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PRIMARY RESEARCH

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Eribulin activity in soft tissue sarcoma monolayer and threedimensional cell line models: could the combination with other drugs improve its antitumoral effect?

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Abstract

Background: Eribulin has shown antitumour activity in some soft tissue sarcomas (STSs), but it has only been approved for advanced liposarcoma (LPS).

Methods: In this study, we evaluated the effect of eribulin on proliferation, migration and invasion capabilities in LPS, leiomyosarcoma (LMS) and fibrosarcoma (FS) models, using both monolayer (2D) and threedimensional (3D) spheroid cell cultures. Additionally, we explored combinations of eribulin with other drugs commonly used in the treatment of STS with the aim of increasing its antitumour activity.

Results: Eribulin showed activity inhibiting proliferation, 2D and 3D migration and invasion in most of the cell line models. Furthermore, we provide data that suggest, for the first time, a synergistic effect with ifosfamide in all models, and with pazopanib in LMS as well as in myxoid and pleomorphic LPS.

Conclusions: Our results support the effect of eribulin on LPS, LMS and FS cell line models. The combination of eribulin with ifosfamide or pazopanib has shown in vitro synergy, which warrants further clinical research.

Keywords: Eribulin, Sarcoma, Proliferation, Migration, Invasion, 2D and 3D models

Background

Soft tissue sarcomas (STSs) represent approximately 1% of all adult cancers. This heterogeneous group of tumours is currently subclassified by the World Health Organization (WHO) into approximately 80 histological subtypes [1]. From a genetic point of view, STSs are classified into 2 categories: those considered to harbour simple genetic alterations and those with complex karyotypes. Liposarcoma (LPS), one of the most common STSs, has been classically subclassified into 4 subtypes: well-differentiated (WDLPS), dedifferentiated (DDLPS), myxoid (MLPS) and pleomorphic (PLPS), although the most recent WHO classification has included a fifth

subtype: myxoid pleomorphic LPS [2]. WDLPS and not representative of a real cancer cell environment [17]. DDLPS together comprise the largest subgroup of liposarcoma. This scenario has led to the emergence of three-dimensional (3D) models, including spheroids, which more faithfully represent the tumour's phenotypic characteristics and sharing a genetic alteration. The amplification of the chromosomal region 12q14-15, tics and mimic its structure [18].

which involves the MDM2 and CDK4 genes [3]. MLPS In this study, we explored the activity of eribulin in a cell line panel: LPS and LMS, which were the target of the phase III clinical trial, and FS, a rare sarcoma, in which eribulin was previously tested in vitro and in xenografts [19]. We investigated the effect on proliferation, high chromosomal instability [5–7]. Finally, adult bone marrow migration and invasion capabilities, in 2D and 3D conditions. Finally, we studied the possible synergistic effect of eribulin with other drugs commonly used in the treatment of STS.

Treatment of advanced STS is mainly based on chemotherapy and other systemic treatments, although surgery

and radiotherapy can also be used with palliative intention in some patients.

Doxorubicin is usually administered as first-line treatment. Other drugs, such as LIPODL221 (MLPS), LPS224 (DDLPS) and LPS246 (DDLPS) cell lines were obtained from the MD Anderson (in combination) and dacarbazine are options for subsequent treatment lines [9]. Additionally, in WDLPS and HT1080 (FS) and 93T449 (WDLPS) were kindly provided by Dr. Carnero from the Biomedicine Institute (Seville, Spain). The cell lines were tested routinely for mycoplasma contamination.

Lines, compounds and cell culture conditions. All culture media were supplemented with a concentration of 10% foetal bovine serum (v/v), 1% L-glutamine (v/v) and antibiotics (100 units/ml of penicillin and 100 µg/ml of streptomycin), all from Merck (Germany), including LPS, LMS, synovial sarcoma and a mixed group of less common STSs [15]. Based on the high activity level in LPS and LMS subtypes, a phase III trial was developed to compare eribulin with dacarbazine in pre-treated patients with these two histologies. This study achieved its primary endpoint, showing a benefit in overall survival (OS) for eribulin. However, regulatory agencies only approved eribulin for advanced LPS based on the results of the subgroup analysis, which did not demonstrate an OS benefit for the LMS cohort [16].

Eribulin is an antitumour agent acting mainly as a microtubule dynamics inhibitor. Other mechanisms of action have also been proposed, such as vascular remodeling, reversion of the epithelial to mesenchymal transition (EMT) and suppression of migration and invasion. Eribulin clinical activity was initially assessed in a phase II trial of patients with STS with several histologies, including LPS, LMS, synovial sarcoma and a mixed group of less common STSs [15]. Based on the high activity level in LPS and LMS subtypes, a phase III trial was developed to compare eribulin with dacarbazine in pre-treated patients with these two histologies. This study achieved its primary endpoint, showing a benefit in overall survival (OS) for eribulin. However, regulatory agencies only approved eribulin for advanced LPS based on the results of the subgroup analysis, which did not demonstrate an OS benefit for the LMS cohort [16].

