nature portfolio

Peer Review File

Gemcitabine plus Selinexor in Selective Advanced Sarcomas: a Phase I of the Spanish Group for Research on Sarcoma Study.

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Muticentric spanish ph I trial of gemcitabine plus selinexor in advanced sarcomas with translational objectives.
Solid rational for the study of association with preclinical datas of synergistic effect in LMS cell lines with a complete loss of survivin inducing an increase of the protein levels of γH2A.X, but an antagonist effetc in osteosarcoma cell lines with clinical relevant results (efficacy in LMS, inefficacy in OS).
Expertise of the group and the PI in conducting studies in sarcomas is recognized.
Well done ph I study in a selected population.
Results are interesting in particular in LMS with manageable toxicities ; but hematologic toxicities can be a concern, in particular with gemcitabin as well as digestive ones (in particular nausea and vomiting, clearly related to selinexor) but authors clearly discuss it in the discussion; how many patients received granulocyte growth factor ? Do authors will recommand systematic association ?
What about other experiences with selinexor and other chemotherapies in other tumors ?
Selinexor have been developped in monotherapy in hematology (Kalakonda N, Lancet Haeematol 2020) and have an approval in diffuse large B-cell lymphoma and myeloma; do authors think that use of selinexor alone in maintenance setting to avoid toxicity after response in combination with gemcitabine could be an option, like it have been tested in TP53 wt endometrial carcinoma ? To be discuss
The next step is a phase II with gemcitabine + selinexor in LMS and MPNST ; the osteosarcoma sub group have been discarbed according to the bad results in the phase I? Why do not performed a randomized phase II gemcitabine +/- selinexor ?

Minors remarks :
Abstract is too long ; regarding the small number of patients, authors should foccus on response rate, duration of response and delay OS.
Avoid using some formulas as « the fact is », « the truth is «

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts

The authors present a timely and interesting manuscript evaluating the safety, efficacy, and biomarker correlation of selinexor combined with gemcitabine in a cohort of patients with soft-tissue sarcomas and osteosarcoma. The combination demonstrates encouraging activity, particularly in leiomyosarcoma (LMS) patients, with a manageable toxicity profile that warrants further clinical investigation. The correlative findings—highlighting associations between response and both survivin expression and nuclear localization of IκBα—are intriguing, although preliminary and limited by the absence of mechanistic validation and the small sample size.

Major Comments

Abstract:
The abstract should be significantly shortened, focusing on the most important clinical and translational findings.

Phase I Section:
• The number of patients treated at each dose level during the escalation phase is unclear. Please specify in both the text and Figure 2A.
• In Figure 2B, include the best overall response per patient and indicate the timepoint of progressive disease.
• The translational analyses are underemphasized. Consider including a summary table of the immunohistochemical expression levels (e.g., survivin, IκBα) stratified by histology. How many patients showed high IκBα expression? High survivin?
• Given the small sample size, response rates may be more appropriate than survival analyses for correlative comparisons.
• Are any genomic profiling data available (e.g., TP53, PTEN, RB1)? This would enrich the translational relevance.
• The univariate survival analyses are statistically underpowered given the sample size. Variables such as sex are unlikely to be informative and should be removed.

Preclinical Section:
• Clarify which cell lines correspond to LMS and which to other subtypes.
• Figure 3A is not well-labeled—please clarify what is being plotted.
• Confirm whether SKUT1 data has a typo error or if a different assay was used.
• Report IC50 values for monotherapies explicitly in the main text (not just supplementary).
• If statistical tests were applied to compare treatment effects, please report p-values.
• Western blot quality (especially for CP0024) is suboptimal and does not clearly demonstrate increased γH2AX expression. Similarly, reductions in γH2AX in SAOS2 and MG-63 are not convincingly shown. Consider quantifying band intensity and including statistical comparisons before drawing firm conclusions.
• Survivin appears to be significantly downregulated by selinexor alone, especially in CP0024. However, this does not correlate with differential sensitivity to selinexor across cell lines, which all appear similarly sensitive. The claim that survivin extinction in CP0024 predicts combination efficacy is overstated and should be tempered.
• Overall, the preclinical findings on survivin and IκBα are heterogeneous and not supported by mechanistic data. The strength of the conclusions should be appropriately moderated.

Minor Comments
The Introduction should be reorganized: begin with the biological rationale, followed by preliminary clinical background.
Line 128: Please clarify if this refers exclusively to the J8 patient.

Extensive English language editing is required throughout. Examples:

Line 87: “this fact increases” → rephrase for clarity.
Lines 94–96, 97–98, 101–102: sentence structure and grammar should be revised.
Line 281, 300: Replace “suffered” with “presented,” “exhibited,” or “experienced.”
Line 292: Replace “considering” with “regarding.”
Line 293: Clarify what “predominant” refers to—histology, metastasis site?
Line 294: Replace “within” with “among.”
Line 295: Replace “relevant” with “frequent” or “common.”
Line 299: Replace “recommendations” with “schedules” or “regimens.”
Replace “series” throughout the manuscript with “cohort,” “subgroup,” or “patients,” as appropriate.
Line 334: “OS” is commonly used for “overall survival”—please use a different abbreviation for “osteosarcoma.”
Table 1: Define “metastatic-free interval” clearly in the legend or methods

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)
This phase I clinical trial evaluated the safety and preliminary efficacy of combining gemcitabine with selinexor, an exportin-1 (XPO1) inhibitor, in patients with advanced sarcomas. Seventeen heavily pretreated patients, primarily with leiomyosarcoma or osteosarcoma, were enrolled in a dose-escalation study using a standard 3+3 design. The recommended phase II dose (RP2D) was determined as gemcitabine 1200 mg/m² (administered at 10 mg/m²/min on days 1 and 8) combined with selinexor 60 mg orally weekly. The most common grade 3-4 toxicities were hematologic, including neutropenia (64.7%) and thrombocytopenia (47.1%), and gastrointestinal side effects were mostly grade 1-2. The overall response rate (ORR) was 31.25%, with a median progression-free survival (mPFS) of 5.6 months and a reported median overall survival (mOS) of 39.5 months in both the overall cohort as well as the leiomyosarcoma subgroup. The leiomyosarcoma subgroup showed the most promising outcomes with an ORR of 44.4% and mPFS of 7.6 months, while the osteosarcoma subgroup demonstrated poorer outcomes. Preclinical studies revealed that the combination was synergistic in

most leiomyosarcoma and MPNST (malignant peripheral nerve sheath tumor) cell lines but antagonistic in osteosarcoma models, mirroring clinical data. Mechanistic studies linked the efficacy of the combination to the depletion of survivin and nuclear accumulation of IκBα, which was associated with enhanced DNA damage and apoptosis. Correlative biomarker analysis in the clinical trial also showed that high survivin or IκBα expression predicted shorter PFS in leiomyosarcoma patients. These findings were reported as support to justify the launch of a phase II trial (NCT06114004) focusing on leiomyosarcoma and MPNST cohorts while excluding osteosarcoma due to lack of efficacy. This study highlights the potential of the gemcitabine-selinexor combination as a potential therapeutic strategy, particularly in select sarcoma subtypes, and identifies survivin and IκBα as candidate predictive biomarkers for response.
While the primary endpoint of the study was clearly delineated with evidence that this is a safe and manageable regimen, analysis of outcomes data including overall survival is not clear and warrants additional revision prior to manuscript acceptance.
Major concerns:
- Overall survival analysis needs statsitical review. For example, it is unclear from the Supplemental Figure 2 demonstrating the Kaplan-Meier curve, given number of patients censored between 18 months (5), how they arrived at median OS timepoint of 39.5 months. This is particularly important as given the markedly lower median OS of 8m reported for the second largest histological subset (osteosarcoma, 35%). it is also not clear how the full cohort has the same reported OS of the more favorable leiomyosarcoma subset (53). Additionally, the reported 36-month OS rate is reported as 50.2%, despite all but 2 patients having died or been censored prior to that time point.
- The relative importance of OS is overstated particularly with a PFS value that is good, but not unheard of for a phase I trial. This is a phase I trial and a phase I population is not a good group to estimate OS off of. This is only a concern here because they put so much emphasis on the improved OS. Advise decreased emphasis on unusually long mOS in the discussion given the above and the lack of power to determine statistical significance of this finding.
- Despite quality of life EORTC QLQ-C30 being included in intended secondary outcomes, this information is not reported in results or discussion; unable to find results in supplementary information.
- The preclinical studies (using cell lines) are interesting and they suggest value to the combination but the idea that the benefit might be synergistic, rather than additive, is not supported by the preclinical studies. Of note, while the preclinical studies are not even mentioned in the abstract, they comprise TWO of the figures. By putting the preclinical studies in the same paper as their phase I there is a bit of an implication that the correlative data is supporting the same hypothesis generated by the preclinical studies. However, it does not - it actually is not clear what the implications of the clinical studies are, particularly in light of the potential relation between survivin and PFS. These would be stronger as two separate manuscripts.
- For a phase I trial there should be a separate section discussing the safety and tolerability with a more fleshed out discussion.
- If preclinical studies are to be discussed in the same manuscript as clinical results (and, to reiterate, I don't think they should be), they should be presented first.
- Need to be careful not over interpret result in a study that includes a known active agent (gemcitabine).
Minor concerns:
- Flow of information in the introduction is disorganized and difficult to follow; rationale for selinexor's individual cytotoxic effects and its potential synergistic effects with gemcitabine is mixed rather than sectioned, and comprehension could be improved by grouping supporting information logically. For example: mechanisms of selinexor mediated apoptosis are detailed in two separate areas separated by discussion of a potential mechanism of selinexor/gemcitabine synergy.
- Line 112 – "progressing to at least an anthracycline-based scheme"; unclear if this indicates that patients had progressed ON an anthracycline-based therapy although that is my assumption
- Line 143 – "If the DLT is" – present tense, should be modified to past tense to match rest of section
- Dose modification/delay protocol may be better represented in an algorithmic figure rather than in text for comprehension and manuscript flow
- Line 376 – "notorious", consider "notable"
- Line 378 – "Thus, "gemcitabine", consider, "for example"
- Line 380 – "in a randomized phase II trial, in a series of individuals with STS" consider omitting the comma
- Line 400 – "hazard", consider "chance"
- Line 405 – "The finding that significantly correlates the expression of survivin… with worse efficacy", consider "Our results indicate a significant correlation between increased survivin expression and reduced efficacy…" followed by substitution of "This could prove crucial" for line 406 "could prove crucial"
- Line 453 – capitalize i.v. and p.o.

