febrile neutropenia (14% *vs.* 5% and 5.5%). This is likely due to a heavier pretreatment with chemotherapy in the cohort of patients with ES compared to the other two populations, taking into account that current management of primary ES consists of high-dose induction chemotherapy to reduce the primary tumor and target microscopic disease, followed by consolidation chemotherapy to remove any residual cells (34). Patients with relapsed ES in this trial received a median of 2 prior chemotherapy-containing regimens. Overall, these results suggest that primary G-CSF prophylaxis should be given to patients with relapsed ES while on treatment with lurbinectedin.

In conclusion, this single-arm phase II study showed signs of antitumor activity with lurbinectedin used as monotherapy at 3.2 mg/m^2 q3wk in patients with relapsed ES, with a manageable safety profile. Lurbinectedin could represent a valuable addition to therapies currently used in the management of these complex diseases, which constitute a highly unmet medical need.

ACKNOWLEDGMENTS

Authors thank all the motivated patients, the caregivers who enrolled on the trial, and all the clinical trial support staff in all the enrolling sites.

AUTHORSHIP CONTRIBUTIONS

V. Subbiah: conceptualization, investigation, resources, writing – original draft, writing – review and editing. **I. Braña:** investigation, resources, writing – review and editing. **A. Longhi:** investigation, resources, writing – review and editing. **V. Boni:** investigation, resources, writing – review and editing. **J-P. Delord:** investigation, resources, writing – review and editing. **A. Awada:** investigation, resources, writing – review and editing. **P. Boudou-Rouquette:** investigation, resources, writing – review and editing. **J. Sarantopoulos:** investigation, resources, writing – review and editing. **G.I. Shapiro:** investigation, resources, writing – review and editing. **A. Elias:** investigation, resources, writing – review and editing. **R. Ratan:** investigation, resources, writing – review and editing. **C. Fernandez:** methodology, formal analysis, writing – review and editing. **C. Kahatt:** conceptualization, methodology, supervision, writing – review and editing. **M. Cullell-Young:** methodology, writing – original draft, writing – review and editing. **M. Siguero:** formal analysis, methodology, writing – review and editing. **A. Zeaiter:** methodology, supervision, writing – review and editing. **S.P. Chawla:** investigation, resources, writing – review and editing.

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TABLES

Table 1. Baseline characteristics of patients with Ewing sarcoma.

	All treated patients (n=28)
Gender	
Male	16 (57%)
Female	12 (43%)
Median age, years (range)	33 (18-74)
ECOG performance status	
0	11 (39%)
1	16 (57%)
2	1 (4%)
Median BSA, m^2 (range)	1.9 (1.6-2.4)
Abnormal LDH (>ULN)	10 (36%)
Disease stage at diagnosis	
Early	14 (50%)
Locally advanced	5 (18%)
Metastatic	9 (32%)
ES anatomical subtype [a]	
Extraosseous	15 (58%)
Osseous	11 (42%)
Median number of tumor sites at baseline (range)	2 (1-6)
≥3 sites	10 (36%)
Most common sites of disease at baseline	
Lung	18 (64%)
Bone	16 (57%)
Pleura	9 (32%)
Lymph nodes	6 (21%)
Skin	5 (18%)
Prior surgery	20 (71%)
Prior radiotherapy	20 (71%)
Median number of prior systemic therapy lines (range) [b]	2 (1-5)
Setting of prior systemic therapy	
Neoadjuvant	9 (32%)
Adjuvant	10 (36%)
Neoadjuvant + adjuvant	2 (7%)
Advanced	23 (82%)
Prior anticancer agents	
Vincristine	26 (93%)
Doxorubicin	25 (89%)
Ifosfamide	25 (89%)
Cyclophosphamide	22 (79%)
Etoposide	20 (71%)
Irinotecan	17 (61%)
Temozolomide	14 (50%)

Data are n (%) of patients or median (range).
[a] Missing data for two patients.
[b] All but one of these lines were chemotherapy-containing lines.
BSA, body surface area; ECOG, Eastern Cooperative Oncology Group; ES, Ewing sarcoma; LDH, lactate dehydrogenase; ULN; upper limit of normal.

Table 2. Overall efficacy of lurbinectedin treatment in patients with Ewing sarcoma.