The rarity of sarcomas makes it difficult to design clinical trials or make clinical decisions regarding specific subtypes. In this context, cellular models are a valuable approach, particularly for drug testing studies, which can provide useful preclinical data to help with the design of further clinical research. Most current studies are performed on monolayer two-dimensional (2D) cell models, which are still the most commonly used due to their high standardisation and cost effectiveness. However, they have a number of limitations, particularly important for drug studies. The main disadvantage is that cells are cultured on flat dishes, where there is no spatial complexity, and they are all equally exposed to compounds, which is

described [20]. Absorbance was measured at 564 nm. For cell cycle and apoptosis assays, cells were seeded as on a Synergy 4 spectrophotometer using the Gen5 pro described previously for proliferation assays (Additional Fig. 1: Table S1), and exposed, 24 h after seeding, to their

For 3D experiments, cells were seeded in ultra-lowcorresponding eribulin GI_{50} concentrations (Table 1) attachment (ULA) round bottom plates. The optimal for 72 h. For analysis, cells are previously fixed with 70% spheroid size relies on the specific assay requirements. Ethanol and nuclei were stained with propidium iodide but is usually within a 200–500 μ m diameter range. We (Merck). The distribution of integrated intensity was established a pre-set density for each cell line, to obtain visualized and quantified by the Celigo S plate cytometer a spheroid diameter around 300 μ m at day 4, as pre (Nexcelom) gating tool, as counts and percentage over previously described in ULA plates [21,22]. Growth was the total cell count. Experiments were performed twice tracked measuring spheroid diameter at days 4, 7, 10 and including at least two replicates.

and 14. Image acquisition and analyses were performed

with a Celigo S plate cytometer (Nexcelom, USA). 2D and 3D migration and invasion assays

The drug screening experiments were performed in. We employed 8-mm pore transwell inserts for migration MW96 plates. The design of the plate was as follows: (Ref. 354578, Corning), and Matrigel coated inserts for all border wells contained phosphate buffered saline invasion experiments (Ref. 354480, Corning). The insert (PBS), to avoid evaporation issues. The rest of the plate was fixed and stained 24 h after seeding using the Di- contained the pre-set number of cells for each cell line. Quick method (QCA, Spain). Two different fields were and condition. With this design, 9 columns with 6 replicates each will be exposed to increasing concentrations of the explored drug, and one column will be used as control.

For the study of migration and invasion capacity in 3D, 24 h after seeding to 1:2 dilutions of drug concentrations. For the study of migration and invasion capacity in 3D, 24 h after seeding to 1:2 dilutions of drug concentrations. 4 day spheroids were placed on a previously solidified Matrigel layer for migration, or embedded in Matrigel for invasion assays. The images were taken and quantified after 3 to 7 days using ImageJ (NIH). Two experiments were performed and the measures obtained in two fields by SRB staining as was performed for the proliferation assays. For the 3D experiments, after 4 days of culture, the spheroids were exposed to 1:2 dilutions of drug

concentrations, for 72 h. Cell viability was measured. Establishment of eribulin-resistant cell lines

after this period using a CellTiter-Glo Luminescent and exploratory antibody array analysis assay (Promega) according to the manufacturer's protocol. Eribulin-resistant cell line was established from 93T449 cells and bright field images were taken. At least two LPS cells by continuous exposure to eribulin (GI_{50} value, 1.24 nM) over a period of three months.

We calculated 2D and 3D growth inhibitory concentrations. Semi-quantitative detection of 1000 human proteins by 50% (GI_{50}) using non-linear regression with. Proteins was performed using the RayBio C-series Human Cytokine Antibody Array C1000 (RayBiotech, USA) following manufacturer's instructions. Dots detection was

Table 1 Eribulin 2D and 3D GI_{50} values (nM). Concentration is displayed as standard deviation. 3D/2D ratio is also shown

Cell line	Sarcoma subtype	Eribulin GI_{50} 2D	Eribulin GI_{50} 3D	GI_{50} 3D/ GI_{50} 2D
HT1080	FS	5.07 2.37	4.67 1.75	0.92
SKUT1	LMS	0.17 0.04	0.39 0.13	2.29
SW872	PLPS	2.18 0.18	3.54 2.49	1.62
LIPODL221	MLPS	1.03 0.22	> 100	NE
93T449	WDLPS	1.24 0.17	47.93 16.92	38.65
LPS224	DDLPS	11.75 0.86	> 100	NE
LPS246	DDLPS	1.25 0.12	> 100	NE

FS Fibrosarcoma, LMS Leiomyosarcoma, PLPS Pleomorphic liposarcoma, MLPS Mixoid liposarcoma, WDLPS Well-differentiated liposarcoma, DDLPS Dedifferentiated liposarcoma, NE Not evaluable

performed with the GenePix 4100A Microarray scan experimental schedule and growth rates are illustrated in Fig. 1B, C, showing that SW872 appears to grow faster (Molecular Devices, USA). The raw numerical intensity compared to the other cell lines, under 2D and 3D conditions, data were extracted and subjected to background subtraction, although it does not reach statistical significance. The data were then normalised against the positive control signals in each array.