Reviewer #4

(Remarks to the Author)
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Reviewer #5

(Remarks to the Author)
Comments to authors
The authors are reporting the outcomes of the phase 1 component of a phase ½ trial with Selinexor and gemcitabine in advanced sarcomas. Per the pre-specified statistical analysis plan, the phase 1 objectives included finding the MTD,

reporting AEs per CTCAE v5, describing the overall response rate, describe a subset who are evaluable using the Choi response criteria, and describing patient qol outcomes. After determining the MTD, the authors were planning to assess efficacy in a randomized phase 2 trial that will accrue specific sarcoma subtypes.
• Though it is a reasonable design, the 3+3 design often chooses a more conservative RP2D (https://pmc.ncbi.nlm.nih.gov/articles/PMC4551156/) as compared to other phase 1 trial designs. The authors state "Although the recommended phase II dose was technically at the +3 dose-level (with gemcitabine at 1200 mg/m² administered at 10 mg/m²/min followed by selinexor at 80 mg weekly), the overall tolerance was noticeably better with selinexor at 60 mg weekly. Therefore, the RP2D was determined to be the +2 dose-level (gemcitabine at 1200 mg/m² at 10 mg/m²/min followed by selinexor at 60 mg weekly)." In essence the authors have chosen a lower dose level (dose level +2) which is even more conservative than the already conservative dose level that would have been selected based on the traditional 3+3 design. Though this may reasonable to take forward in the phase two setting, it does deviate from the pre-specified statistical analysis plan.
o This may be more relevant as the authors state G-CSF was used as part of the study (which makes sense) to prevent prolonged neutropenia and subsequent complications like febrile neutropenia. It is possible that a higher dose level like dose level 3 would have been well tolerated.

• Though the authors do discuss the primary and secondary objectives of the phase 1 study including the RP2D, the safety profile, and the response rates (ORR and a subset with Choi), they also dedicate a large portion of the manuscript describing efficacy data. It is reasonable to describe the outcomes of the 17 patients that were enrolled, but the authors should consider limiting the discussion and conclusions to fit the scope of the phase one study.

• For the overall cohort of 17 patients, the median overall survival was longer than the median follow-up. This can happen in cases where there is a single patient contributing a large amount of survival time
o https://www.sciencedirect.com/science/article/pii/S0006497118638236#:~:text=Median OS was not reached,the median OS estimate upward.
• The CONSORT-like diagram in supplementary Figure 1 shows an efficacy population of 17 but the authors should highlight that although 17 may have been assessed for PFS, only 16 were available for response evaluation.

• Supplementary Table 2
o Lack of uniformity: Some values do not have 95% CI (e.g. median OS for survivin expression)
o No clear idea of a dose-response relationship based on survivin or IκBα expression as data stratified by dose level or sarcoma subtype was not provided. Though this may provide some evidence overall that the combination of gemcitabine and Selinexor reduces expression of survivin and IκBα, it is unclear if this is subtype specific.

• Supplementary Table 1
o In the manuscript the authors state that a log-rank test was used to test differences and in the pre-specified statistical analysis plan they state "For continuous efficacy variables such as PFS, their medians will be used. Association between variables will be measured and tested by conventional statistical analysis: Pearson and/or Spearman tests." Please clarify
What statistical tests were used to derive p-values in each table or figure so that a reviewer can understand.
If there was a deviation from the pre-specified plan, what was the reasoning.

• Supplementary Figure 2 (Panels B and C)
o Panel B refers to the 9 LMS cases that were enrolled and Figure 2B shows the PFS of all cases. For LMS, one of the patients still has follow-up ongoing (non-protocol specified) and has not progressed at ~30 months whereas the Supplementary Figure 2B shows a longer PFS of around 36 months. As this is likely the same patient, please clarify the difference between these two figures.
o For osteosarcoma patients enrolled, there is one patient who had progression around 36 months (per Figure 2B) but in SF2 Panel C the KM curve is cutoff around 30 months. As this is likely the same patient, please clarify the difference between these two figures.

• Supplementary Figure 3 (Panels A and B)
o The authors state: "In the leiomyosarcoma series of patients, high expression of survivin (>50%) correlated significantly with worse PFS [3.4 months(95% CI 0-7.6) vs 15.4 months (95% CI 3.1-27.7), p=0.022], whereas the high intensity of IκBα expression (level 4) was significantly associated with worse PFS [3.4 months (95% CI 0-7.6) vs 15.4 months (95% CI NR), p=0.007], (Supplementary Table S2 and Supplementary Figure S3)."
The p-values are presumably generated from log-rank tests reflect the whole curve in both Panels A and B of SF3 are not reflective of the median PFS which the authors report and overlap with each other.

• The discussion has more than one statement that is grammatically incorrect, unclear, or too assertive given the limited sample size.
o Unclear and too assertive: The authors state "The activity detected in this series, with an ORR of 31% according to RECIST criteria and central assessment, and a mPFS of 5.7 months, suggests signs of notorious activity with this scheme in the context of second lines in patients with advanced progressing sarcoma."
What does notorious activity mean?
The authors describe the median PFS for the 17 patients without at least giving the 95% CI to help the reader understand the precision of the median PFS.
o Conclusions not related to the phase 1 component of the study: The authors state "A phase II trial has been activated (NCT06114004) exploring the combination of gemcitabine 1,200 mg/m2 in a 10 mg/m2/min i.v. infusion on days 1 and 8, followed by selinexor p.o. at a 60 mg flat dose on days 1, 8, and 15 in cycles repeated every 21 days, in two different cohorts:

leiomyosarcoma, and MPNST. The inclusion of a cohort of MPNST patients was supported by the preclinical evidence, in which we observed synergy between selinexor and gemcitabine in the majority of the cell lines used. The osteosarcoma cohort was discarded for the phase II part following the insights gained in this phase I trial, where the mPFS was just 1.2 months, and also because of the antagonism observed in the preclinical experiments."
Though the authors do give some preclinical data that may show some activity in MPNST, this was not the focus of the phase 1 component based on the statistical analysis plan.

• Editorial comments
o Throughout the manuscript, it is often unclear what the underlying denominator is for the respective percentages. The authors should provide more granular descriptions so that a reviewer does not have to assume what the denominator is for each result.
o Some terminology is not clear (Table 1- Extension at baseline) however could be inferred. This again reiterates the recommendation to have a more clearly written manuscript.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Reviewer 1: thank you for the responses; few remarks

Response 3 : I would rather suggest mentioning the study in endometrial cancer in the subgroup of patients with p53 wild-type tumors.(Vergote I J Clin Oncol ref 33)

Response 6: last sentence p 17 "And this paragraph in the Discussion section has been also modified:
Page 17: "Despite the prophylactic recommendations for ondansetron and olanzapine drugs in the protocol, the truth is, it was evident that they were not universally adopted." I would have rather suggested: Prophylactic ondansetron and olanzapine recommanded in the protocol, were not systematicaly prescribed.

Reviewer #2

(Remarks to the Author)
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Reviewer #3

(Remarks to the Author)
I think they have addressed out concerns

Reviewer #5

(Remarks to the Author)
The revised manuscript does read better and the investigators have addressed most of my questions. It is appreciated that the investigators now include "n=xx" throughout the manuscript so the reader understands the sample size associated with each effect estimate. The authors have toned down any conclusions based on survival endpoints, added "disclaimers" about interpreting OS, and highlighted the reason for moving towards a phase 2 with the dose level that was chosen.

Please address the following
1) The investigators still need to address the inclusion of the survivin analysis in the abstract as it was not pre-specified. If the investigators chose to include this in the manuscript, it should be removed from the abstract, noted this was exploratory and any comparison of groups (survivin >= 50%) was not-prespecified and should be interpreted with caution.
2) For Supp Table S3, please include the number of patients in each group so that a reader could understand why there is no 95%CI as the authors rightly mentioned could happen in a small subgroup of a phase 1 trial. On a re-read of the statistical analysis plan, the statistician clearly states "P-values are only going to be reported for Efficacy Analysis." Table S3 does not fall into the planned efficacy analysis.

Dear editorial team,

We would like to thank you for the opportunity to strengthen our manuscript by addressing the reviewers' comments and suggestions. Please find below our point-by-point responses to their feedback. The page numbers referenced in this rebuttal letter correspond to those in the tracked changes version of the manuscript.