	All treated patients (n=28)
Response by RECIST	
Complete response	.
Partial response	4 (14%)
Stable disease	12 (43%)
≥4 months [a]	7 (25%)
<4 months	5 (18%)
Progressive disease	9 (32%)
Not evaluable [b]	3 (11%)
ORR (%) (95%CI)	14.3% (4.0-32.7%)
CBR (%) (95%CI) [c]	39.3% (21.5-59.4%)
DCR (%) (95%CI) [d]	57.1% (37.2-75.5%)
Duration of response	
Events, n/N (%)	3/4 (75%) [e]
Median DoR, months (95%CI)	4.2 (2.9-5.5)
Patients still responding at 4 months (95%CI)	50.0% (1.0-99.0%)
Progression-free survival	
Events, n/N (%)	22/28 (79%)
Median PFS, months (95%CI)	2.7 (1.4-4.3)
4-month PFS (95%CI)	46.2% (27.0-65.3%)
6-month PFS (95%CI)	23.1% (5.9-40.3%)
Overall survival	
Events, n/N (%)	17/28 (61%)
Median OS, months (95%CI)	12.0 (8.5-18.5)
6-month OS (95%CI)	88.2% (75.7-100.8%)
12-month OS (95%CI)	48.5% (27.8-69.2%)

[a] Includes one patient who had an unconfirmed partial response.
[b] Three patients were not evaluable because they had no radiological assessments during treatment, either due to symptomatic deterioration caused by disease progression (n=2) or early death from malignant disease (n=1).
[c] Partial response or stable disease for ≥4 months.
[d] Partial response or stable disease.
[e] One patient with confirmed partial response discontinued treatment after showing clinical deterioration following an episode of disease-related cognitive disorder; this was a decision by the Investigator. No radiological disease progression was observed at the time of discontinuation, and hence the patient was censored for DoR assessment.
CBR, clinical benefit rate; CI, confidence interval; DoR, duration of response; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; RECIST, Response Evaluation Criteria In Solid Tumors.

Table 3. Characteristics of patients with clinical benefit (confirmed response or disease stabilization for ≥4 months).

Baseline characteristics						Study treatment characteristics					
Age (years)/ gender / ECOG PS	**Location sites**	**Disease subtype**	**No. of prior lines**	**Last therapy / Best response**	**TTP to last prior therapy (mo)**	**Cycles received**	**Sum of target lesions at baseline (mm)**	**Best response**	**DoR (mo)**	**PFS (mo)**	**OS (mo)**
30/M/0	Lung Pleura	Extra-osseous	2	Vincristine, dactinomycin, cyclophosphamide, ifosfamide and etoposide / SD	16.6	6	43	SD≥4	-	4.2	38.1+
58/M/0	Lung Pleura Bone	Extra-osseous	3	Irinotecan, temozolomide / UK	7.8	14	29	SD≥4	-	8.2+	9.9+
37/M/0	Lung Lymph nodes Bone	Extra-osseous	2	Vincristine, irinotecan, temozolomide / UK	3.2	14	35	PR (54% reduction)	4.2+	8.3+	9.8+
22/M/1	Skin Subcutaneous tissue Bone	Osseous	2	Vincristine, irinotecan, temozolomide / UK	6.4	9	71	PR (75% reduction)	5.5	7.1	13.4
24/F/0	Skin Subcutaneous tissue	Osseous	2	Vincristine, irinotecan, temozolomide / NA	22.2	9	32	SD≥4	-	6.4	26.7+
30/F/0	Lung Bone	Extra-osseous	2	Vincristine, irinotecan / SD	4.8	6	53	SD≥4	-	5.1	14.9
74/M/1	Lung Lymph nodes Pleura	Extra-osseous	2	Vincristine, irinotecan, temozolomide / SD	2.6	5	82	SD≥4	-	4.0+	18.5
37/F/0	Bone	Osseous	2	Cyclophosphamide, topotecan / CR	16.7	12	43	SD≥4	-	8.8	20.1+
30/F/1	Lymph nodes	Extra-osseous	2	Irinotecan, temozolomide / SD	24.7	6	32	SD≥4	-	4.1	9.3
49/M/1	Lymph nodes Pleura Pericardial effusion	Extra-osseous	2	Irinotecan, temozolomide / PD	2.4	6	85	PR (52% reduction)	2.9	4.1	12.0
54/M/1	Lung Lymph nodes Pleura	Osseous	1	Vincristine, dactinomycin, ifosfamide / SD	8.4	6	76	PR (57% reduction)	2.9	4.3	19.1

CR, complete response; DoR, duration of response; ECOG, Eastern Cooperative Oncology Group; F, female; M, male; mo, months; NA, not available; OS, overall survival; PD, disease progression; PFS, progression-free survival; PR, partial response, PS, performance status; SD; stable disease; UK, unknown; TTP, time to progression.