Effect of eribulin and other drugs on proliferation. Duplicate results were eliminated for the analysis. The most sensitive cell line to eribulin in 2D and 3D conditions was SK-UT-1 (LMS). HT1080 (FS) and SW872 (PLPS) keep similar values of sensitivity in both conditions, while the rest of the cells showed increases in their GI_{50} when grown in 3D conditions. Values in 2D were in the nanomolar range for all cell lines, and also for 4 of them in 3D conditions. The other 3 lines did not reach their GI_{50} values with a 100 nM exposure (Table 1). A fold change over 2 times and p -value ≤ 0.05 were used as cut-off.

Drug combinations

Drug combination studies were performed in pairs, combining eribulin with the other drugs. Treatment was established for 72 h, with a maximum concentration up to 10 mM (specific concentrations for each cell line are based on single drug experiments and summarized in Additional file 1: Table S1), and 1:3 dilutions. According to a 6 × 6 dose-response matrix design, both, the effect of the single drugs, as well as the combination, were tested in all lines except LPS246 (DDLPS), changing their basal morphology to a more rounded one (Additional file 2: Fig. S1).

Experiments were first analysed using Synergy-Finder package (<http://bioconductor.org/packages/release/bioc/html/synergyfinder.html>) [23, 24]. Additionally, eribulin promoted morphological changes in all lines except LPS246 (DDLPS), changing their basal morphology to a more rounded one (Additional file 3: Fig. S2).

We used 4 available algorithms to test the synergies between drugs: the Bliss model (BLISS), Highest-Sinrations (up to 10 mM). Pazopanib and palbociclib (HSA), Loewe additivity model (LOEWE) and presented GI_{50} at a micromolar range. Doxorubicin, Zero Interaction Potency (ZIP) [25]. The effect was considered synergistic only when the 4 algorithms produced a positive scores [26]. Additionally, to evaluate the overall effect of a drug combination, drug combination sensitivity scores were determined by using a Combination Sensitivity Score (CSS) approach, which is based on the area under their dose-response curves at relative GI_{50} values of compounds [27]. A top drug combination was assumed only if all the synergy scores had a positive value and CSS was ≥ 60 . Experiments for each combination were performed at least two times.

Effect of eribulin on cell cycle

Cell cycle and apoptosis were also explored by DNA content quantification of cells. A general increase in the percentage of cells in sub G0 and S-phase was observed in all cell lines, indicating that eribulin treatment led to apoptosis and S-phase arrest. Regarding checkpoints, a decreased G0/G1-phase was observed, and G2/M-phase variations seemed to be dependent on the cell line (Additional file 5: Fig. S3).

Statistical analysis

The averages and standard deviations of the data, as well as the Student T tests, were performed using Microsoft Office Excel 2007 (Microsoft, USA). As previously indicated, GI_{50} calculation was performed by non-linear interpolation using GraphPad Prism 7 software (Graph Pad Software).

Results

Cell characterisation

The morphology of the cells is shown in Fig. 1A. All cell lines grew with a mesenchymal morphology under 2D culture. Regarding 3D, SK-UT-1 (LMS) and all the LPS cell lines grew as compact spheroids. LIPODL221 and LPS246, both DDLPS, showed a high increase in their GI_{50} values in 3D, so they were as well excluded from this part. In the 2 remaining lines, 93T449 and HT1080 (FS) formed less compact spheroids. The effect of eribulin on migration and invasion in both 2D and 3D cultures

In the LMS and FS cell lines, an overall decrease in migration and invasion was observed. Migration decreased between 40 and 90%, and invasion was abolished in 20% to 90%. The effects were greater in 3D than in monolayer cultures. A representative experiment is shown in Fig. 2.

However, decreased migration and invasion was less visible in LPS cell lines, and not statistically significant.

Some cells did not migrate or invade in basal conditions (LIPODL221, MLPS), whereas others (LPS224 and LPS246, both DDLPS), showed a high increase in their GI_{50} values in 3D, so they were as well excluded from this part. In the 2 remaining lines, 93T449 and HT1080 (FS) formed less compact spheroids.

SW872, only a modest decrease in both properties was observed (data not shown).

Exploratory identification of pathways implicated in eribulin action

We next sought to determine potential action modes for eribulin. To do so, we used 93T449 LMS cell line to generate an eribulin-resistant line through continuous drug exposure. An exploratory analysis of deregulated proteins included and enriched pathways, pointed out candidate pathways to explore, as is protein metabolism (Additional file 6: Fig. S4).

Effect of eribulin combinations in 2D cultures

Combinations of eribulin and a second drug were studied to establish potential synergies. We selected drugs commonly used in STS (doxorubicin, ifosfamide, trabectedin, pazopanib and gemcitabine) plus palbociclib, as possible agents for LPS.

Of all the tested combinations, 2 showed synergy for eribulin. To do so, we used 93T449 LMS cell line to generate an eribulin-resistant line through continuous drug exposure. An exploratory analysis of deregulated proteins included and enriched pathways, pointed out candidate pathways to explore, as is protein metabolism (Additional file 6: Fig. S4).

eribulin and ifosfamide showed consistent synergistic action in all the cell lines tested (Fig. 3B and Table 2). These results were confirmed with Tidycomb, with the exception of the LIPODL221 (MLPS) cell line (Additional file 7: Fig. S5).