REVIEWER COMMENTS

Reviewer #1 (eREF; sarcoma, clinical):

Muticentric spanish ph I trial of gemcitabine plus selinexor in advanced sarcomas with translational objectives.
Solid rational for the study of association with preclinical datas of synergistic effect in LMS cell lines with a complete loss of survivin inducing an increase of the protein levels of γH2A.X, but an antagonist effect in osteosarcoma cell lines with clinical relevant results (efficacy in LMS, inefficacy in OS).
Expertise of the group and the PI in conducting studies in sarcomas is recognized.
Well done ph I study in a selected population.
Results are interesting in particular in LMS with manageable toxicities; but hematologic toxicities can be a concern, in particular with gemcitabine as well as digestive ones (in particular nausea and vomiting, clearly related to selinexor) but authors clearly discuss it in the discussion;

1.- How many patients received granulocyte growth factor? Do authors will recommend systematic association?

Answer: There have been 11 patients requiring, at any time beyond the 1st cycle, the co-administration of GCSF. We have modified accordingly in the text (in the Results section, page 15):

"Granulocyte-colony stimulating factors (G-CSF) were ~~**commonly**~~ used throughout the study, **beyond the first cycle,** to prevent delays and infections **in 11 of 17 (65%) accrued patients.** The most frequent recommendations were 3 doses of G-CSF from day 2 and 5-7 doses from day 9 of each cycle. Only 2 (11%) patients suffered febrile neutropenia. No toxic death was reported in this trial.''

Moreover, we have added a systematic recommendation in the following paragraph in the Discussion section, Page 17:

"**We recommend the systematic use of G-CSF for 3 and 5 consecutive days from days 2 and 9 of each cycle**."

2.- What about other experiences with selinexor and other chemotherapies in other tumors?

Answer: Selinexor have been developed in monotherapy in hematology (Kalakonda N, Lancet Haeematol 2020) and have an approval in diffuse large B-cell lymphoma and myeloma. We have added the following paragraph in the Introduction section:

Page 3: ‘’**Selinexor as a monotherapy has received FDA approval for the treatment of relapsed or refractory diffuse large B-cell lymphoma and triple-class refractory myeloma, based on clinical trials that demonstrated overall response rates of 28%[8] and 26%[9], respectively.**’’

Two new citations were added to the manuscript:

8. Kalakonda, N., Kauffman, M. & Shah, J. Selinexor for relapsed or refractory diffuse large B-cell lymphoma: examining the artifact – Authors' reply. *The Lancet Haematology* **7**, e707-e708, doi:10.1016/S2352-3026(20)30300-8 (2020).

9. Chari, A. *et al.* Oral Selinexor–Dexamethasone for Triple-Class Refractory Multiple Myeloma. *New England Journal of Medicine* **381**, 727-738, doi:doi:10.1056/NEJMoa1903455 (2019).

3.- Do authors think that use of selinexor alone in maintenance setting to avoid toxicity after response in combination with gemcitabine could be an option, like it have been tested in TP53 wt endometrial carcinoma?

Answer: Thanks for the suggestion. It has been added the following paragraph in the Discussion section, Page 20:

‘’The potential application of selinexor as a maintenance therapy represents a promising future strategy, particularly following its combination with gemcitabine during the induction phase. This approach has proven effective in the first-line treatment of advanced leiomyosarcoma, as demonstrated by the use of doxorubicin combined with trabectedin, followed by trabectedin maintenance.[34]”

A new reference (Ref. 34) has been added to the manuscript:

34. Pautier, P. *et al.* Doxorubicin–Trabectedin with Trabectedin Maintenance in Leiomyosarcoma. *New England Journal of Medicine* **391**, 789-799, doi:doi:10.1056/NEJMoa2403394 (2024).

4.- The next step is a phase II with gemcitabine + selinexor in LMS and MPNST; the osteosarcoma sub group have been discarded according to the bad results in the phase I?

Answer: The Phase II portion is currently in progress for two cohorts, namely leiomyosarcoma and MPNST, as noted in the concluding section of the Discussion. The osteosarcoma cohort was excluded based on clinical and preclinical evidence.

5.- Why do not performed a randomized phase II gemcitabine +/- selinexor?

Answer: This was the original proposal. After the completion of Phase I, a randomized Phase II-III trial comparing Gemcitabine plus Selinexor to Gemcitabine alone was planned. Unfortunately, Karyopharm Therapeutics reduced the budget, and as a result, we were only able to continue with a single-arm Phase II study supported by Menarini.

6.- A) Minors remarks: Abstract is too long; regarding the small number of patients, authors should focus on response rate, duration of response and delay OS.

Answer: The abstract has been shortened following your recommendations from 344 to 243 words (Page 2):

~~**Patients with advanced sarcoma have limited therapeutic options.**~~ Exportin-1 (XPO-1), a mediator of nuclear export, has been related to drug resistance and poor prognosis **in solid tumors**. Selinexor, an XPO-1 inhibitor, has shown preclinical and clinical activity in sarcomas. In this Phase I study, ~~we explore~~ the combination of gemcitabine and selinexor **has been explored in a classic 3 + 3 design.** ~~**This Phase I study had a classic 3+3 design.**~~ Adult patients with selected advanced sarcomas received gemcitabine on days 1,8, and weekly selinexor in 21-day cycles. The main endpoint was to determine the recommended phase 2 dose (RP2D). Secondary end-points included, **among others,** ~~**toxicity,**~~ overall response rate (ORR), overall survival (OS), ~~**quality of life,**~~ and translational objectives. Preclinical studies of synergy and dynamic biomarkers were performed in sarcoma cell lines. Seventeen patients were included in this study. Although only one dose-limiting toxicity (grade 4 thrombocytopenia) was detected in dose-level +3, the R2PD was determined to be the dose-level +2 (gemcitabine at 1200 mg/m² at 10 mg/m²/min followed by 60 mg weekly selinexor) based on its better tolerability. The most frequent adverse events ~~**(AEs)**~~ (all grades) were neutropenia (82.4%) and thrombocytopenia (76.5%). ~~**Neutropenia (64.7%) and thrombocytopenia (47.1%) were the most frequent grade 3-4 AEs.**~~ The ORR was 31.25 %, ~~**With a median follow-up of 30 months (95% CI, 18-41),**~~ **and** the median OS (mOS) was 39.5 months (95% CI, 12.4-67) with a 36-month OS rate of 50.2%. ~~**The median progression-free survival (mPFS) was 5.6 months (95% CI, 1.6-9.5) for the whole series and 7.6 months (95% CI, 1.9-13.2) among the patients with leiomyosarcoma (n=9). Among patients with osteosarcoma (n=6), the mPFS was 1.2 months (95% CI, 1-1.3).**~~ In leiomyosarcoma patients, high expression of survivin (>50%) significantly correlated with worse PFS [3.4 months (95% CI 0-7.6) vs 15.4 months (95% CI 3.1-27.7), p=0.022]. ~~**In vitro cell viability studies showed that the combination of selinexor with gemcitabine was synergistic in the majority of leiomyosarcoma cell lines tested, and antagonistic in osteoarcoma cells. The combination of gemcitabine 1200 mg/m² at 10 mg/m²/min days 1 and 8, followed by selinexor at 60 mg weekly, is a tolerable regimen with interesting activity in leiomyosarcoma.**~~ **Based on preclinical and clinical findings, a phase II is currently running exploring this combination in two different cohorts, leiomyosarcoma and malignant peripheral nerve sheath tumors.**

6.-B) Avoid using some formulas as « the fact is », « the truth is ».

Answer: This paragraph in the Discussion section has been modified from:

Page 17: "**Despite G-CSF not being recommended from the treatment initiation, the fact is that it was incorporated in the majority of patients throughout the study. Certainly, its use could explain the low proportion of patients, 11%, with febrile neutropenia**."

Into:

Page 17: "**Although our study followed the standard Phase I trial design, where the RP2D is selected based on a 20-33% dose-limiting toxicity (DLT) rate in the first cycle, we recommended dose level +2 (below the suggested level by DLTs) due to observed cumulative toxicity.[20] While several patients (65%) required G-CSF after the first cycle, 65% and 47% experienced grade 3 or 4 neutropenia and thrombocytopenia, respectively."**."

And this paragraph in the Discussion section has been also modified:

Page 17: "Despite the prophylactic recommendations for ondansetron and olanzapine drugs in the protocol, ~~**the truth is,**~~ **it was evident** that they were not universally adopted."

Reviewer # 2

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

The authors present a timely and interesting manuscript evaluating the safety, efficacy, and biomarker correlation of selinexor combined with gemcitabine in a cohort of patients with soft-tissue sarcomas and osteosarcoma. The combination demonstrates encouraging activity, particularly in leiomyosarcoma (LMS) patients, with a manageable toxicity profile that warrants further clinical investigation. The correlative findings—highlighting associations between response and both survivin expression and nuclear localization of IκBα—are intriguing, although preliminary and limited by the absence of mechanistic validation and the small sample size.

Major Comments

Abstract:

1.- The abstract should be significantly shortened, focusing on the most important clinical and translational findings.

Answer: The abstract has been shortened following your recommendations from 344 to 243 words. (See the reply for the request 6.-A of Reviewer #1).

Phase I Section:

2.-A)• The number of patients treated at each dose level during the escalation phase is unclear. Please specify in both the text and Figure 2A.

Answer: The following information has been added to the RESULTS section to enhance clarity.

Page 14: "No DLTs were detected from 0 to +2 dose-levels, **with 3 patients treated at each level,** while one DLT (grade 4 thrombocytopenia) was detected in a patient accrued at +3 dose-level."

The paragraph following this sentence clarified that the dose level +3 was expanded to include up to eight patients due to the occurrence of two non-compliant patients during the first cycle.

