Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-21-828

-Reviewer A-

We would like to thank Reviewer A for her/his constructive comments on our manuscript. Below are our answers.

Comment #1: *First, the authors discuss the failure of anti-angiogenic therapy in human MPM at length in the introduction. However, neither the results of the experiments outlined nor the discussion address this.*"

Reply #1: The Reviewer's comment is well taken. As discussed in the manuscript, although there is a strong rationale for inhibiting angiogenesis in MPM, antiangiogenic agents failed so far to generate meaningful anti-tumor activity in subsequent phase III studies (1). Nevertheless, in well-selected patient subpopulations, combination of cisplatin and pemetrexed with bevacizumab might result in a significant improvement of survival outcomes (1,2). In order to implement personalized therapeutic approaches concerning these antiangiogenic agents in mesothelioma patients, evaluating the mechanisms of vascularization of MPM is crucial. Therefore, in the current study we aimed to gain insights into the motility, invasion and vascularization of intrapleurally implanted human MPM cell lines and also to assess the role of VEGF-A in vascular plexus formation. Besides identifying two distinct angiogenic growth patterns, we also found significant differences in VEGF-A expression concerning the investigated cell lines, thus suggesting that VEGF-A levels might indeed have a meaningful impact on the mechanisms of vascularization. Additionally, to the best of our knowledge, this is the first study to describe two widely different angiogenic growth patterns in MPM. These fundamental differences between the examined cell lines might explain the divergent therapeutic response to bevacizumab seen in our previous *in vivo* models concerning the same MPM cell lines (3). Altogether, our findings might partly explain why not all patients, and not all settings, are appropriate for anti-angiogenic therapy in MPM. These considerations are now included in the "*Introduction*" and "*Discussion*" chapters.

Changes in the text #1: The following sentences were inserted to the respective chapters.

-page 6 (Introduction), lines 103-107: "Nevertheless, in well-selected patient subpopulations, combination with bevacizumab might result in a significant improvement of survival outcomes (1,4). Therefore, in order to implement personalized therapeutic approaches concerning antiangiogenic agents in mesothelioma patients, identifying novel biomarkers of response are crucial, as well as evaluating the mechanisms of vascularization of MPM (4)."

-page 6 (Introduction), lines 117-122: "Despite the strong rationale, a truly effective anti-angiogenic strategy has not been developed for MPM therapy, and little is known about the

specific vascularization mechanisms which are crucial to explore for the implementation personalized therapeutic approaches in the future. In the present study, we aimed to gain insights into the motility, invasion and vascularization of intrapleurally implanted human MPM cell lines, and to assess the role VEGF-A in vascular plexus formation."

-page 22 (Discussion), lines 531-535: "Given the widely different angiogenic growth patterns and VEGF-A levels described in the examined cell lines, our results might explain the divergent therapeutic response to bevacizumab seen in our previous *in vivo* models (3). Moreover, our data might further strengthen the hypothesis that not all patients, and not all settings, are appropriate for anti-angiogenic therapy in MPM."

Comment #2: *"The kinetics of the animal model needs to be more detailed. Did the duration of the experiments differed per cell line. Did it take more time for mice to become moribund when P31 or SCP11 were employed? Did the pattern of mesothelial implantation/proliferation differ between the two? Did both groups show diffuse vascular deposition along the pleural surface and were these deposits homogenous between cell lines?"*

Reply #2: We thank Reviewer A for raising this point. As already highlighted in the "*Results*" chapter (page 15, lines 353) of the manuscript, colonies formed by both cell lines were allowed to grow until the animals became moribund, and the number of days elapsed until the mice became moribund was visibly lower in case of SPC111 cell line (vs. P31 cells, 28-35 days vs. 42-52 days, respectively) (for further details concerning the main time points of sacrificing the research animals please see answer #2 of Reviewer C). Neither the pattern of mesothelial implantation nor the morphological aspects differed between the two cells. Specifically, as shown in Figure 1C-1D of the manuscript (and detailed on page 15, lines 363-365), both MPM cell lines induced dense, tortuous vascular proliferations bulging into the pleural space and covering large areas of the diaphragm. In case of both MPM cell lines, the size of the nodules ranged between 1 mm and 3 mm in diameter. Of note, however, SPC111 cells reached this nodule size slightly faster (vs. P31 cells), thus explaining why the mice inoculated with this cell line became moribund earlier than the ones inoculated with P31. Tumor-independent vascular plexus growth along the pleural surface was observed in both groups and these deposits were homogenous between the cell lines.

Changes in the text #2: For a better understanding, we have revised the first paragraph of the Results chapter:

-page 15, lines 353-355: "Colonies formed by both cell lines were allowed to grow until the animals became moribund. Notably, the number of days elapsed until the mice became moribund was lower in case of the SPC111 cell line (vs. P31 cells, 28-35 days vs. 42-52 days, respectively)."