Discussion

Fewer new therapies or drug combinations with clinically relevant efficacy have been identified for STS than for other tumours, in part because these tumours occur less frequently and are highly heterogeneous. Consequently, most clinical trials have included all (or many) STS subtypes together. These entities are different from a histological and molecular point of view, and they do not necessarily share the same drug sensitivity [28].

Cellular models are a useful tool with which to perform drug testing, especially in rare entities such as STS, given that they can serve as the basis for new clinical trial designs. Traditionally, the most commonly performed experiments have been based on cell proliferation or apoptosis in 2D conditions. Currently, we can analyse the effect of drug exposure on other aspects that are important for cancer progression, such as migration and invasion. Moreover, we already know that the classical monolayer culture, although it has a low cost and is easy to handle, does not reflect the architectural situation in the tumour. Spheroids are tridimensional structures that mimic the spatial architecture of tumour cells, and they

allow the study not only of proliferation, but also migration and invasion [21–22]. We have employed all of these approaches to better characterise the action of eribulin in these cell lines. Eribulin has been previously studied in sarcomas in 2D culture models. There are positive data with preclinical models of Ewing's sarcoma, rhabdomyosarcomas and osteosarcoma cell lines [29]. Regarding the subtypes examined in this study, eribulin has been reported to exert an antiproliferative effect and to induce apoptosis in primary and established DDLPS lines grown in monolayer. An effect on cell growth in cell lines correspond to LMS (SK-L-MS1) and FS (HT1080) has also been observed. Similarly, in our study eribulin also produced an inhibitory effect on cell growth in LPS, LMS and FS cell lines [30–32].

A modification of cell morphology was observed in the majority of cell lines, which is consistent with one of the mechanisms of action of eribulin. This mechanism involves the suppression of microtubule dynamics and EMT, which can prompt changes in cell morphology

[33]. These changes have also been observed in other osteosarcoma and breast cell lines [367]. In our cellular models, and particularly in LPS and LMS cell models, eribulin led to significantly increased apoptosis rates, it has been related to cellular differentiation [34], G1/S arrest, while G2/M arrest seemed to be [35]. Effects on apoptosis and cell cycle have been also reported, and associated to apoptosis and mitotic arrest

Table 2 Combination scores for eribulin-ifosfamide and eribulin-pazopanib calculated by Synergy nder. Mean of two experiments and SD are included for each algorithm

Cell line	Combination	ZIP	HSA	BLISS	LOEWE
HT1080	Eribulin Ifosfamide	7.27 2.41	10.08 1.94	8.22 2.37	4.39 1.29
SKUT1		3.74 0.76	7.07 1.26	4.18 0.84	0.7 2.63
SW872		7.49 4.24	8.75 5.07	7.8 4.32	4.48 4.31
LIPODL221		1.31 1.55	6.07 1.33	2.90 0.34	1.99 0.50
93T449		5.19 0.28	7.74 2.48	6.48 2.38	2.55 1.61
LPS224	Eribulin Pazopanib	6.98 0.04	7.77 0.99	7.81 0.44	4.26 0.34
LPS246		2.27 1.15	5.28 0.17	2.68 1.25	1.69 0.32
SKUT1		4.08 4.34	10.25 0.50	5.17 2.49	5.53 1.31
SW872		8.81 1.92	13.97 0.03	9.19 1.41	8.00 0.03
LIPODL221		3.38 2.29	11.07 1.40	3.37 2.24	7.67 1.98

ZIP Zero Interaction Potency, HSA Highest Single Agent, LOEWE Loewe additivity model, BLISS Bliss model

There have been few studies of the effect of eribulin on 3D STS cell models. A previous report with a 3D LM82D and 3D conditions. There are no previous reports on osteosarcoma model suggested an impact on proliferation [32]. These findings are consistent with the findings [38]. Eribulin's action has not been explored previously morphological changes observed under eribulin exposure under 3D conditions in LMS, FS or LPS, nor has its effect involving a mesenchymal to epithelial switch, which has been compared with 2D. While the HT-1080 FS cell line could be related to a decrease in migration and invasion, HT-1080 maintained similar GI_{50} values as the monolayer culture, on STS models [35].

the LPS and LMS models showed an increase in their GI_{50} values, primarily to nanomolar values, and even to micromolar values in some cases. This increase could be due to the spheres' architecture, given that HT-1080 had been already implicated, as the activation of PI3K [41], a less compact structure. A loose aggregate is more similar to a 2D culture, in which all cells are equally exposed to oxygen, nutrients and drugs. The rest of the cell lines, dysregulated molecules under a pathway enrichment which had a more compact structure, were hindered by approach. Our findings suggest that protein metabolism is an over-represented process in this resistant cell line. We have generated a resistant cell line from the parental 93T449 by eribulin exposure and we have explored a diffusion gradient through the inner layers. They probably required a subsequent increase in GI_{50} values, given that not all the cells were equally exposed. Nowadays is considered that cellular metabolic rewiring is essential for tumor development, and that metabolic alterations are related to sensitivity (or resistance) to chemotherapeutic agents. Our preliminary findings suggest that this process could be implicated in Eribulin response, although further experiments will be required that would better represent the situation in the tumour to confirm this data [42].