Additionally, the information of 16 evaluable patients for radiological assessment was implemented in Figure 2A.

2.-B)• In Figure 2B, include the best overall response per patient and indicate the time point of progressive disease.

Answer: The requested information has been implemented in Figure 2B.

2.- C)• The translational analyses are underemphasized. Consider including a summary table of the immunohistochemical expression levels (e.g., survivin, IκBα) stratified by histology. How many patients showed high IκBα expression? High survivin?

Answer: A new Supplementary Table (Supplementary Table S4) with IHQ findings stratified by patient and histology has been added and mentioned on the Results section, page 15. Additionally, the number of cases per antibody was correctly added to the sentence.

Page 15: ''Protein expression analyses by immunostaining were fully assessable for all the biomarkers in 15 out of 17 cases **for p53, Calbindin 1 and survivin and 14 out of 17 for IκBα antibodies (Supplementary Table S2).**''

2.- D) • Given the small sample size, response rates may be more appropriate than survival analyses for correlative comparisons.

Answer: A specific correlation has been made between potential prognostic factors and response. The first analysis clusters response vs progressive disease plus stabilization, and the second clusters progressive disease vs response and stabilization. This analysis is depicted in Supplementary Table S6. A sentence has been added to the Results section, page 16, and Methods, page 12:

''**No clinical or molecular factors showed a correlation with ORR or clinical benefit (defined as PR or SD), as shown in Supplementary Table S4.**''

'' **Prognostic factors related to response were assessed using binary logistic regression.**''

2.-E)• Are any genomic profiling data available (e.g., TP53, PTEN, RB1)? This would enrich the translational relevance.

Answer: Regrettably, genomic analysis was not conducted due to budgetary constraints.

2.-F)• The univariate survival analyses are statistically underpowered given the sample size. Variables such as sex are unlikely to be informative and should be removed.

Answer: The variable sex has been deleted from the univariate analysis.

Preclinical Section:

3.-A) • Clarify which cell lines correspond to LMS and which to other subtypes.

Answer: The correspondence between cell line and histology has been written in the Methods section, Page 5 and Results section, Page 12. However, we added this information to the Figure legends, Page 24:

''Quantification of necrotic and apoptotic cell populations is presented as percentages of total LMS **(CP0024, SK-UT-1, AA and IEC005), ~~OS~~ osteosarcoma (MG-63, U2OS and SAOS-2),** and MPNST **(ICP060, S462, sNF96.2 and 88-14)** cells after 72-hour treatment with either 1 μM selinexor, 1.5 nM gemcitabine, or the combination of both (n=3).''

And

‘’ The expression of survivin and yH2A.X was evaluated by western blot in A) CP0024 **(leiomyosarcoma)**; B) IEC005 **(leiomyosarcoma)**; C) MG-63 **(osteosarcoma)**; and D) SAOS-2 **(osteosarcoma)** cells after 48 hours of treatment with selinexor and/ or gemcitabine.’’

3.-B)• Figure 3A is not well-labeled—please clarify what is being plotted.

Answer: The Figure 3A plots the concentration of Gemcitabine (YY Axis) or Selinexor (XX Axis). This information has been explained in the Figure legend (Page 24):

‘’A) Isobologram displays the IC50 ~~**value**~~ **concentration** of selinexor on the X-axis and the IC50 ~~**value**~~ **concentration** of gemcitabine on the Y-axis.

3.-C)• Confirm whether SKUT1 data has a typo error or if a different assay was used.

Answer: It is a typo error. The label has been corrected.

3.-D) • Report IC50 values for monotherapies explicitly in the main text (not just supplementary).

Answer: This information has been added to the main text as requested:

Page 12: ‘’ ~~**The IC50 values for single-agent treatments can be found in Supplementary Table S3.**~~ **Leiomyosarcoma cell lines exhibited IC_{50} values for selinexor ranging from 0.050 µM (AA) to 0.256 µM (CP0024), whereas IC_{50} values for gemcitabine ranged from 0.342 nM (SK-UT-1) to 9.70 nM (IEC005) within the same histologic subtype. In osteosarcoma cells, selinexor IC_{50} values ranged from 0.130 µM (SAOS-2) to 0.146 µM (U2OS), while gemcitabine IC_{50} values ranged from 0.399 nM (SAOS-2) to 0.914 nM (U2OS). For MPNST cells, selinexor IC_{50} values ranged from 0.102 µM (ICP060) to 1.60 µM (S462), and gemcitabine IC_{50} values ranged from 14.5 nM (ICP060) to 35.1 nM (S462) in this histology (Supplementary Table S5).** ~~**On the other hand, t**~~**T**hree leiomyosarcoma…’’

3.-E)• If statistical tests were applied to compare treatment effects, please report p-values.

Answer: The p-values had been added to the main text.

3.-F)• Western blot quality (especially for CP0024) is suboptimal and does not clearly demonstrate increased γH2AX expression. Similarly, reductions in γH2AX in SAOS2 and MG-63 are not convincingly shown. Consider quantifying band intensity and including statistical comparisons before drawing firm conclusions.

Answer: Western blots had been quantified as recommended by the reviewer and the ratio y-H2AX: A-Tubulin added to the Figure. The methods section was modified to include this sentence: ‘’ **Blot protein quantification was performed with ImageJ**.’’

3.-G)• Survivin appears to be significantly downregulated by selinexor alone, especially in CP0024. However, this does not correlate with differential sensitivity to selinexor

across cell lines, which all appear similarly sensitive. The claim that survivin extinction in CP0024 predicts combination efficacy is overstated and should be tempered.

Answer: We agree with the reviewer and therefore we added a new sentence to the Discussion section, Page 19:

‘’**However, we cannot rule out the possibility that survivin expression is related to selinexor activity rather than to the combination, since selinexor monotherapy abrogated this protein’s expression in several cell lines that showed similar sensitivity to the compound in our preclinical experiments**.’’

3.-H)• Overall, the preclinical findings on survivin and IκBα are heterogeneous and not supported by mechanistic data. The strength of the conclusions should be appropriately moderated.

Answer: We agree with the reviewer that we may had overstated the conclusions. Therefore, we changed the following sentence:

Page 13: ‘’Immunofluorescence analysis revealed selinexor-induced nuclear accumulation of IκBα in the CP0024 cell line (Figure 4E), which was absent in IEC005, MG-63, and SAOS-2 cells (Figure 4F-H), ~~**indicating**~~ **suggesting** that IκBα nuclear localization ~~**is**~~ **may be** critical for achieving complete survivin depletion and mediating the synergistic effect of selinexor with gemcitabine.’’

Moreover, we adapted our mechanistic hypothesis to moderate it:

Page 19: ‘’ The complete loss of survivin seem**eds** to be associated with the nuclear accumulation of IκBα, which was only observed in our preclinical experiments in the CP0024 cell line. IκBα **had been reported to** regulate**s** survivin by sequestering NF-κB in the nucleus, after selinexor treatment, thereby suppressing its transcriptional activation of survivin, an anti-apoptotic protein.[30] It is worth noting that the low intensity of IκBα expression, before treatment, was associated with better PFS in our series. A possible hypothesis **to be further tested** is that survivin binds the IKKβ promoter, increasing its transcriptional activity. This **could** elevate**s** IKKβ expression, leading to Iκ-Bα phosphorylation/degradation and subsequent NF-κB nuclear translocation. NF-κB then **could** upregulate**s** survivin, creating a feed-forward loop.31 Future preclinical studies should address these hypotheses in order to improve clinical study design.’’

Minor Comments:

*4.- The Introduction should be reorganized: begin with the biological rationale, followed by preliminary clinical background***.**

Answer: Thanks for the suggestion. The introduction has been arrange following the suggested order as follows:

Gral Introduction

Patients with advanced sarcomas represent a vulnerable population with a poor prognosis. The median overall survival has increased in the last decade due, at least partially, to the emergence of new treatment options in second lines. Specifically, in advanced soft tissue sarcomas (STS) the survival expectancy is now above one and a half years if we consider the recent randomized phase III trials.[1,2] In the case of osteosarcoma patients, the figures are even worse, with life expectancy below one year in metastatic relapsed disease, except for surgically rescued cases.[3-5]

Therefore, new strategies or new drugs with different mechanisms of action need to be incorporated into the therapeutic arsenal of advanced sarcoma patients.