-page 15, lines 357-360: "In case of both MPM cell lines, the size of the nodules ranged between 1 mm and 3 mm in diameter. Of note, however, SPC111 cells reached this nodule size slightly faster, thus explaining why the mice inoculated with this cell line became moribund earlier than the ones inoculated with P31."

-page 15, lines 362-365: "Importantly, neither the pattern of mesothelial implantation nor the morphological aspects of proliferation differed between the two cells. Specifically, as shown in Figure 1C-1D, both MPM cell lines induced dense, tortuous vascular proliferations bulging into the pleural space and covering large areas of the diaphragm."

-page 16, lines 375-376: "Tumor-independent vascular plexus growth along the pleural surface was observed in both groups and these deposits were homogenous between cell lines."

Comment #3: *Second, the authors spend a great deal of effort demonstrate that VEGF overexpression alone was sufficient for vascular deposition to the point of fatality for the mouse. Did this correlate with lesion formation? I ask because the authors don't provide evidence that the vascular plexuses are required for tumor formation, just associated with their appearance. Does blocking VEGF affect tumor development differentially in both cell lines?*

Overall this seems like a good although incomplete study. I feel that at least some characterization of the tumor behavior is necessary, as the data presented is entirely descriptive leaving me confused as to the relevance of the study as a whole. Depending on the scope of the study/journal, I feel that additional experiments clarifying the role of VEGF at a minimum is required.

Reply #3: In order to elucidate the role of VEGF-A in vascular plexus formation, we aimed to investigate how VEGF-A overexpression in MPM cells influences vascularization. Indeed, we found that increased VEGF-A production by VEGF-A-transduced MPM cells resulted in accelerated capillary plexus formation and soon the entire surface of the diaphragm was covered by tortuous microvascular structures (please see Figure 2F and G of the manuscript). Importantly, this latter phenomenon ultimately led to the premature death of the mice, allowing no time for adequate tumor development. Hence, no aspects of tumor formation could be studied in detail in these mice and the degree to which VEGF-A expression and the resulting plexus formation contribute to lesion formation remains unknown.

However, in our previous study (3), we have examined the *in vivo* growth-inhibitory potential of bevacizumab in both cell models. We found that in line with the findings of Li Q et al. (5), bevacizumab alone was effective only against P31 tumors with high baseline VEGF-A levels and could not provide therapeutic benefit in the SPC111 model where tumor cells had markedly low baseline VEGF-A levels, as measured by ELISA.

To our knowledge, the current study is the first to report two distinct vascular growth patterns of orthotopically implanted human MPM xenografts. Of note, these two cell models have widely different VEFG-A levels, and according to our previous findings (3), they also respond differently to the anti-angiogenic agent bevacizumab. Unfortunately, due to the premature death of SPC111-RFP-VEGF-A-inoculated mice, the exact role of VEGF-A levels on the resulting tumor vascularization patterns could not be assessed. Nevertheless, besides providing potential explanations for distinct therapeutic responses seen in MPM patients concerning anti-angiogenic agents, our results are hypothesis-generating for following biomarker studies and might lay the framework for future personalized therapeutic strategies

in these patients. The translational relevance of our findings is further detailed in Reply #3 (Reviewer B).

Changes in the text #3: The aspects mentioned by the Reviewer are now briefly discussed in the Discussion chapter:

-page 21, lines 505-507: "Unfortunately, due to the premature death of SPC111-RFP-VEGF-A-inoculated mice, the exact role of VEGF-A levels on the resulting tumor vascularization patterns could not be assessed."

-page 22, lines 535-538: "Of note, in this previous study, bevacizumab alone was effective only against P31 tumors and could not provide therapeutic benefit in the SPC111 model where tumor cells had markedly low baseline VEGF-A levels, as measured by ELISA(3)."

-page 23, lines 569-572: "Besides providing insights into MPM nodule formation and vascularization, our results are hypothesis-generating for following biomarker studies and might lay the framework for future personalized therapeutic strategies in MPM patients. "

-Reviewer B-

We are pleased that Reviewer #2 is positive about our paper and we thank her/him for providing the below suggestions.

The authors have submitted a MS to demonstrate two distinct growth profiles of orthotopic human MPM xenografts: one shows direct involvement of peritumoral micro-vessels whereas the other is characterised by the initial "push away" of the micro-vessels followed by desmoplastic response and subsequent induction of desmoplastic tissue where neo-angiogenesis occurs

The MS certainly addresses a relevant matter with potential clinical implications. It is clearly written and the discussion is consistent with the results

Comment #1: *The authors should provide evidence if the two distinct mechanisms described are not dependent by the histological origin the cells tested have been originated from.*

