nature portfolio

Corresponding author(s): Javier Martin-Broto

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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ | A description of all covariates tested |
| ☒ | ☐ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | N/A |
| Data analysis | SPSS (version 29.0.2.0) was utilized to analyze clinical data and to investigate the associations between protein levels and clinical outcomes. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Included in the manuscript: The clinical dataset, with anonymized participant data regarding demographics, treatment information and outcomes will be available upon request to corresponding author for related research and following the signature of an specific agreement to this end. The study protocol and the statistical analysis plan will be available in the Supplementary data. In addition, data regarding the preclinical experiments generated in this study are provided in the Supplementary Information and Source Data file.

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

| | |
|---|---|
| Reporting on sex and gender | Participants provided consent for reporting their self-identified sex (M/F), which is included in Table 1 (Patient Demographics) as frequencies and percentages. The variable Sex was analyzed in relation to clinical outcomes, and the findings applied to both males and females. However, this data was removed at the request of one of the reviewers. We are happy to reintroduce it into the manuscript if required. |
| Reporting on race, ethnicity, or other socially relevant groupings | Data were not stratified by race, ethnicity, or other sociodemographic characteristics, given that sarcomas occur across all populations irrespective of such variables |
| Population characteristics | The reported characteristics include median age (with range). Disease extension was recorded both at diagnosis—classified as localized, locally advanced, or metastatic—and at baseline, distinguishing between locally advanced and metastatic cases. Baseline performance status was assessed using the ECOG scale, with values of 0–1. The metastatic-free interval was reported as a median with range for all patients, as well as separately for those with metastatic disease. The number of previous treatment lines was also given as a median with range. Histological subtypes included leiomyosarcoma, osteosarcoma, alveolar soft part sarcoma, and synovial sarcoma. |
| Recruitment | Patients were recruited according to the inclusion and exclusion criteria. |
| Ethics oversight | Ethics approval was obtained from each participating institution before study initiation and the name and code of approval of Refernce Ethics Commitee is provided in the Manuscript (Ethics Committee from Hospital Clínico San Carlos, approval code C.I. 20/152- EC_M). Patients signed the informed consent to participate in the study. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
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| Sample size | This phase I trial had a 3 + 3 design. Any observed adverse event during the first 21-day period of observation (from day 1 to 21) was considered dose-limiting toxicity (DLT) if the following conditions were met. For hematologic toxicities, grade 4 neutropenia for ≥7 days, grade 4 thrombocytopenia or grade 3 complicated by hemorrhage higher than grade 1, or febrile neutropenia with documented grade ≥ 3 infection or sepsis. For non-hematological toxicities, this was any grade ≥ 3, excluding nausea/vomiting or diarrhea unless uncontrolled by appropriate antiemetic or antidiarrheal therapy. Additionally, the following were not considered as DLT: grade 3 fatigue persisting less than 14 days, grade 3 increased serum creatinine or electrolyte abnormalities deemed not clinically significant and not requiring treatment, and grade 3 transaminitis unless this would delay the treatment administration. If the DLT rate is > 0.33 among the patients with the initial dose level, the treatment dose will be lowered to dose -1. |
| Data exclusions | Two patients in the +3 dose level followed a noncompliant treatment regimen and were excluded from the dose-limiting toxicity analysis. All the patients were included in the safety and efficacy population. |
| Replication | Not applicable |
| Randomization | Not applicable |
| Blinding | Not applicable |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☐ | ☒ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

# Antibodies

Antibodies used

Calbedin-1 (D1I4Q; Cell Signalling; Danvers, MA, USA)
IκBα (66418-1-Ig; Proteintech; Rosemont, IL, USA)
Survivin (10508-1-AP; Proteintech; Rosemont, IL, USA)
Anti-p53 (DO-7) primary antibody (Roche)
Rabbit Anti-Mouse IgG–Peroxidase (Sigma-Aldrich)
Goat Anti-Rabbit IgG H&L Peroxidase-conjugated (Abcam).

Validation

For immunohistochemistry, a positive and negative control was used. These controls were selected according to datasheet and/ or literature. For western blot, protein bands were selected according to the protein weight reported in the datasheet of each antibody.

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Leiomyosarcoma cell lines included CP0024 (female patient), IEC005 (established in the Martin-Broto lab from a female patient), AA (provided by Dr. Carnero, from IBiS, Seville, Spain), and SK-UT-1 (ATCC HTB-114). Osteosarcoma cell lines were MG-63 (ATCC CRL-1427), U2OS (ATCC HTB-96), and SAOS-2 (ATCC HTB-85). MPNST cell lines comprised ICP060 (established in the Dr. Martin-Broto lab from a male patient), S462, 88-14 (provided by Dr. Romagosa, from Hospital Vall d'Hebron, Barcelona, Spain), and sNF96.2 (ATCC CRL-2884).

Authentication

Cell line purity was verified by the provider for commercial cell lines, and by a pathologist through morphological evaluation and/or molecular assays in the case of primary cell lines.

Mycoplasma contamination

All cell lines were negative for mycoplasma

Commonly misidentified lines
(See ICLAC register)

Not applicable

# Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

NCT04595994

Study protocol

The full trial protocol can be accessed through request in the private area of the Sponsor's website: https://grupogeis.org/en/scientific-activity/clinical-trials/open-trials and is also included in Supplementary Information.

Data collection

Clinical data was collected using a web-based Electronic Data Capture (EDC) system. This data was entered into the EDC platform by the clinical sites treating the patients. The period of recruitment was 22 months (November 2020 to September 2022) and data entry and cleaning was performed during this period.

Outcomes

The primary objective was to determine the maximum tolerated dose (MTD) or the recommended dose for phase II of selinexor plus gemcitabine, based on dose-limiting toxicities (DLTs) observed during the first cycle (days 1-21). The secondary objectives included the evaluation of the safety profile according to CTCAE 5.0, the overall response rate (ORR), the efficacy according to Choi response, and the assessment of the patient's quality of life (QoL), measured with EORTC QLQ-C30.

## Plants

| | |
|---|---|
| Seed stocks | N/A |
| Novel plant genotypes | N/A |
| Authentication | N/A |

## Flow Cytometry

### Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

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| Sample preparation | Cells were stained using a FITC Annexin V/propidium iodide (PI) Apoptosis Detection Kit (Immunostep, Salamanca, Spain) following the manufacturer's protocol. |
| Instrument | BD FACSCanto II or BD Accuri C6 Plus system (BD Biosciences) |
| Software | Data analysis was conducted using BD FACS Diva (RRID: SCR_001456) and Floreada.io software (RRID: SCR_025286). |
| Cell population abundance | A total of 20,000 singlets (gate in FSC-A/FSC-H) were evaluated per sample in all experiments. |
| Gating strategy | Cells were first gated on FSC/SSC by selecting events with FSC values above 2M to exclude cellular debris. Singlets were identified using FSC-A/FSC-H, selecting the main diagonal population and excluding aggregates or clumps based on deviations below the linear relationship. For analysis of Annexin V-FITC and propidium iodide (PI) staining, appropriate fluorescence minus one (FMO) controls were used. Specifically, negative controls stained with only Annexin V-FITC or PI were used to establish thresholds for negative signal in each respective channel, for accurate quadrant gating in FITC/PerCP-Cy5.5 plots. |

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.