PRECLINICAL INFO

Overexpression of XPO-1 has been related to therapy resistance and poor survival in solid tumors.[6] Selinexor is a first-in-class, highly specific small-molecule inhibitor of exportin-1 (XPO-1, also known as CMR1). This protein is the main mediator of nuclear export in many cell types, and it mediates the leucine-rich nuclear export signal. Inhibiting this function hinders the escape of various tumor suppressor proteins, thereby strengthening tumor suppression.[7]

In preclinical studies, Selinexor's efficacy was evaluated across multiple sarcoma subtypes through in vitro and in vivo models, encompassing 17 cell lines and 9 xenograft models. Most sarcoma cell lines showed sensitivity to selinexor with IC_{50} values ranging from 28.8 nM to 218.2 nM. Selinexor suppressed sarcoma xenograft growth, including an alveolar soft part sarcoma model that was resistant *in vitro*.[8]

Selinexor combined with gemcitabine, among other drugs, demonstrated enhanced activity in mouse xenograft models in pancreatic carcinoma.[9] The synergistic action may derive from the biological effect of the selinexor reducing mRNA and the protein expression of DNA damage-repairing gene products. Thus, selinexor prevents different repair signaling, providing rationale for a combination with DNA damage agents, such as gemcitabine.[10] Importantly, the sequence turned out to be relevant, and cytotoxicity was higher if the DNA damage agent was first administered and then selinexor. Theoretically, selinexor could induce apoptosis not only through the blockade of DNA repair gene products but also by nuclear retention of p27, induction of Bax protein, and depletion of survivin.[9] The Fas pathway can represent another potential synergistic mechanism between gemcitabine, a Fas sensitizer,[11] and selinexor, a Fas activator.[12]

Selinexor has been tested in a phase I trial including patients with solid tumors. In this trial, among the 157 patients evaluated for efficacy, 1 patient had a complete response (CR), 6 patients had partial responses (PR), and 67 patients had stable disease (SD), with 27 (17%) patients reaching stabilization at least for 4 months. Interestingly, a patient with STS had stable disease for almost two years.[13] The toxicity profile, most frequently low-grade gastrointestinal (nausea, vomiting, anorexia) and general (weight loss and fatigue), is manageable with supporting drugs. This fact increases tolerability as demonstrated in patients with long-term use of selinexor and facilitates the combination with other drugs. In addition, the metabolism of selinexor is independent of the cytochrome P450 isoenzymes, making its combination with other drugs more feasible. Additionally, selinexor was tested in a phase Ib trial involving 54 patients with STS and bone tumors that had progressed after at least one prior line of treatment. SD was reported in 30 out of 52 evaluable patients (58%), with 17 (33%) remaining stable for at least 4 months. The median progression-free survival (PFS) was 4.2 and 3.7 months for liposarcoma and leiomyosarcoma patients, respectively.[14] In the correlative studies linked to the aforementioned phase I trials, selinexor reduced proliferation and increased apoptosis, due to the nuclear accumulation of tumor suppressor proteins such as p53 and FOXO3.[13,14]

Based on previous considerations, a phase I trial was designed to explore the combination of gemcitabine plus selinexor in patients with sarcomas. Here we present the results of this trial.

Various:

5.-A) Line 128: Please clarify if this refers exclusively to the J8 patient.

Answer: Yes, this referred to day 8 of each cycle.

The phrase (Page 8) in the context… "For day 8, if the neutrophil count was ≥ 1,000/μl and the platelet count ≥ 100,000/μl, then both drugs were administered at full dose. If the neutropenia was grade 3 or thrombocytopenia was ≤ grade 2, then gemcitabine was administered at 80% of the total dose, and selinexor at the investigator's discretion, while if thrombocytopenia was ≥ grade 3 or neutropenia was grade 4, gemcitabine was omitted or delayed and selinexor delayed until grade 2 or less."…

…has been modified as follows:

"**The rules and recommendations for day 8 are as follows:** if the neutrophil count was ≥ 1,000/μl and the platelet count ≥ 100,000/μl, then both drugs were administered at full dose; if the neutropenia was grade 3 or thrombocytopenia was ≤ grade 2, then gemcitabine was administered at 80% of the total dose, and selinexor at the investigator's discretion,

while if thrombocytopenia was ≥ grade 3 or neutropenia was grade 4, gemcitabine was omitted or delayed and selinexor delayed until grade 2 or less."

5.-B) Line 87: "this fact increases" → rephrase for clarity.

Answer: The phrase (Page 4) in the context… "The toxicity profile, most frequently low-grade gastrointestinal (nausea, vomiting, anorexia) and general (weight loss and fatigue), is manageable with supporting drugs. This fact increases tolerability as demonstrated in patients with long-term use of selinexor and facilitates the combination with other drugs"..

…has been modified as follows:

"The toxicity profile, predominantly characterized by mild gastrointestinal symptoms such as nausea, vomiting, and anorexia, as well as general effects including weight loss and fatigue, is manageable with supportive medications. This enhances tolerability, as evidenced in patients undergoing long-term treatment with selinexor, and supports its use in combination with other therapies."

6.- Extensive English language editing is required throughout. Examples: 5.-C) Lines 94–96, 97–98, 101–102: sentence structure and grammar should be revised.

Answer: We have rephrased the requested sentences to gain clarity.

5.-A) Lines 94-96: "In the correlative studies linked to the aforementioned phase I trials, selinexor reduced proliferation and increased apoptosis, due to the nuclear accumulation of tumor suppressor proteins such as p53 and FOXO3"

It has been rephrased to…

"In the correlative studies associated with the previously mentioned phase I trials, selinexor was shown to decrease cellular proliferation and enhance apoptosis, attributable to the nuclear accumulation of tumor suppressor proteins including p53 and FOXO3."

5.-B) Lines 97-98: "Selinexor combined with gemcitabine, among other drugs, demonstrated enhanced activity in mouse xenograft models in pancreatic carcinoma"

It has been rephrased to…

"Selinexor, in combination with gemcitabine and other agents, exhibited increased efficacy in mouse xenograft models of pancreatic carcinoma."

5.-C) Lines 101-102: "Importantly, the sequence turned out to be relevant, and cytotoxicity was higher if the DNA damage agent was first administered and then selinexor."

It has been rephrased to…

"Importantly, the order of administration proved to be important, with cytotoxicity being greater when the DNA-damaging agent was given prior to selinexor."

5.-D) Line 281, 300: Replace “suffered” with “presented,” “exhibited,” or “experienced.”

Line 281: “At the time of inclusion, 15 (88%) of the patients **presented** ~~**suffered**~~ metastasis while…”

Line 300: “Only 2 (11%) patients **experienced** ~~**suffered**~~ febrile neutropenia…”

5.-E) Line 292: Replace “considering” with “regarding.”: See below 5.-F the new sentence.

5.-F) Line 293: Clarify what “predominant” refers to—histology, metastasis site?

Line 293: “Considering the highest toxicity events per patient, treatment-related, hematologic side effects were predominant and were distributed as follows…”

Predominant refers to the treatment-related side effects. However, we propose the following modification to gain clarity:

“When examining the highest toxicity events experienced by each patient, treatment-related hematologic side effects were the most common and occurred in the following distribution...”

5.-G) Line 294: Replace “within” with “among.”

Line 294: “~~**Within**~~ **Among** the grade 3 or 4 toxicities, the hematologic…”

5.-H) Line 295: Replace “relevant” with “frequent” or “common.”

Line 295: “the hematologic ones were still the most ~~**relevant**~~ **common**…”

5.-I) Line 299: Replace “recommendations” with “schedules” or “regimens.”

Line 299: “The most frequent ~~**recommendations**~~ **schedules** were 3 doses…”

5.-J) Replace “series” throughout the manuscript with “cohort,” “subgroup,” or “patients,” as appropriate.

Line 49: “for the whole series” was eliminated after the abstract reedition.

Line 277: “The median age of patients in the series was 50 years” has been modified to “the median age of patients was 50 years”.

Line 311: “for the whole series of 17 patients” has been modified to “for the whole **cohort** of 17 patients”

Line 321: “with clinical outcomes in the complete series” has been modified to “with clinical outcomes in the complete **cohort**”

Line 323: “in the whole series” has been modified to "in the whole **cohort**”

Line 323: “In the leiomyosarcoma series…” has been modified to "In the leiomyosarcoma **subgroup**…”

Line 375 The sentence “The activity detected in this series, with an ORR of 31%” has been completely modified to: ‘’**This study shows notable activity with an ORR of 31% based on RECIST criteria and central review, and a mPFS of 5.7 months. These**

results warrant further investigation of this treatment regimen as a second-line option for patients with advanced, progressing sarcoma."

Line 380 "in a series of individuals with STS" has been modified to "in a **cohort** of individuals with STS"

Line 430 "was associated with better PFS in our series" has been modified to "was associated with better PFS in our **cohort**"

Line 437 "However, in our series, we could not detect calbindin 1 protein expression…" has been modified to "However, in our **cohort**, we could not detect calbindin 1 protein expression"

Line 444 "but in our series, it lacked significant…" has been modified to "but in our **patients**, it lacked significant…"

5.-K) Line 334: "OS" is commonly used for "overall survival"—please use a different abbreviation for "osteosarcoma."

We have chosen to preserve to whole name of "osteosarcoma" to avoid misunderstanding.

Line 334 "In OS cell lines" has been modified to "In **osteosarcoma** cell lines"

Line 551 (Figure 3) "…OS, and MPNST cells after 72-hour…" has been modified to "…**osteosarcoma**, and MPNST cells after 72-hour…"

5.-L) Table 1: Define "metastatic-free interval" clearly in the legend or methods.