Reply #1: Although some key differences might indeed exist between MPM cell lines concerning their histological origin, the impact of these histological features on tumor vascularization has not yet been demonstrated (6,7). In order to address the above question of the Reviewer, additional MPM and other tumor (fibrosarcoma and melanoma) cell lines were injected orthotopically into the pleural cavity of 63 SCID mice. Unfortunately, even after multiple attempts, none of the inoculated MPM cells (including VMC40, CRL5915, Meso100, M38K, SPC212 and I2) reached the adequate nodule size to perform in-depth analysis on tumor vascularization. Notably, however, the inoculated fibrosarcoma and melanoma cell lines (HT1080 and A2058, respectively) grew out properly in our SCID mice and both tumor types presented vascular growth patterns similar to the SPC111 colonies. Even though we could not assess the vascular features of our other MPM cells due to their poor growth

properties *in vivo*, our findings concerning HT1080 and A2058 suggest that the two distinct vascular patterns are not histotype specific, and moreover, they might not be tumor-specific either. The potential influence of different histological subtypes on vascular features is now briefly highlighted in the manuscript.

Changes in the text #1: The need for additional studies investigating the correlation between the histological subtypes and vascular growth patterns is now highlighted in the Discussion chapter.

-page 23, lines 561-564: "Finally, although the impact of histological subtypes on vascular features has not yet been demonstrated in MPM (6,7), further studies are needed to clarify whether there is a correlation between these newly described vascular growth patterns and the histological origin of the tumor cells."

Comment #2: *Have the authors looked at other transcripts other than Collagen-1?*

Reply #2: We thank Reviewer B for bringing this up. In our study, we found that late-stage SPC111 tumor nodules were largely negative for human-specific collagen I, which plays a key role in extracellular matrix (ECM) formation. In contrast, the centers of P31 tumors contained a large amount of collagen type I of human origin. To validate these differences at the mRNA level, we examined the expression of the human COL1A1 gene with real-time PCR in both cell lines, and we found that the relative expression of the COL1A1 was indeed significantly higher in the P31 (vs. SPC111).

Besides COL1A1, we have also examined the relative expression of several other transcripts of interest concerning ECM, angiogenesis, ECM production, tumor cell invasiveness, cell-cell and cell-matrix adhesion, tumor suppression, and semaphorin signaling (Figure 1 of the rebuttal letter). Notably, the relative expression of the other examined ECM components (i.e. FN1 and LOX) were also higher in the P31 than in the SPC111 tumors (nevertheless, the differences were not statistically significant). No such trendlines were observed in case of the other factors examined. Given that all examined ECM components of human origin were notably higher in P31 models (vs. SPC111 nodules), our results might partly explain the earlier development of tumor vasculature in these nodules.