Table 4. Laboratory abnormalities and treatment-related adverse events in patients with Ewing sarcoma treated with lurbinectedin.

	All treated patients (n=28)			
NCI-CTCAE grade	**1-2**	**3**	**4**	**Total**
Hematological laboratory abnormalities				
Anemia	20 (71%)	4 (14%)	.	24 (86%)
Leukopenia	11 (39%)	10 (36%)	3 (11%)	24 (86%)
Neutropenia	3 (11%)	4 (14%)	12 (43%)	19 (68%)
Thrombocytopenia	12 (43%)	3 (11%)	1 (4%)	16 (57%)
Biochemical laboratory abnormalities				
Creatinine increased	23 (82%)	1 (4%)	.	24 (86%)
ALT increased	19 (68%)	2 (7%)	.	21 (75%)
GGT increased	17 (61%)	.	.	17 (61%)
AST increased	15 (54%)	.	.	15 (54%)
AP increased	14 (50%)	.	.	14 (50%)
Bilirubin increased	3 (11%)	.	.	3 (11%)
CPK increased	2 (7%)	1 (4%)	.	3 (11%)
Treatment-related adverse events				
Fatigue	11 (39%)	.	.	11 (39%)
Nausea	8 (29%)	.	.	8 (29%)
Decreased appetite	4 (14%)	.	.	4 (14%)
Febrile neutropenia	.	3 (11%)	1 (4%)	4 (14%)
Diarrhea	3 (11%)	.	.	3 (11%)
Headache	3 (11%)	.	.	3 (11%)
Peripheral neuropathy	3 (11%)	.	.	3 (11%)
Gastroesophageal reflux disease	2 (7%)	.	.	2 (7%)
Arthralgia	1 (4%)	.	.	1 (4%)
Constipation	1 (4%)	.	.	1 (4%)
Pyrexia	1 (4%)	.	.	1 (4%)
Upper respiratory tract infection	1 (4%)	.	.	1 (4%)
Vomiting	1 (4%)	.	.	1 (4%)

Data are n (%) of patients.

Hematological and biochemical abnormalities are shown regardless of relationship to treatment.

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; GGT, gamma-glutamyltransferase; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events.