Additionally, in compact spheroids, only the outer ring proliferates, while the rest of the cells are quiescent, and some drugs need proliferation of cells for their activity. These findings could favour an increase in GI_{50} values, although further experiments will be required that would better represent the situation in the tumour to confirm this data [42]. Differences in 2D and 3D sensitivities have been reported previously in sarcomas, which generally grow in a very tight structure in 3D. For example, chondrosarcoma cell line monolayers sensitive to doxorubicin have been previously explored in STS cell line models [32, 43]. Regarding eribulin combinations, a recent study was published suggesting activity with an AKT inhibitor (MK-2206) in FS and LMS cell lines [31].

Eribulin has been reported to affect migration and invasion of breast cancer cell lines in 2D conditions [13]. Synergy indicates that 2 drugs combined exert a greater effect than that expected from the 2 drugs individually. It has also been reported to inhibit migration in scratch assays in primary WDLPS and DDLPS lines. We achieved similar results in these subtypes, but no effect was observed on the PLPS cell line subtype. The most impressive results were observed in the LMS and FS cell lines, developed to evaluate synergy between drugs in vitro,

and using more than 1 scientific evaluation method should provide more consistent results. In our study, we have explored the combination of eribulin with a complete panel of drugs commonly used in STS with restrictive synergy requirements. All of the 4 scores obtained by the Synergy Finder analysis had to show a positive value, whereas other studies usually consider synergy to have occurred when only one of the algorithms is positive [45, 46]. Our approach helped us obtain greater certainty in the results.

We found 2 interesting combinations. First, eribulin and pazopanib appeared to be synergistic for LMS, MLPS and PLPS, but not for other LPSs. Pazopanib is a potent and selective multitargeted receptor tyrosine kinase inhibitor that showed efficacy in a phase III trial in STS, and it is currently approved for the treatment of advanced STS (except LPS) pretreated with anthracyclines [47,48]. Additionally, the combination of eribulin and ifosfamide was consistently synergistic in all lines and subtypes tested. Ifosfamide is an alkylating agent classically used in neo/adjuvant treatment or for first line treatment of advanced STS in combination with an anthracycline. It is also used at high doses as monotherapy in second-line treatment of STS. Due to the consistency of results among the STS subtypes analysed in our study, this combination needs further exploration. From a clinical point of view, the eribulin-pazopanib combination would need careful investigation due to the toxicity profile of each drug. In addition, ifosfamide is associated with high haematological toxicity, which could be increased with eribulin. Therefore, a hypothetical phase I clinical trial to explore this combination should start with a low dose of ifosfamide.

Conclusions

We have proved eribulin's effect on proliferation, migration and invasion in STS cell line models. The preclinical results we have obtained are sufficiently robust to consolidate its antitumour effect in this context. Additionally, the combination of eribulin with pazopanib or ifosfamide has a synergistic effect in STS, which warrants further clinical research.

Abbreviations

BLISS: Bliss model algorithm; CSS: Combination Sensitivity Score; DDLPS: Differentiated liposarcoma; DMEM: Dulbecco's Modified Eagle Medium; EMT: Epithelial to mesenchymal transition; FS: Fibrosarcoma; GI50: Growth Inhibitory Concentration by 50%; HEPES: 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid; HSA: Highest Single Agent algorithm; LMS: Leiomyosarcoma; LOEWE: Loewe additivity model algorithm; LPS: Liposarcoma; MLPS: Myxoid liposarcoma; MW96: 96 well plates; NEAA: Essential amino acids; OS: Overall survival; PLPS: Pleomorphic liposarcoma; PyrNa: Sodium pyruvate solution; SRB: Sulphorhodamine B; STS: Soft tissue sarcomas; ULA: Ultra-low

attachment; WDLPS: Well-differentiated liposarcoma; ZIP: Zero Interaction Potency algorithm.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12935-021-02337-5>.

Additional file 1: Table S1. Detailed experimental conditions for employed cell lines, covering seeding densities and drug dose ranges for 2- and 3D conditions.

Additional file 2: Figure S1. Eribulin dose response curves for cell lines studied. A representative experiment is shown for A) 2D and B) 3D culture conditions. Red line represents the value of IC_{50} . Scatter plots. Cells have been split in two plots according to their values for a better representation. In 3D experiments, only 4 cell lines are presented since the other 3 are over the tested drug range, and considered as resistant. A representative experiment is shown for A) 2D and B) 3D culture conditions. Red line represents the value of IC_{50} . Scatter plots. Cells have been split in two plots according to their values for a better representation. In 3D experiments, only 4 cell lines are presented since the other 3 are over the tested drug range, and considered as resistant.

Additional file 3: Figure S2. Eribulin effect on morphology. Phenotypical changes of cell lines exposed to eribulin for 72h.