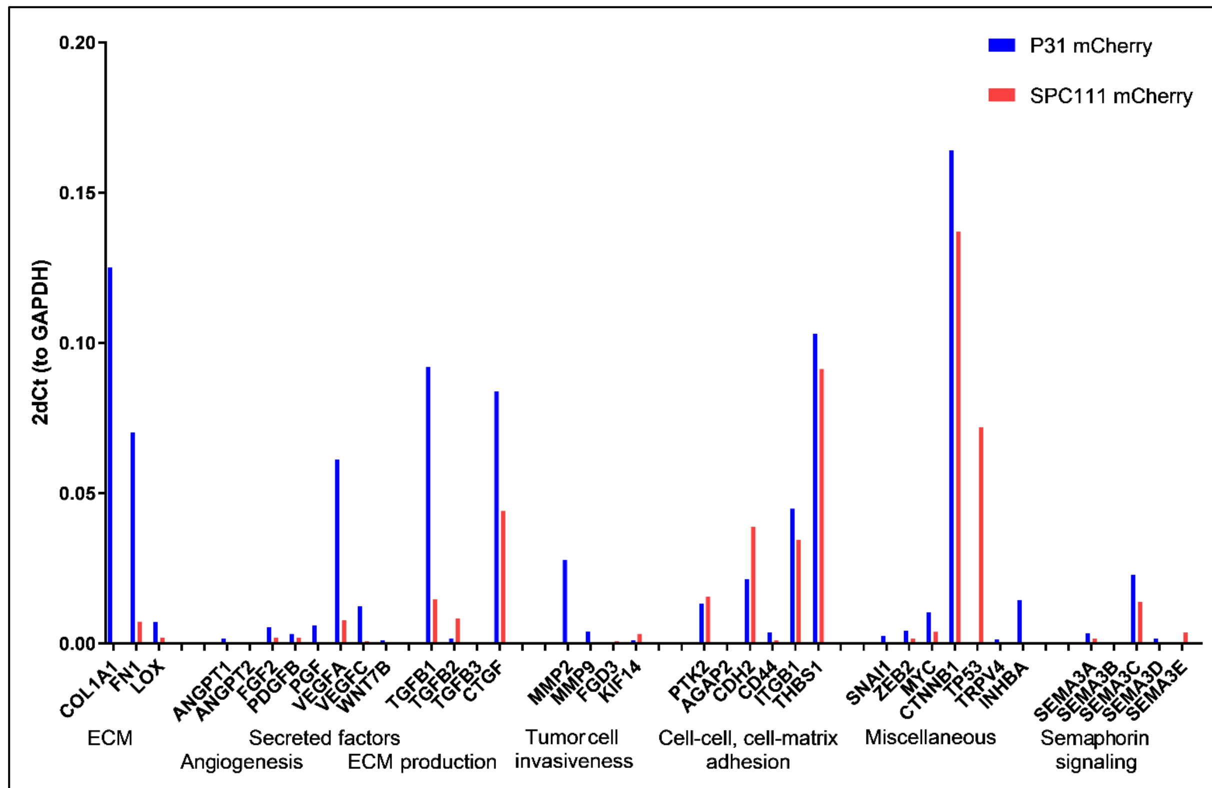

Figure 1. Real-time PCR of genes of interest concerning the ECM, angiogenesis, ECM production, tumor cell invasiveness, cell-cell and cell-matrix adhesion, tumor suppression, and semaphorin signaling.

Changes in the text #2: The manuscript was supplemented with the aforementioned additional findings.

-page 19, lines 455-460: "In addition, we have also examined the relative expression of several other transcripts of interest concerning ECM, angiogenesis, ECM production, tumor cell invasiveness, cell-cell and cell-matrix adhesion, tumor suppression, and semaphoring signaling. Notably, the relative expression of the other examined ECM components (i.e. FN1 and LOX) were also higher in the P31 than in the SPC111 tumors (nevertheless, the differences were not statistically significant) (data not shown). No such trendlines were observed in case of the other genes examined."

-page 21, lines 514-516: "Moreover, the other examined ECM components of human origin were as well notably higher in P31 models, further explaining the earlier development of tumor vasculature in these nodules."

Comment #3: *The authors should provide more insight into the potential translational impact of their findings.*

Reply #3: The Reviewer's comment is well taken. Therapeutic approaches for MPM have not yet benefited considerably from the paradigm shift of personalized medicine. Antiangiogenic agents have been trialed in this devastating disease for two decades, yet none of these have shown efficacy which has warranted further development as single agents in any line of therapy (2,8-10). Additionally, the resulting toxicities and the costs further hindered the widespread implementation of these approaches. Nevertheless, in appropriately

selected patients, some anti-angiogenic combinations undoubtedly show a clear efficacy in MPM (1). Despite extensive efforts, biomarker studies have failed so far to identify a unique biomarker that predicts the efficacy of bevacizumab or other anti-angiogenic agents, either in MPM or in other cancers. We believe that instead of finding a single biomarker for predicting the therapeutic response, we will likely need to move toward harmonizing multiple markers of vascularization in order to better reflect the complex interactions between the host and tumor.

Until recently, little was known about the specific tumor vasculature of MPM. To the best of our knowledge, the current study is the first to report two distinct vascular growth patterns of orthotopically implanted human MPM xenografts. Our findings are of translational relevance since these distinct vascular patterns might provide an explanation for the inconsistent therapeutic efficacies of different anti-angiogenic agents seen in previous trials, and moreover might contribute to the development of future biomarker panels. Additionally, our results might also have potential therapeutic implications since the penetration of the majority of anticancer agents is largely dependent on tumor vasculature. Accordingly, due to poor penetration into the tumoral tissue, anticancer drugs might be less effective in MPMs with avascular, pushing growth patterns than in well-vascularized tumors characterized by invasive patterns.

Changes in the text #3: The translational relevance is further discussed in the Introduction and Discussion chapters.

-page 6, lines 90-91: “Therapeutic approaches for MPM have not yet benefited considerably from the paradigm shift of personalized medicine.”

-page 6, lines 103-107: “Nevertheless, in well-selected patient subpopulations, combination with bevacizumab might result in a significant improvement of survival outcomes (1,4). Therefore, in order to implement personalized therapeutic approaches concerning antiangiogenic agents in mesothelioma patients, identifying novel biomarkers of response are crucial, as well as evaluating the mechanisms of vascularization of MPM (4).”

-page 22, lines 539-548: “Until recently, little was known about the specific tumor vasculature of MPM. To the best of our knowledge, the current study is the first to report two distinct vascular growth patterns of orthotopically implanted human MPM xenografts. Our findings are of translational relevance since these distinct vascular patterns might provide an explanation for the inconsistent therapeutic efficacies of different anti-angiogenic agents seen in previous trials and, moreover, might contribute to the development of future biomarker panels. Additionally, our results might also have potential therapeutic implications since the penetration of the majority of anticancer agents is largely dependent on tumor vasculature. Accordingly, due to poor penetration into the tumoral tissue, anticancer drugs might be less effective in MPMs with avascular, pushing growth patterns than in well-vascularized tumors characterized by invasive patterns.”

-page 23, lines 569-572: “Besides providing insights into MPM nodule formation and vascularization, our results are hypothesis-generating for following biomarker studies and might lay the framework for future personalized therapeutic strategies in MPM patients.”

-Reviewer C-

We appreciate the suggestions provided by the Reviewer to improve the manuscript's quality. Below are our answers to her/his comments.