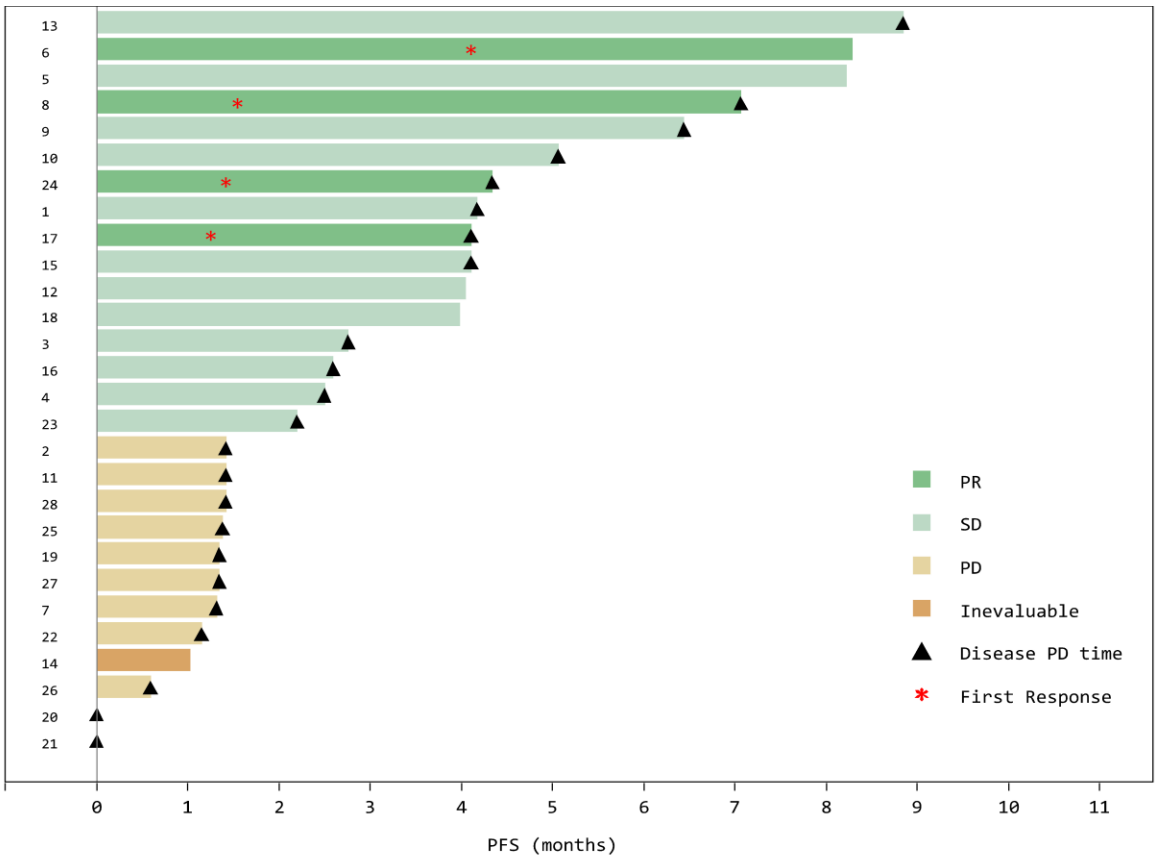
FIGURE LEGENDS

Figure 1. (A) Progression-free survival. Each numbered bar represents a patient with Ewing sarcoma treated with lurbinectedin (n=28). The times when each patient experienced disease progression as per RECIST, and response to treatment, are shown with triangles and asterisks, respectively. (B) Maximum variation of target lesions in patients with measurable disease and at least one radiological tumor assessment. Each patient is identified using the same number as in Figure 1A. PD, progressive disease; PFS, progression-free survival; PR, partial response; RECIST, Response Evaluation Criteria In Solid Tumors; SD, stable disease.

Figure 2. (A) Tumor shrinkage observed in a 54-year-old male with osseous Ewing sarcoma who achieved partial response with lurbinectedin. The patient had been pre-treated with two lines of chemotherapy for advanced disease. Three target lesions were present at baseline: two in the lung and one in a right hilar lymph node. (B) After two cycles of treatment with lurbinectedin all target lesions were smaller, with the sum of longest diameters decreasing from 76 mm to 35 mm (i.e., a tumor shrinkage of 53.9%). In both images, the red arrow shows the location of the lesions.