Answer: It has been added to the legend of Table 1: **The metastasis-free interval is the time elapsed from initial localized diagnosis until the disease becomes advanced (metastatic or locally inoperable). If advanced at diagnosis, this interval is zero.**

Reviewer #2 (ECR):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3 (eREF; sarcoma, clinical):

This phase I clinical trial evaluated the safety and preliminary efficacy of combining gemcitabine with selinexor, an exportin-1 (XPO1) inhibitor, in patients with advanced sarcomas. Seventeen heavily pretreated patients, primarily with leiomyosarcoma or osteosarcoma, were enrolled in a dose-escalation study using a standard 3+3 design. The recommended phase II dose (RP2D) was determined as gemcitabine 1200 mg/m² (administered at 10 mg/m²/min on days 1 and 8) combined with selinexor 60 mg orally weekly. The most common grade 3-4 toxicities were hematologic, including neutropenia (64.7%) and thrombocytopenia (47.1%), and gastrointestinal side effects were mostly grade 1-2. The overall response rate (ORR) was 31.25%, with a median progression-free survival (mPFS) of 5.6 months and a reported median overall survival (mOS) of 39.5 months in both the overall cohort as well as the leiomyosarcoma subgroup. The leiomyosarcoma subgroup showed the most promising outcomes with an ORR of 44.4% and mPFS of 7.6 months, while the osteosarcoma subgroup demonstrated poorer outcomes. Preclinical studies revealed that the combination was synergistic in most leiomyosarcoma and MPNST (malignant peripheral nerve sheath tumor) cell lines but antagonistic in osteosarcoma models, mirroring clinical data. Mechanistic studies linked the efficacy of the combination to the depletion of survivin and nuclear accumulation of IκBα, which was associated with enhanced DNA damage and apoptosis. Correlative biomarker analysis in the clinical trial also showed that high survivin or IκBα expression predicted shorter PFS in leiomyosarcoma patients. These findings were reported as support to justify the launch of a phase II trial (NCT06114004) focusing on leiomyosarcoma and MPNST cohorts while excluding osteosarcoma due to lack of efficacy. This study highlights the potential of the gemcitabine-selinexor combination as a potential therapeutic strategy, particularly in select sarcoma subtypes, and identifies survivin and IκBα as candidate predictive biomarkers for response.
While the primary endpoint of the study was clearly delineated with evidence that this is a safe and manageable regimen, analysis of outcomes data including overall survival is not clear and warrants additional revision prior to manuscript acceptance.

1.- **Major concerns:**

- *Overall survival analysis needs statistical review. For example, it is unclear from the Supplemental Figure 2 demonstrating the Kaplan-Meier curve, given number of patients censored between 18 months (5), how they arrived at median OS timepoint of 39.5 months. This is particularly important as given the markedly lower median OS of 8m reported for the second largest histological subset (osteosarcoma, 35%). It is also not clear how the full cohort has the same reported OS of the more favorable leiomyosarcoma subset (53). Additionally, the reported 36-month OS rate is reported as 50.2%, despite all but 2 patients having died or been censored prior to that time point.*

Answer: Regarding the presence of censored cases, the study database cutoff was April-24. At that time, 4 patients had no longer follow-up: two were lost due to a change of treatment center (April-22 and June-23), one was lost for unknown reasons in November-22 and one withdrew consent in December-23. It is clear that if one or more of these

patients had died before the cutoff date, the estimated median OS would have occurred earlier. For this reason, we agree with the reviewer that the emphasis on the improved (estimated) OS for the overall trial population should be reduced. Accordingly, we have modified the discussion and included this point in the limitations of the study.

Page 20: ‘’**Another limitation of the study is that, at the April 2024 database cutoff, four patients were lost of follow-up; any death among them before this date could have led to a shorter median OS, so the estimated OS for the trial population should be interpreted with caution**.”

2.- The relative importance of OS is overstated particularly with a PFS value that is good, but not unheard of for a phase I trial. This is a phase I trial and a phase I population is not a good group to estimate OS off of. This is only a concern here because they put so much emphasis on the improved OS. Advise decreased emphasis on unusually long mOS in the discussion given the above and the lack of power to determine statistical significance of this finding.

Answer: I agree with your perspective regarding the OS in a phase I part. Actually, I do not believe we emphasized this result. On the contrary, when discussing the median overall survival (OS) in lines 399-401, “The unexpected long-term survival in our study could be in part by hazard, due to the limited number of patients, but we can speculate about an immunomodulation effect with XPO1 inhibition.” we were quick to suggest that chance may have played a role in a small subset of cases. Nevertheless, we have further downplayed this by stating the following:

Page 18: “**The long-term survival observed in our study may largely be attributed to chance, given the limited number of patients. We can speculate about a potential immunomodulatory effect associated with XPO1 inhibition.[23,24] This is an area that requires further investigation**.”

3.- Despite quality of life EORTC QLQ-C30 being included in intended secondary outcomes, this information is not reported in results or discussion; unable to find results in supplementary information.

Answer: You are right, we appreciate your request because this information was lacking in the manuscript. We have implemented the following information:

Page 8: In the Methods section, we have added more details: “, quality of life measured with EORTC QLQ-C30 **core, version 3, focusing on items 29 and 30**,”.

Page 15: In the Results section, it has been added the following sentence: ‘**’The global health status was analyzed using items 29 and 30 of the QLQ-C30, following the EORTC recommendations. There was a moderate improvement in the perception of global health status (GHS)/ quality of life (QoL), throughout the treatment, especially after 4 cycles of treatment (Supplementary Table S2).”**

The methods were included in the manuscript, Page 8: ‘’… quality of life measured with EORTC QLQ-C30 core, version 3, focusing on items 29 and 30**. The analyses of these**

items [Global Health Status (GHS)/ Quality of Life (QoL)] followed two steps. Firstly, the raw data of the average of the two items was estimated according to the equation: *RawScore*: RS = (I_1+I_2+…I_n)/n. Then, the GHS/QoL was transformed to a standardized score (S; 0-100), according to the equation: S = [(RS-1/range]x100. Other secondary endpoints included post hoc correlative studies searching for potential predictive biomarkers…"

Page 17: In the Discussion section it has been added the following information: "**Quality of life analyses indicated that patients perceived general health status did not significantly worsen throughout the study**."

In the Supplementary, it has been added an additional Supplementary Table S2.

4.-A) The preclinical studies (using cell lines) are interesting and they suggest value to the combination but the idea that the benefit might be synergistic, rather than additive, is not supported by the preclinical studies.

Answer: We analyzed synergy in 11 cell lines (4 leiomyosarcomas, 4 MPNSTs, and 3 osteosarcomas) by assessing cell viability through isobolographic analysis. Synergy was observed in 3 of 4 LMS lines and 3 of 4 MPNST lines, while antagonism was found in all 3 osteosarcoma lines.

The clinical study was supported by preclinical analyses conducted on 17 sarcoma cell lines and 9 xenograft models (Ref 10, Nakayama et al.).

To better specify the observed synergy and moderate conclusions, we modified the following sentence in the Discussion section:

Page 18: ''This data is in line with the observations of our preclinical study, where we detected signals of synergy, **in cell viability studies,** between both drugs in 3 out of 4 leiomyosarcoma cell lines…".

4.-B) Of note, while the preclinical studies are not even mentioned in the abstract, they comprise TWO of the figures. By putting the preclinical studies in the same paper as their phase I there is a bit of an implication that the correlative data is supporting the same hypothesis generated by the preclinical studies.

Answer: The following phrase has been added in the abstract (Some reviewers suggested shortening the abstract).

Page 2: "**Based on preclinical and clinical findings, a phase II is currently running exploring this combination in two different cohorts, leiomyosarcoma and malignant peripheral nerve sheath tumors**."

Although the clinical study was supported by preclinical sarcoma research (see previous response), further investigation into the synergy between gemcitabine and selinexor, as well as specific sarcoma subtypes, was required. Therefore, in the Phase I study budget, we included *in vitro* studies.

4.-C) However, it does not - it actually is not clear what the implications of the clinical studies are, particularly in light of the potential relation between survivin and PFS. These would be stronger as two separate manuscripts.

Answer: The preclinical analysis performed alongside the clinical study aimed to guide the phase II trial.

We believe it adds value to Phase I, especially since both preclinical and clinical findings have been consistent. In the ongoing Phase II, survivin is being measured in a more homogeneous population (in LMS and MPNST cohorts) to provide a more robust evaluation of this biomarker.

5.- For a phase I trial there should be a separate section discussing the safety and tolerability with a more fleshed out discussion.

Answer: Thanks for the request. We have added a subheading, and we have added more information concerning safety and tolerability throughout this subsection.

Page 13: A ‘’Clinical results’’ subheading has been added to the Results section.

Page 14: A “Safety and Tolerability” subheading has been added to the Results section.

Page 15: A ‘’Efficacy results’’ subheading has been added to the Results section.

Page 15: A sentence has been added in the Results section: ‘’**Granulocyte-colony stimulating factors (G-CSF) were commonly used throughout the study, beyond the first cycle, to prevent delays and infections in 11 of 17 (65%) accrued patients.**”

Page 15: The following sentence has been added: **“Notably, no patients left the study because of toxicity. This shows that managing bone marrow and gastrointestinal side effects, as well as following dose delay and reduction guidelines, was effective in keeping patients in the study**.”

Page 15: Additionally, at the end of “Safety and Tolerability” section, it has been added the lacking information from QoL:

‘’**The global health status was analyzed using items 29 and 30 of the QLQ-C30, following the EORTC recommendations. There was a moderate improvement in the perception of global health status (GHS)/ quality of life (QoL), throughout the treatment, especially after 4 cycles of treatment (Supplementary Table S2)**.”

The methods were included in the manuscript (Page 8): ‘’… quality of life measured with EORTC QLQ-C30 core, version 3, focusing on items 29 and 30**. The analyses of these items [Global Health Status (GHS)/ Quality of Life (QoL)] followed two steps. Firstly, the raw data of the average of the two items was estimated according to the equation: *RawScore*: RS = (I_1+I_2+…I_n)/n. Then, the GHS/QoL was transformed to a standardized score (S; 0-100), according to the equation: S = [(RS-1/range]x100. Other secondary endpoints included** post hoc correlative studies searching for potential predictive biomarkers…”

6.- If preclinical studies are to be discussed in the same manuscript as clinical results (and, to reiterate, I don't think they should be), they should be presented first.

Answer: This has been implemented in the text (Methods and Results section). Figures, Figure Legends and Supplementary Tables were renumbered.