This study by Kovacs and colleagues nicely demonstrated mechanisms of blood vessel formation for malignant pleural mesothelioma using orthotopic mouse models. They further identified the role of VEGF-A, extracellular matrix and cell motility as major factors accelerating the formation of blood vessels.

I have a few minor points:

Comment #1: *Method part: please specify spheroid size used. Spheroids most likely develop central necrosis at larger size and this may influence the experiment outcomes.*

Reply #1: We agree with the Reviewer that the core of spheroids with high cellular density can become hypoxic and necrotic since only about the 100 µm thick outer layer can be considered well-supplied by either oxygen or nutrients (11,12). In our experiments, the HUVEC aggregates had a 200 µm diameter, while MPM aggregates had a somewhat larger size (between 300 and 400 µms of diameter). This size difference was chosen in order to be able to distinguish the spheroids based on their phase-contrast image during long-term imaging. However, we do not expect necrosis to be a major factor in the repulsive effect shown in Figure 6A and B of the manuscript. First, the aggregates usually have an oblate ellipsoid shape: they are wider in the horizontal than in the vertical direction. According to our physical sections, this effect often reduces the vertical extension of our MPM spheroids by 50%. Second, physical sections often reveal a loose core in the MPM aggregates: in our experience, the presence of an ECM environment often makes the aggregates less compact than they are in an adhesion-limited culture surface. Our physical sections never indicated the presence of extended necrosis in the core of our aggregates (Figure 2, of the rebuttal letter). Third, vascular sprouts also avoid smaller aggregates. Finally, in our study, we present a significant difference between the effects of P31 and SPC111 spheroids which were seeded at the same aggregate size and thus likely would have similar hypoxic cores. The spheroid size is now specified in the Methods section. In addition, accessory data concerning spheroid formation, time-lapse videomicroscopy and endothelial sprouting anisotropy analysis were as well added to the Methods chapter.

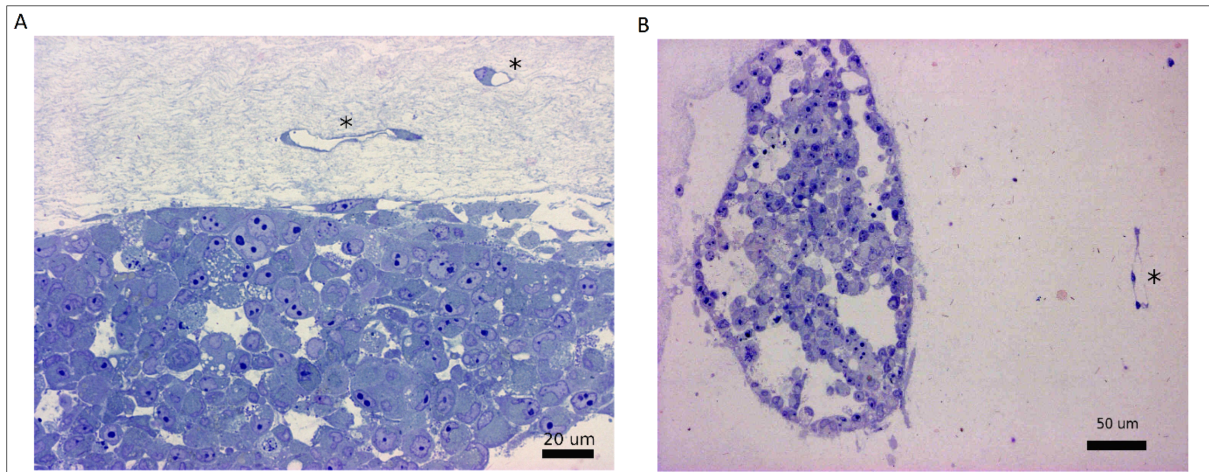

Figure 2. SPC111 spheroids have an oblate ellipsoid shape, a loose-packed interior, without obvious signs of necrosis. Semithin sections of spheroids and adjacent vascular sprouts that were cultured in fibrin (a) and collagen I (b) hydrogel. The 0.5 um thick sections are stained by toluidine blue, and were processed as described previously (13). Vascular tubes are marked by asterisks.

Changes in the text #1: Two additional subsections were added to the Methods chapter regarding spheroid size and formation, and time-lapse videomicroscopy. Additionally, the *Endothelial sprouting anisotropy analysis* subsection was also supplemented with additional data.

-page 8-9, lines 152-168: ***Spheroid formation*** subsection

-page 9, lines 169-179: ***Time-lapse videomicroscopy*** subsection

-page 11, lines 242-245: "We used these identified sprout segments to create vectors pointing from the center to a given cylinder. Vectors were then normalized into the unit range and averaged to yield the anisotropy value for each sprout arbor. Thus, the value 0 corresponds to a fully isotropic arbor morphology while 1 corresponds to a fully anisotropic arbor where all sprouts extend in the same direction."