Additional file 4: Table S2. $2D\ GI_{50}$ values for studied drugs. The concentration value is displayed as mean ± standard deviation.

Additional file 5: Figure S3. Effect of eribulin in Cell cycle. Bar plots show Cell cycle distribution for subG0, G0/G1, S and G2/M phases comparison in untreated and eribulin exposed cells. * $p < 0.05$, ** $p < 0.005$.

Additional file 6: Figure S4. Pathway enrichment analysis for deregulated processes between parental and resistant 93T449. NES: Normalized enrichment score; pval: p value.

Additional file 7: Figure S5. Tidycomb Analysis. Eribulin combination with A. Ifosfamide, B. Pazopanib and C. Doxorubicin, on LMS and LPS cell lines. The area highlighted in red corresponds to the score values considered as indicators of synergy.

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Author's contributions

These are the main contributions by authors: Conceptualisation and resources (JT, AR, MM). Data curation (JE, VHS, YW, YH, AG, JPK, AB, VMM, DB). Software and visualisation (YW, YH, JT). Methodology and Formal analysis (JE, VHS, PR, AR, MM). Supervision and funding acquisition (DH, JF, EOC, AR, MM). Writing and editing original draft (JE, VHS, JT, AR, MM). All authors read and approved the final manuscript.

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Availability of data and materials

Data are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests

AG reports having received honoraria (Clovis, MSD, AstraZeneca, GSK, PharmaMar and Roche) and travel/accommodation/expenses (Merck Sharp & Dohme, PharmaMar, Roche, Eisai, P zer, Fibre and Tesaro, GSK Company) outside the submitted work. VM reports having received honoraria as speaker from Roche, P zer, Novartis, Lilly, Rovi and Bayer. JF has received consulting and advisory honoraria from Amgen, Ipsen, Eisai, Sirtex, Merck, Roche and Novartis; research funding from Merck; and travel and accommodation expenses from Amgen and Servier. AR reports having received honoraria and providing advisory/consultancy services (MSD, AstraZeneca, Roche, GSK, Clovis, PharmaMar, Lilly, Amgen), as well as having received research grant/funding to his institution (Eisai, PharmaMar, Roche), travel/accommodation/expenses (AstraZeneca, Tesaro: A GSK Company, PharmaMar, Roche), and participating in a speakers bureau (MSD, AstraZeneca, Roche, GSK, Clovis, PharmaMar) outside the submitted work. MM reports having received honoraria (MSD, AstraZeneca and GSK), research grant/funding to his institution (Eisai and PharmaMar), and travel/accommodation/expenses (AstraZeneca, GSK, PharmaMar, Roche and P zer) outside the submitted work. The remaining authors declare no conflicts of interest.