7.- Need to be careful not over interpret result in a study that includes a known active agent (gemcitabine).

Answer: The results of activity have been contextualized with gemcitabine combinations in sarcomas in the Discussion section, as gemcitabine monotherapy achieves an ORR below 5% and a median PFS of 2 to 3 months.

To maintain caution in our conclusions, we have added the following statement to the Discussion section, Page 19:

"In any case, the results of activity in this study, including survival, should be interpreted with caution due to the small number of patients analyzed."

Minor concerns:

8.- Flow of information in the introduction is disorganized and difficult to follow; rationale for selinexor's individual cytotoxic effects and its potential synergistic effects with gemcitabine is mixed rather than sectioned, and comprehension could be improved by grouping supporting information logically. For example: mechanisms of selinexor mediated apoptosis are detailed in two separate areas separated by discussion of a potential mechanism of selinexor/gemcitabine synergy.

Answer: Please see the reply to Query #4 of Reviewer 2. The Introduction has been rearranged accordingly.

9.- Line 112 – "progressing to at least an anthracycline-based scheme"; unclear if this indicates that patients had progressed ON an anthracycline-based therapy although that is my assumption.

Answer: The condition requires that patients have previously undergone an anthracycline-based regimen and experienced disease progression at some point, not necessarily during anthracycline treatment but possibly during the follow-up period. The condition for progression is that it should be within 6 months of enrolment.

For greater clarity, the phrase (Page 7) "Patients diagnosed with sarcoma, preferably leiomyosarcoma or osteosarcoma, progressing in the previous 6 months to at least an anthracycline-based scheme, …"

has been modified to: "**Patients diagnosed with sarcoma, preferably leiomyosarcoma or osteosarcoma, previously treated with at least one previous line based on anthracyclines, and progressing in the previous 6 months**,…".

10.- Line 143 – "If the DLT is" – present tense, should be modified to past tense to match rest of section.

Answer: The phrase (Page 8) "If the DLT rate is > 0.33 among the patients with the initial dose level, the treatment dose will be lowered to dose -1." has been modified to "**If the DLT rate was greater than 0.33 among the patients with the initial dose level, the treatment dose was lowered to dose -1**."

11.- Dose modification/delay protocol may be better represented in an algorithmic figure rather than in text for comprehension and manuscript flow.

Answer: The protocol displays 6 tables summarizing the recommendations for dose modifications and delays, for different days and both drugs. I would rather suggest consulting the uploaded protocol for this aim.

In line with this, the following phrase already included in the manuscript (Page 8) "The protocol details recommendations for dose modifications in both drugs in the case of non-hematological toxicities." has been modified to:

"**The protocol provides detailed recommendations for dose adjustments and delays for both drugs in response to hematological and non-hematological toxicities**."

12.- Line 376 – "notorious", consider "notable".

Answer: The phrase (Page 17) "…suggests signs of notorious activity…" has been modified to "**This study shows notable activity with an ORR of 31% based on RECIST criteria and central review, and a mPFS of 5.7 months. These results warrant further investigation of this treatment regimen as a second-line option for patients with advanced, progressing sarcoma**…"'.

13.- Line 378 – "Thus, "gemcitabine", consider, "for example".

Answer: The phrase (Page 18) "Thus, gemcitabine plus docetaxel achieved…" has been modified to "**For example**, gemcitabine and docetaxel achieved…".

14.- Line 380 – "in a randomized phase II trial, in a series of individuals with STS" consider omitting the comma.

Answer: The comma has been omitted from the phrase.

15.- Line 400 – "hazard", consider "chance".

Answer: Actually, this phrase has been modified (query #2 Reviewer 3), and now "chance" is the word used.

16.- Line 405 – "The finding that significantly correlates the expression of survivin… with worse efficacy", consider "Our results indicate a significant correlation between increased survivin expression and reduced efficacy…" followed by substitution of "This could prove crucial" for line 406 "could prove crucial".

Answer: Both suggestions have been implemented. The phrase (Page 19) "The finding that significantly correlates the expression of survivin… with worse efficacy" has been modified to "**Our results indicate a significant correlation between increased survivin**

expression and reduced efficacy of the combination of gemcitabine and selinexor. This could prove crucial…"

17.- Line 453 – capitalize i.v. and p.o.

Answer: Both corrections have been implemented in the text as I.V. and P.O (Page 20).

Reviewer #4 (ECR):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #5 (Stats, trial design, biomarkers):

Comments to authors

The authors are reporting the outcomes of the phase 1 component of a phase ½ trial with Selinexor and gemcitabine in advanced sarcomas. Per the pre-specified statistical analysis plan, the phase 1 objectives included finding the MTD, reporting AEs per CTCAE v5, describing the overall response rate, describe a subset who are evaluable using the Choi response criteria, and describing patient qol outcomes. After determining the MTD, the authors were planning to assess efficacy in a randomized phase 2 trial that will accrue specific sarcoma subtypes.

1.- Though it is a reasonable design, the 3+3 design often chooses a more conservative RP2D (https://pmc.ncbi.nlm.nih.gov/articles/PMC4551156/) as compared to other phase 1 trial designs. The authors state "Although the recommended phase II dose was technically at the +3 dose-level (with gemcitabine at 1200 mg/m² administered at 10 mg/m²/min followed by selinexor at 80 mg weekly), the overall tolerance was noticeably better with selinexor at 60 mg weekly. Therefore, the RP2D was determined to be the +2 dose-level (gemcitabine at 1200 mg/m² at 10 mg/m²/min followed by selinexor at 60 mg weekly)." In essence the authors have chosen a lower dose level (dose level +2) which is even more conservative than the already conservative dose level that would have been selected based on the traditional 3+3 design. Though this may reasonable to take forward in the phase two setting, it does deviate from the pre-specified statistical analysis plan. This may be more relevant as the authors state G-CSF was used as part of the study (which makes sense) to prevent prolonged neutropenia and subsequent complications like febrile neutropenia. It is possible that a higher dose level like dose level 3 would have been well tolerated.

Answer: The final recommendation has indeed deviated from the initial statistical plan. The decision to recommend dose level +2 was based on the difference between cumulative toxicity and that observed in cycle 1, which was used for DLT assessment. No patients received G-CSF during the first cycle; however, 80% required G-CSF at some point after cycle 1. We have added a sentence (a new reference) at the beginning of the discussion to clarify why dose level +2 was selected as the RP2D:

Page 17: "**Although our study followed the standard Phase I trial design, where the RP2D is selected based on a 20-33% dose-limiting toxicity (DLT) rate in the first cycle, we recommended dose level +2 (below the suggested level by DLTs) due to observed cumulative toxicity.[20] While most patients (65%) required G-CSF after the first cycle, 65% and 47% experienced grade 3 or 4 neutropenia and thrombocytopenia, respectively.**"

2.- Though the authors do discuss the primary and secondary objectives of the phase 1 study including the RP2D, the safety profile, and the response rates (ORR and a subset with Choi), they also dedicate a large portion of the manuscript describing efficacy data. It is reasonable to describe the outcomes of the 17 patients that were enrolled, but the

authors should consider limiting the discussion and conclusions to fit the scope of the phase one study.

Answer: Thanks, we agree with you. We have balanced the information, adding more information about safety and tolerability (please see reply 4 of reviewer#3), and have modulated/smoothened the statements about efficacy (please see reply 2 of reviewer#3).

Part of the discussion on the efficacy of this combination focused on the observation that the results suggest effectiveness in leiomyosarcoma but not in osteosarcoma. We believe it is important to identify efficacy signals within subgroups to minimize the inherent heterogeneity due to the diversity of sarcomas.

3.- For the overall cohort of 17 patients, the median overall survival was longer than the median follow-up. This can happen in cases where there is a single patient contributing a large amount of survival time: https://www.sciencedirect.com/science/article/pii/S0006497118638236#:~:text=Median OS was not reached,the median OS estimate upward.

Answer: Regarding the presence of censored cases, the study database cutoff was April-24. At that time, 4 patients had no longer follow-up: two were lost due to a change of treatment center (April-22 and June-23), one was lost for unknown reasons in November-22 and one withdrew consent in December-23. It is clear that if one or more of these patients had died before the cutoff date, the estimated median OS would have occurred earlier. For this reason, we agree with the reviewer that the emphasis on the improved (estimated) OS for the overall trial population should be reduced. Accordingly, we have modified the discussion and included this point in the limitations of the study.

Page 20‘’**Another limitation of the study is that, at the April 2024 database cutoff, four patients were lost of follow-up; any death among them before this date could have led to a shorter median OS, so the estimated OS for the trial population should be interpreted with caution.**’’

4.- The CONSORT-like diagram in supplementary Figure 1 shows an efficacy population of 17 but the authors should highlight that although 17 may have been assessed for PFS, only 16 were available for response evaluation.

Answer: The Consort-like diagram has been modified accordingly.

5.- Supplementary Table 2: Lack of uniformity. Some values do not have 95% CI (e.g. median OS for survivin expression).

Answer: Yes, this occurs in subgroups with very small sample size when the last observation is an event, leaving no right-hand tail for CI estimation. In such cases, statistical software cannot compute the 95% CI interval for the median.

6.- No clear idea of a dose-response relationship based on survivin or IκBα expression as data stratified by dose level or sarcoma subtype was not provided. Though this may

provide some evidence overall that the combination of gemcitabine and Selinexor reduces expression of survivin and IκBα, it is unclear if this is subtype specific.

Answer: As shown in the current Supplementary Table S5, the significant value of these biomarkers was verified just in the leiomyosarcoma cohort, but not in the remaining subset.