Comment #2: *Please specify numbers of animal used per group*

Reply #2: Thank you for pointing this out. In total, 53 SCID mice were used for the SPC111 model, whereas P31 cells were injected into 30 mice. Notably, an additional 18 mice were used for the SPC111-VEGF-A model. Details concerning the timepoints of animal termination are shown in Figure 3 of the rebuttal letter. Of note, the figure only contains the animals which were suitable for tumor examination (i.e. immunofluorescence staining and in-depth tumor analysis).

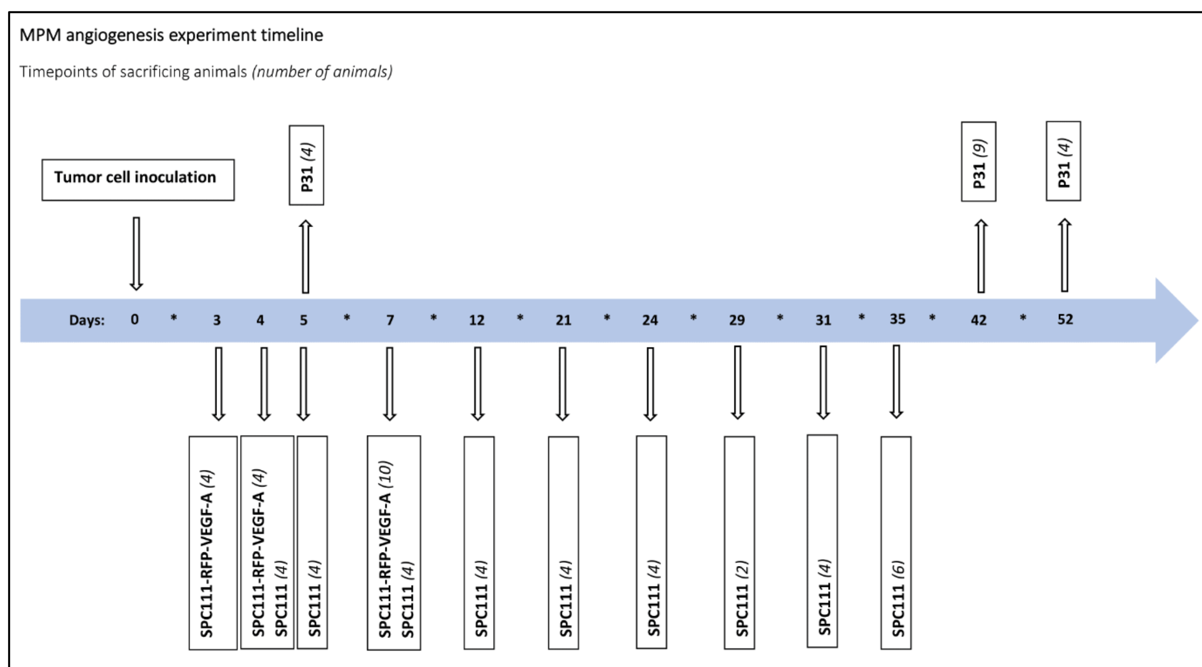

Figure 3. Timeline of angiogenesis experiment showing the main time points of sacrificing the research animals.

Changes in the text #2: The number of animals used per each group is now specified in the Animals subsection.

-page 11, lines 250-252: **"**In total, 53 SCID mice were used for the SPC111 model, whereas P31 cells were injected into 30 mice. Notably, an additional 18 mice were also used for the SPC111-VEGF-A model."

Comment #3: *Figure 1C, 1D: Please also indicate tumor free area (referred to in the result section) in the figure. This is not quite clear as all the irregular vessels are seen around wherever mCherry is present.*

Reply #3: As elaborated in the figure legend of Figure 1 of the manuscript, the mCherry expression of cells was somewhat low in the colonies, therefore, for better orientation, we marked the peripheral margins of the tumoral nodules with arrows. Accordingly, everything outside this tumorous area is considered tumor-free. Nevertheless, as requested by the Reviewer, representative parts of the tumor-free areas are now marked with asterisks on the updated Figures 1C and D.

Changes in the text #3: Representative parts of the tumor-free areas are marked with asterisks on the Figures 1C and D. The corresponding figure legends were as well updated:

-page 31 lines 738-739 and lines 746-747: "Representative parts of the tumor-free areas are marked with asterisks."

Comment #4: *Figure 2: It will be clearer if authors show comparison of vascularization of SPC111-RFP and SPC111-RFP-VEGF-A from the same time point and show overall survival (eg. Kaplan-Meier plot) of the 2 groups plus P31 group.*

Reply #4: Thank you for bringing this up. With regards to SPC111-RFP-VEGF-A model, the representative images of the tumor vasculature were captured on days 4 and 7 after inoculation (Figure 2F and G of the manuscript, respectively). Importantly, no further images are available from this subgroup since all mice became moribund by day 7. Meanwhile, the characteristic images of SPC111 (mCherry) nodules originate from days 5, 21 and 29 (Figure 3 of the manuscript). Unfortunately, no images were captured on the same day regarding the aforementioned two subpopulations. Nevertheless, we believe that these minor differences of 1 and 2 days between the representative images of SPC111-RFP-VEGF-A and non-VEGF-A-transduced SPC111 nodules are irrelevant.