Figure 1

A



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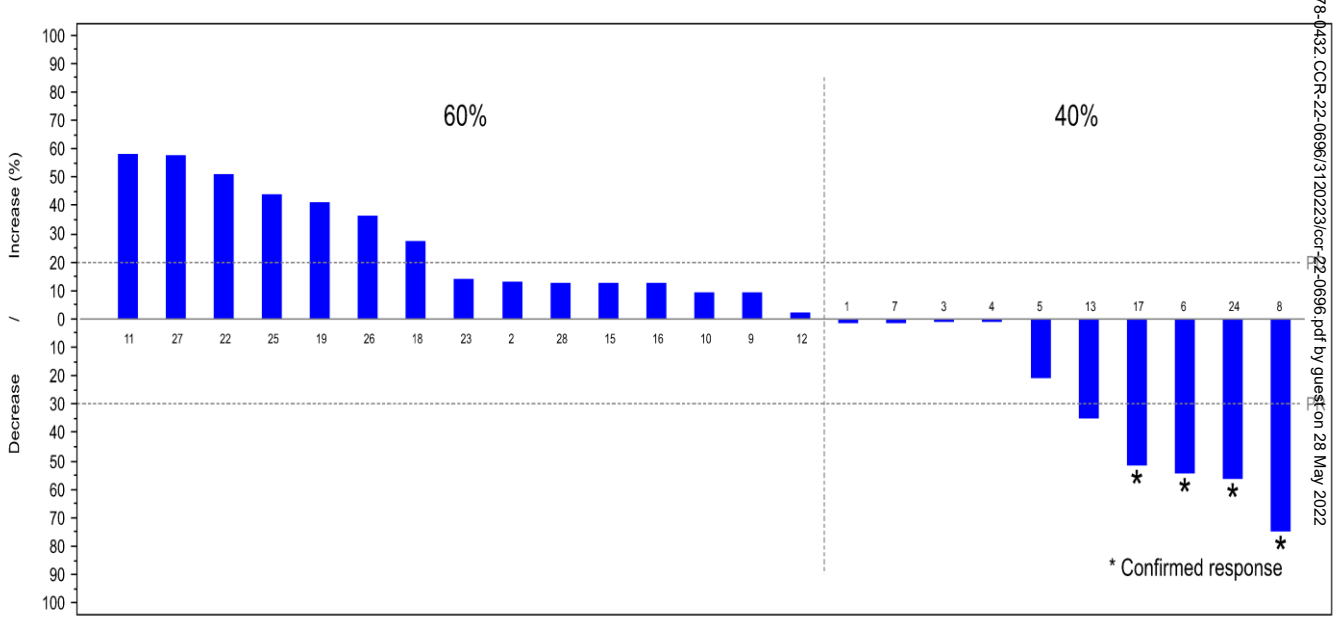
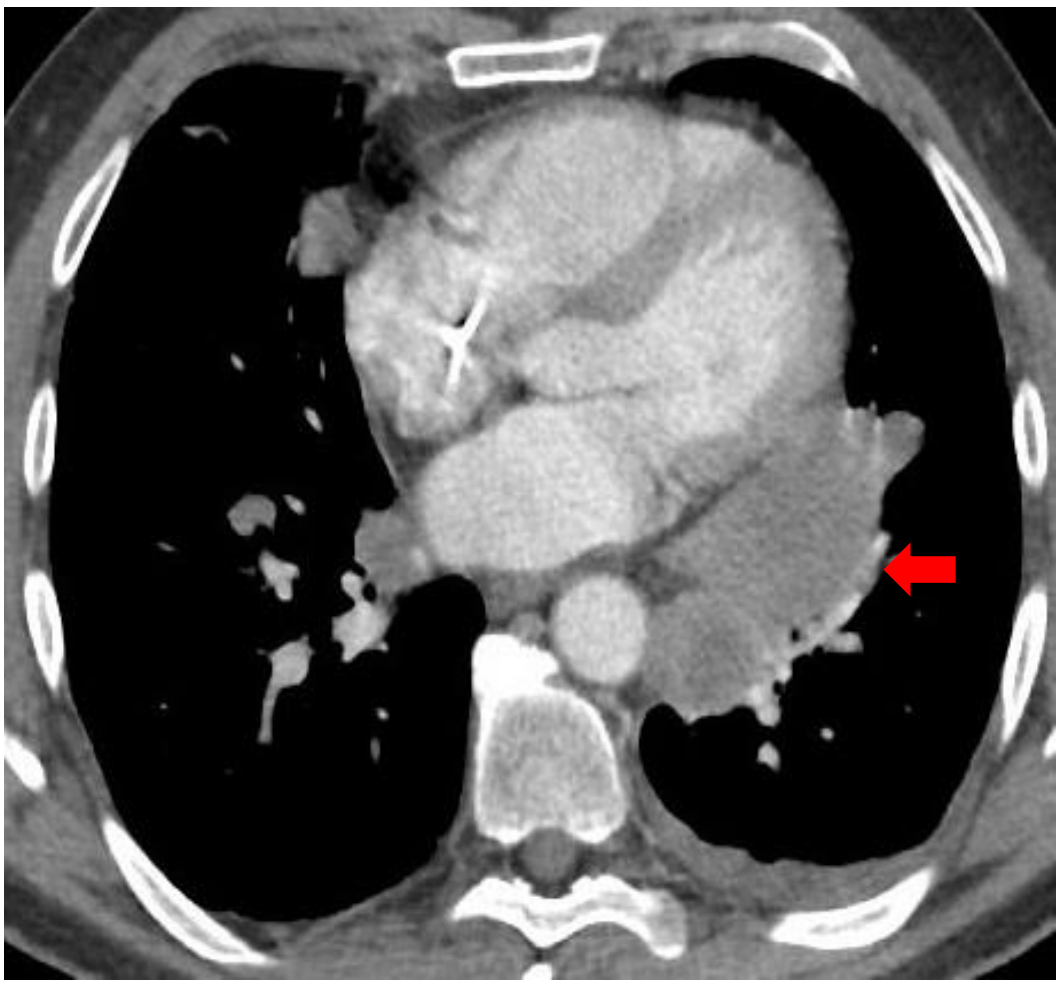


Figure 2

A



B