7.- Supplementary Table 1. In the manuscript the authors state that a log-rank test was used to test differences and in the pre-specified statistical analysis plan they state "For continuous efficacy variables such as PFS, their medians will be used. Association between variables will be measured and tested by conventional statistical analysis: Pearson and/or Spearman tests." Please clarify.

Answer: The correct test used is the one stated in the manuscript: the log-rank test, which is appropriate for this type of analysis. The reference to a different test in the Statistical Analysis Plan is an error.

8.- What statistical tests were used to derive p-values in each table or figure so that a reviewer can understand.

Answer: We add this information to any table or figure as a footnote.

9.- If there was a deviation from the pre-specified plan, what was the reasoning.

Answer: Except for the error noted in point 7, there have been no deviations from the initial statistical plan. The plan also covers the ongoing phase II.

10.- Supplementary Figure 2 (Panels B and C). Panel B refers to the 9 LMS cases that were enrolled and Figure 2B shows the PFS of all cases. For LMS, one of the patients still has follow-up ongoing (non-protocol specified) and has not progressed at ~30 months whereas the Supplementary Figure 2B shows a longer PFS of around 36 months. As this is likely the same patient, please clarify the difference between these two figures.

Answer: The patient mentioned is a censured case with a PFS of 33.8 months and it is the same in Supplementary Figure S2B and A (note that A is the whole series and C, the osteosarcoma patients).

11.- For osteosarcoma patients enrolled, there is one patient who had progression around 36 months (per Figure 2B) but in SF2 Panel C the KM curve is cutoff around 30 months. As this is likely the same patient, please clarify the difference between these two figures.

Answer: Note that osteosarcoma patients are shown in Supplementary Figure S2C and the whole population in Supplementary Figure S2A. In both figure we can see a censored patient at 30 months.

12.- Supplementary Figure 3 (Panels A and B). The authors state: "In the leiomyosarcoma series of patients, high expression of survivin (>50%) correlated significantly with worse PFS [3.4 months (95% CI 0-7.6) vs 15.4 months (95% CI 3.1-

27.7), p=0.022], whereas the high intensity of IκBα expression (level 4) was significantly associated with worse PFS [3.4 months (95% CI 0-7.6) vs 15.4 months (95% CI NR), p=0.007], (Supplementary Table S2 and Supplementary Figure S3).” The p-values are presumably generated from log-rank tests reflect the whole curve in both Panels A and B of SF3 are not reflective of the median PFS which the authors report and overlap with each other.

Answer: The p-values reported correspond to log-rank tests comparing the entire Kaplan-Meier survival distributions between groups. The statistical significance reflects differences across the whole follow-up period, not solely at the median survival point. This can result in a significant p-value even when median values and their confidence intervals partially overlap.

13.- The discussion has more than one statement that is grammatically incorrect, unclear, or too assertive given the limited sample size, or unclear and too assertive: The authors state “The activity detected in this series, with an ORR of 31% according to RECIST criteria and central assessment, and a mPFS of 5.7 months, suggests signs of notorious activity with this scheme in the context of second lines in patients with advanced progressing sarcoma.” What does notorious activity mean?

Answer: The discussion has been revised according to Reviewers comments. In regards to the sentence ‘’…notorious activity’’, we had smoothed it:

Page 17: ‘’**This study shows notable activity with an ORR of 31% based on RECIST criteria and central review, and a mPFS of 5.7 months. These results warrant further investigation of this treatment regimen as a second-line option for patients with advanced, progressing sarcoma**.’’

We also have modified notorious to notable also following the recommendation of the Reviewer 3.

14.-A) The authors describe the median PFS for the 17 patients without at least giving the 95% CI to help the reader understand the precision of the median PFS.

Answer: In line 310, from the original submission, the 95% CI was given in the sentence: ‘’The median PFS (mPFS) was 5.6 months (95% CI, 1.6-9.5) for the whole series of 17 patients.’’. Moreover, the 95% CI of subgroups and other estimations were reported through the manuscript (lines 312, 313, 322, 324 and 326).

14.-B) Conclusions not related to the phase 1 component of the study: The authors state “A phase II trial has been activated (NCT06114004) exploring the combination of gemcitabine 1,200 mg/m2 in a 10 mg/m2/min i.v. infusion on days 1 and 8, followed by selinexor p.o. at a 60 mg flat dose on days 1, 8, and 15 in cycles repeated every 21 days, in two different cohorts: leiomyosarcoma, and MPNST. The inclusion of a cohort of MPNST patients was supported by the preclinical evidence, in which we observed synergy between selinexor and gemcitabine in the majority of the cell lines used. The osteosarcoma cohort was discarded for the phase II part following the insights gained in this phase I trial, where the mPFS was just 1.2 months, and also because of the

antagonism observed in the preclinical experiments." Though the authors do give some preclinical data that may show some activity in MPNST, this was not the focus of the phase 1 component based on the statistical analysis plan.

Answer: We intended to note, as an epilogue, that a new Phase II trial was initiated partly based on the findings of this Phase I study. In line with your recommendations, we have partially revised the final sentence:

Page 20: ''**In summary, the combination of Gemcitabine 1,200 mg/m2 in a 10 mg/m2/min I.V. infusion on days 1 and 8, followed by selinexor P.O. at a 60 mg flat dose on days 1, 8, and 15 in cycles repeated every 21 days warrants investigation in phase II and III trials for certain sarcomas. We have currently initiated a phase II trial to further evaluate activity in selected sarcoma groups**.''

Editorial comments:

1.- Throughout the manuscript, it is often unclear what the underlying denominator is for the respective percentages. The authors should provide more granular descriptions so that a reviewer does not have to assume what the denominator is for each result.

Answer: The denominator for each result has been added to the text, according to the Editorial comment.

Page 15: ‘’There were only 9 **out of 17** cases with sufficient quality in the CT scan to be considered evaluable by Choi criteria.’’

Page 16: The high intensity of IκBα nuclear expression (score 4) was significantly associated with worse PFS [1.4 months (95% CI 0-3.9) vs 9.6 months (95% CI 0-25.2), p=0.047] in the whole cohort **(n=14)**. In the leiomyosarcoma subgroup of patients, high expression of survivin (>50%) correlated significantly with worse PFS [**(n=9);** 3.4 months (95% CI 0-7.6) vs 15.4 months (95% CI 3.1-27.7), p=0.022], whereas the high intensity of IκBα expression (level 4) was significantly associated with worse PFS [**(n=8);** 3.4 months (95% CI 0-7.6) vs 15.4 months (95% CI NR), p=0.007],..’’

2.- Some terminology is not clear (Table 1- Extension at baseline) however could be inferred. This again reiterates the recommendation to have a more clearly written manuscript.

Answer: The meaning of ‘’Baseline’’ has been explained in the Table 1 footnote.

Dear editorial team,

We want to thank you for the opportunity to strengthen our manuscript by addressing the new reviewers' comments and suggestions. Please find below our point-by-point responses to their feedback.

Reviewer #1 (Remarks to the Author):

Reviewer 1: Thank you for the responses; a few remarks

1) Response 3: I would rather suggest mentioning the study in endometrial cancer in the subgroup of patients with p53 wild-type tumors. (Vergote I J Clin Oncol ref 33).

Answer:
Thank you for your suggestion. We have slightly modify the discussion to include this information:
"The potential application of selinexor as a maintenance therapy has already showed activity in patients with *p53* wild-type endometrial carcinoma, and represents a promising future strategy also in sarcoma, particularly following its combination with gemcitabine during the induction phase."

2) Response 6: last sentence p 17 "And this paragraph in the Discussion section has been also modified:
Page 17: "Despite the prophylactic recommendations for ondansetron and olanzapine drugs in the protocol, the truth is, it was evident that they were not universally adopted."
I would have rather suggested: Prophylactic ondansetron and olanzapine recommanded in the protocol, were not systematicaly prescribed.

Answer:

We have modified this sentence to: "Prophylactic ondansetron and olanzapine recommended in the protocol, were not systematically prescribed in all patients".

Reviewer #5 (Remarks to the Author):

The revised manuscript does read better and the investigators have addressed most of my questions. It is appreciated that the investigators now include "n=xx" throughout the manuscript so the reader understands the sample size associated with each effect estimate. The authors have toned down any conclusions based on survival endpoints, added "disclaimers" about interpreting OS, and highlighted the reason for moving towards a phase 2 with the dose level that was chosen.

Please address the following

1) The investigators still need to address the inclusion of the survivin analysis in the abstract, as it was not pre-specified. If the investigators chose to include this in the manuscript, it should be removed from the abstract, noted this was exploratory and any comparison of groups (survivin >= 50%) was not-prespecified and should be interpreted with caution.

Answer:

We have deleted from the abstract the following sentence: " In leiomyosarcoma patients, high expression of survivin (>50%) significantly correlated with worse PFS [3.4 months (95% CI 0-7.6) vs 15.4 months (95% CI 3.1-27.7), p=0.022]."

2) For Supp Table S3, please include the number of patients in each group so that a reader could understand why there is no 95%CI as the authors rightly mentioned could happen in a small subgroup of a phase 1 trial. On a re-read of the statistical analysis plan, the statistician clearly states "P-values are only going to be reported for Efficacy Analysis." Table S3 does not fall into the planned efficacy analysis.

Answer:

For a better understanding, the number of patients has been added to Table S3 following your suggestion.

In addition, we have reviewed the Authors Checklist and we have made the requested modifications through the manuscript in order to align with the Journal Guidelines.