As for the survival outcomes, the number of days elapsed until the mice became moribund was 28-35 days and 42-52 days in the SPC111 and P31 models, respectively. All animals inoculated with SPC111-RFP-VEGF-A cells died by the end of day 7. Given that the survival outcomes of mice are already detailed in the corresponding subchapters (for details please see Changes in the text #2 (Reviewer #1) and also lines 354-355 of the manuscript), we did not include the Kaplan-Meier plots in the manuscript but show the below Figure for the Reviewer to examine (Figure 4 of the Rebuttal letter). Of note, the Kaplan-Meier plots contain only the animals which were sacrificed due to moribundity.

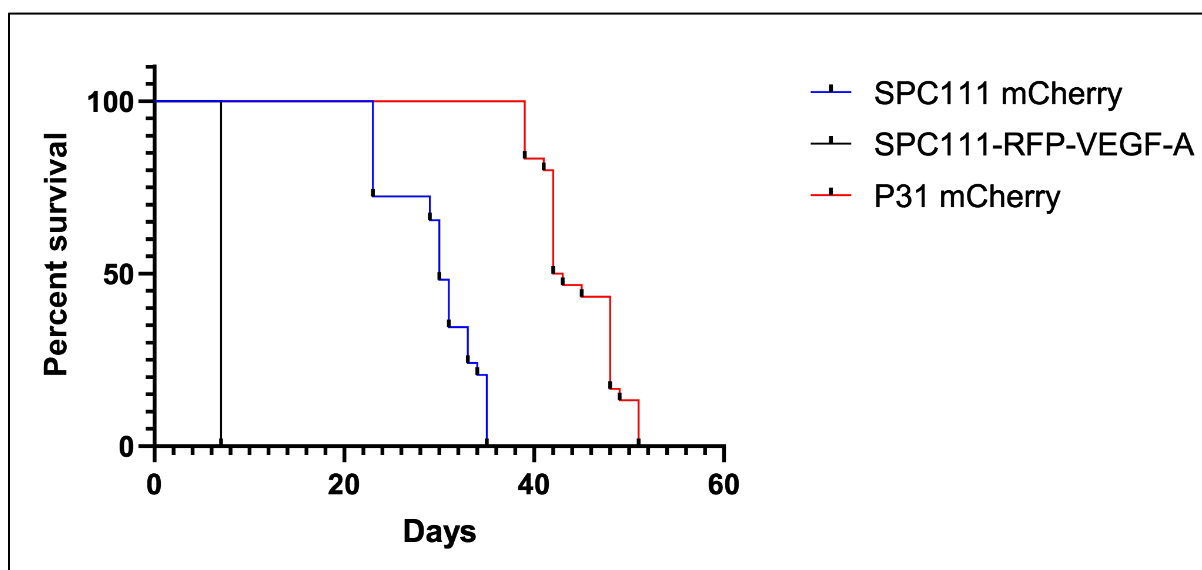

Figure 4. Kaplan-Meier estimates of survival outcomes according to different study models. SPC111 mCherry (blue); SPC111-RFP-VEGF-A (black), P31 mCherry (red). Only the animals which were sacrificed due to moribundity were included in the Kaplan-Meier plots.

Changes in the text #4: No changes in the text.

Comment #5: *Discussion: Another limitation of the study is the use of cell lines and immune-deficient mice. The vascular formation might be different from asbestos induced mesothelioma and in an immune competent setting.*

Reply #5: We agree with the Reviewer that using MPM cell lines and immune-deficient mice have their own disadvantages and thus the immunological contexts cannot be fully

reconstructed in these models. Nevertheless, they are widely used by the scientific community to study the pathogenesis of MPM, and undoubtedly, without these models, our knowledge of MPM, its origin, and treatment would be much less advanced (14). Of note, the vast majority of early angiogenesis studies were conducted on cell line-derived xenograft (CDXs), and moreover, the pharmacodynamics, efficacy, and toxicity of bevacizumab were as well first assessed in these models (14,15). In our study, we used two well-characterized and widely used cell lines that were engrafted into immunosuppressed mice. Given the heterogeneity of human MPM, by using these well-known cell lines, our results can be easily reproduced and validated. Additionally, our results can be easily compared with the findings of others concerning the same cell lines.