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References

- Kallen ME, Hornick JL. The 2020 WHO classification: what's new in soft tissue tumor pathology? *Am J Surg Pathol*. 2021;45(1):e1–23.
- Fletcher CBJ, Anonescu C, Merens F: WHO classification of tumours: soft tissue and bone tumours, Vol. 3, 5th ed. WHO Classification of Tumours Editorial Board; 2020. ISBN: 978-92-832-4502-5. <https://publications.iarc.fr/BookAndReportSeries/WHOClassificationOfTumoursSoftTissueAndBoneTumours-2020>
- Thway K. Well-differentiated liposarcoma and dedifferentiated liposarcoma: an updated review. *Semin Diagn Pathol*. 2019;36(2):112–21.
- Anderson WJ, Jo VY. Pleomorphic liposarcoma: updates and current differential diagnosis. *Semin Diagn Pathol*. 2019;36(2):122–8.
- Serrano C, George S. Leiomyosarcoma. *Hematol Oncol Clin North Am*. 2013;27(5):957–74.
- George S, Serrano C, Hensley MLC, Hayward I. Soft tissue and uterine leiomyosarcoma. *J Clin Oncol*. 2018;36(2):144–50.
- Mangla A, Yadav U. Leiomyosarcoma. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021. <https://www.ncbi.nlm.nih.gov/pubmed/31869131>
- Toro JR, Travis LB, Wu HJ, Zhu K, Fletcher CD, Devesa SS. Incidence patterns of soft tissue sarcomas, regardless of primary site, in the surveillance, epidemiology and end results program, 1978–2001: an analysis of 26,758 cases. *Int J Cancer*. 2006;119(12):2922–30.
- Casali PG, Abecassis N, Aro HT, Bauer S, Biagini R, Bielack S, Bonvallet Boukovinas I, Bovee J, Brodowicz T, et al. Soft tissue and visceral sarcomas: ESMO/EURACAN clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2018;29(Suppl 4):iv268–9.
- Dickson MA, Schwartz GK, Keohan ML, D'Angelo SP, Gounder MM, Cristofari, et al. Progression-free survival among patients with well-differentiated or dedifferentiated liposarcoma treated with CDK4 inhibitor palbociclib: a phase 2 clinical trial. *JAMA Oncol*. 2016;2(7):937–40.
- Smith JA, Wilson L, Azarenko O, Zhu X, Lewis BM, Littlefield BA, Jordan MA. Eribulin binds at microtubule ends to a single site on tubulin to suppress dynamic instability. *Biochemistry*. 2010;49(6):1331–7.
- Funahashi Y, Okamoto K, Adachi Y, Semba T, Uesugi M, Ozawa Y, Tohyama O, Uehara T, Kimura T, Watanabe H, et al. Eribulin mesylate reduces tumor microenvironment abnormality by vascular remodeling in preclinical human breast cancer models. *Cancer Sci*. 2014;105(10):1334–42.
- Yoshida T, Ozawa Y, Kimura T, Sato Y, Kuznetsov G, Xu S, Uesugi M, Agoulnik S, Taylor N, Funahashi Y, et al. Eribulin mesylate suppresses experimental metastasis of breast cancer cells by reversing phenotypic transition (EMT) to mesenchymal transition (MET) states. *Br J Cancer*. 2014;110(6):1497–505.
- Swami U, Shah U, Goel S. Eribulin in cancer treatment. *Mar Drugs*. 2015;13(8):5016–58.
- Schöski P, Raoult IL, Cio A, Bui NB, Bauer S, Hartmann JT, Krarup-Hansen A, Grünwald V, Sciort R, Dumez H, et al. Activity of eribulin mesylate in patients with soft tissue sarcoma: a phase 2 study in four independent histological subtypes. *Lancet Oncol*. 2011;12(11):1045–52.
- Schöski P, Chawla S, Maki RG, Italiano A, Gelderblom H, Choy E, Grignani G, Camargo V, Bauer S, Rha SY, et al. Eribulin versus dacarbazine in previously treated patients with advanced liposarcoma or leiomyosarcoma: a randomised, open-label, multicentre, phase 3 trial. *Lancet*. 2016;387(10028):1629–37.
- Lamhamed Cherradi SE, Santoro M, Ramamoorthy V, Menegaz BA, Bartholomeusz G, Iles LR, Amin HM, Livingston JA, Mikos AG, Ludwig JA. 3D tissue-engineered model of Ewing's sarcoma. *Adv Drug Deliv Rev*. 2014;79–80:155–71.
- Colella G, Fazioli F, Gallo M, De Chiara A, Apice G, Ruosi C, Cimmino A, de Nigris F. Sarcoma spheroids and organoids-promising tools in the era of personalized medicine. *Int J Mol Sci*. 2018;19(2):615.
- Towle MJ, Nomoto K, Asano M, Kishi Y, Yu MJ, Littlefield BA. Broad spectrum preclinical antitumor activity of eribulin (Halaven(R)): optimal effectiveness under intermittent dosing conditions. *Anticancer Res*. 2012;32(5):1611–9.
- Orellana EA, Kasinski AL. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. *Bio Protoc*. 2016. <https://doi.org/10.21769/BioProtoc.1984>.
- Vinci M, Gowan S, Boxall F, Patterson L, Zimmermann M, Court W, Lomas C, Mendiola M, Hardisson D, Eccles SA. Advances in establishment and analysis of three-dimensional tumor spheroid-based functional assays for target validation and drug evaluation. *BMC Biol*. 2012;10:29.
- Heredia-Soto V, Redondo A, Kreilinger JJP, Martínez-Marín V, Berjón A, Mendiola M. 3D culture modelling: an emerging approach for translational cancer research in sarcomas. *Curr Med Chem*. 2020;27(29):4778–88.
- Ianevski A, He L, Aittokallio T, Tang J. SynergyFinder: a web application for analyzing drug combination dose-response matrix data. *Bioinformatics*. 2017;33(15):2413–5.
- He L, Kuleskiy E, Saarela J, Turunen L, Wennerberg K, Aittokallio T, Tang J. Methods for high-throughput drug combination screening and synergy scoring. *Methods Mol Biol*. 2018;1711:351–98.
- Yadav B, Wennerberg K, Aittokallio T, Tang J. Searching for drug synergy in complex dose-response landscapes using an interaction potency model. *Comput Struct Biotechnol J*. 2015;13:504–13.
- Tang J, Wennerberg K, Aittokallio T. What is synergy? The Saariselkä agreement revisited. *Front Pharmacol*. 2015;6:181.
- Malyutina A, Majumder MM, Wang W, Pessia A, Heckman CA, Tang J. Drug combination sensitivity scoring facilitates the discovery of synergistic and efficacious drug combinations in cancer. *PLoS Comput Biol*. 2019;15(5):e1006752.
- Lee DY, Staddon AP, Shabason JE, Sebro R. Phase I and phase II clinical trials in sarcoma: implications for drug discovery and development. *Cancer Med*. 2019;8(2):585–92.
- Kolb EA, Gorlick R, Reynolds CP, Kang MH, Carol H, Lock R, Keir ST, Maris JM, Billups CA, Desjardins C, et al. Initial testing (stage 1) of Eribulin, a novel tubulin binding agent, by the pediatric preclinical testing program. *Pediatr Blood Cancer*. 2013;60(8):1325–32.
- Yahiro K, Matsumoto Y, Fukushima JI, Kawaguchi KI, Endo M, Setsu N, Ohashi K, Fukushima S, Nakagawa M, Kimura A, et al. Class III -

overexpression induces chemoresistance to Eribulin in a leiomyosarcoma cell line. *Anal Cell Pathol* 2018;2018:1-18. doi:<https://doi.org/10.1155/2018/8987568>.

31. Hayasaka N, Takada K, Nakamura H, Arihara Y, Kawano Y, Osuga T, Murase K, Kikuchi S, Iyama S, Emori M, et al. Combination of eribulin plus AKT inhibitor evokes synergistic cytotoxicity in soft tissue sarcoma cells. *Sci Rep*. 2019;9(1):5759.
32. De Vita A, Miserocchi G, Recine F, Mercatali L, Pieri F, Medri L, Bongiovanni A, Cavaliere D, Liverani C, Spadazzi C, et al. Activity of Eribulin in a primary culture of well differentiated/dedifferentiated adipocytic sarcoma. *Molecules*. 2016;21(12):1662.
33. Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat Rev Drug Discov*. 2010;9(10):790–803.
34. Takezaki Y, Namikawa T, Koyama T, Munekeg E, Munekeg M, Maeda H, Kitagawa H, Hanazaki K. Antitumor effects of Eribulin Mesylate in gemcitabine-resistant pancreatic cancer cell lines. *Anticancer Res*. 2016;36(11):6077–82.
35. Kawano S, Asano M, Adachi Y, Matsui J. Antimitotic and non-mitotic effects of Eribulin Mesilate in soft tissue sarcoma. *Anticancer Res*. 2016;36(4):1553–61.
36. Sampson VB, Vetter NS, Zhang W, Patil PU, Mason RW, George E, Gorlick R, Kolb EA. Integrating mechanisms of response and resistance against the tubulin binding agent Eribulin in preclinical models of osteosarcoma. *Oncotarget*. 2016;7(52):86594–607.
37. Goto W, Kashiwagi S, Asano Y, Takada K, Takahashi K, Fujita H, Takashima T, Shibutani M, Amano R, Tomita S, et al. The effects of Eribulin on breast cancer microenvironment identified using Eribulin-resistant breast cancer cell lines. *Anticancer Res*. 2019;39(8):4031–41.
38. Watanabe K, Yui Y, Sasagawa S, Suzuki K, Kanamori M, Yasuda T, Kimura T. Low-dose eribulin reduces lung metastasis of osteosarcoma. *Oncotarget*. 2019;10(2):161–74.
39. Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay Drug Dev Technol*. 2014;12(4):207–18.
40. Monderer D, Luseau A, Bellec A, David E, Ponsolle S, Saiagh S, Bercegeay S, Piloquet P, Denis MG, Lodé L, et al. New chondrosarcoma cell lines and mouse models to study the link between chondrogenesis and chemoresistance. *Lab Invest*. 2013;93(10):1100–14.
41. Gris-Oliver A, Ibrahim YH, Rivas MA, García C, Sánchez M, Ruiz-Pace F, Viaplana C, Pérez JM, Lombardussac A, Grueso J, et al. PI3K activation promotes resistance to eribulin in HER2-negative breast cancer. *Br J Cancer*. 2021;124(9):1581–91.
42. Zaal EA, Berkers CR. The influence of metabolism on drug response in cancer. *Front Oncol*. 2018;8:500.
43. Perez M, García-Heredia JM, Ferrer B, Muñoz-Galván S, Martín-Broto J, Carnero A. Sarcoma stratification by combined pH2AX and MAP17 (PDZK1IP1) levels for a better outcome on doxorubicin plus olaparib treatment. *Signal Transduct Target Ther*. 2020;5(1):195.
44. Zheng S, Aldahdooh J, Shadbahr T, Wang Y, Aldahdooh D, Bao J, Wang W, Tang J. DrugComb update: a more comprehensive drug sensitivity data repository and analysis portal. *Nucleic Acids Res*. 2021. <https://doi.org/10.1093/nar/gkab438>.
45. Bernard M, Cardin GB, Cahuzac M, Ayad T, Bissada E, Guertin L, Bahig H, Nguyen-Tan PF, Filion E, Ballivy O, et al. Dual inhibition of autophagy and PI3K/AKT/MTOR pathway as a therapeutic strategy in head and neck squamous cell carcinoma. *Cancers (Basel)*. 2020;12(9):2371.
46. Margue C, Philippidou D, Kozar I, Cesi G, Felten P, Kulms D, Letellier E, Haan C, Kreis S. Kinase inhibitor library screening identifies synergistic drug combinations effective in sensitive and resistant melanoma cells. *J Exp Clin Cancer Res*. 2019;38(1):56.
47. Miyamoto S, Kakutani S, Sato Y, Hanashi A, Kinoshita Y, Ishikawa A. Drug review: Pazopanib. *Jpn J Clin Oncol*. 2018;48(6):503–13.
48. van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, Schöski P, Aglietta M, Staddon AP, Beppu Y, et al. Pazopanib for metastatic soft tissue sarcoma (PALETTE): a randomised, double-blind placebo-controlled phase 3 trial. *Lancet*. 2012;379(9829):1879–86.

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