In order to gain insights into the specific aspects of MPM in an immunocompetent microenvironment, syngeneic models or genetically engineered mouse models (GEMMs) are needed which also have their own disadvantages (14,16). In syngeneic models, murine cell lines are implanted usually into the immunocompetent host subcutaneously or intraperitoneal and not into the pleural space of mice (16). In addition, all commercially available murine mesothelioma cell lines were in fact established from peritoneal mesothelioma (16). While peritoneal and pleural mesothelioma might share some of the same biological aspects, this has yet to be proved. Meanwhile, GEMMs are usually generated to obtain "spontaneous" MPMs, without exposure to asbestos fibers, by heterozygous or homozygous conditional mutation of *Ink4a* and/or *Nf2* and/or *Trp53*, or by injection of Ad*Cre* to mimic the human condition (17). However, in this system, the rate and histological subtype of MPMs are highly dependent on the type of inactivated genes, and the mutational landscape does not correspond entirely with asbestos-induced human tumors (14). MPM can also be induced through murine exposure to asbestos fibers (16). Indeed, they reflect better the natural history of the disease, however, it takes several months to more than 1 year before the development of mesothelioma (18). In addition, most asbestos-induced murine models are being developed by intraperitoneal injection of asbestos (16,19), and the ones developed by asbestos inhalation usually result in a low pleural tumor burden (16,20).

Altogether, while CDXs are undoubtedly not ideal model systems, they also offer many advantages, and the other available models have their own limitations as well. Nevertheless, we understand the concerns of the Reviewer, and as requested, we now mention the limitations of CDX models in the corresponding subsection.

Changes in the text #5: The potential study limitations arising from the use of MPM cell lines and immune-deficient mice are now briefly discussed in the end of the Discussion chapter.

-page 23, lines 555-561: “The use of MPM cell lines and immune-deficient mice might as well constitute another potential study limitation. Although these cell line-derived xenografts are widely used by the scientific community to study the biological features of MPM and therefore, our results can be easily reproduced, the immunological contexts of tumorigenesis cannot be fully reconstructed in these models. Accordingly, our results require further validation in additional experimental models such as genetically engineered mouse- or asbestos-induced murine models.”

References

1. Zalcman G, Mazieres J, Margery J, et al. Bevacizumab for newly diagnosed pleural mesothelioma in the Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS): a randomised, controlled, open-label, phase 3 trial. Lancet 2016;387:1405-14.
2. Nowak AK, Brosseau S, Cook A, et al. Antiangiogeneic Strategies in Mesothelioma. Frontiers in oncology 2020;10:126-.
3. Laszlo V, Valko Z, Kovacs I, et al. Nintedanib Is Active in Malignant Pleural Mesothelioma Cell Models and Inhibits Angiogenesis and Tumor Growth In Vivo. Clinical Cancer Research 2018;24:3729-40.
4. Nowak AK, Brosseau S, Cook A, et al. Antiangiogeneic Strategies in Mesothelioma. Front Oncol 2020;10:126.
5. Li Q, Yano S, Ogino H, et al. The therapeutic efficacy of anti vascular endothelial growth factor antibody, bevacizumab, and pemetrexed against orthotopically implanted human pleural mesothelioma cells in severe combined immunodeficient mice. Clin Cancer Res 2007;13:5918-25.
6. Pulford E, Hocking A, Griggs K, et al. Vasculogenic mimicry in malignant mesothelioma: an experimental and immunohistochemical analysis. Pathology 2016;48:650-9.
7. Edwards JG, Cox G, Andi A, et al. Angiogenesis is an independent prognostic factor in malignant mesothelioma. Br J Cancer 2001;85:863-8.
8. Dubey S, Jänne PA, Krug L, et al. A phase II study of sorafenib in malignant mesothelioma: results of Cancer and Leukemia Group B 30307. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer 2010;5:1655-61.
9. Jahan TM, Gu L, Wang X, et al. Vatalanib (V) for patients with previously untreated advanced malignant mesothelioma (MM): A phase II study by the Cancer and Leukemia Group B (CALGB 30107). Journal of Clinical Oncology 2006;24:7081-.
10. Baas P, Boogerd W, Dalesio O, et al. Thalidomide in patients with malignant pleural mesothelioma. Lung Cancer 2005;48:291-6.
11. Langan LM, Dodd NJF, Owen SF, et al. Direct Measurements of Oxygen Gradients in Spheroid Culture System Using Electron Parametric Resonance Oximetry. PloS one 2016;11:e0149492-e.

12. Grimes DR, Kelly C, Bloch K, et al. A method for estimating the oxygen consumption rate in multicellular tumour spheroids. Journal of the Royal Society, Interface 2014;11:20131124-.
13. Tarnoki-Zach J, Stockhammer P, Isai DG, et al. Multicellular contractility contributes to the emergence of mesothelioma nodules. Sci Rep 2020;10:20114.
14. Blanquart C, Jaurand M-C, Jean D. The Biology of Malignant Mesothelioma and the Relevance of Preclinical Models. Frontiers in oncology 2020;10:388-.
15. Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res 2005;65:671-80.
16. Shamseddin M, Obacz J, Garnett MJ, et al. Use of preclinical models for malignant pleural mesothelioma. Thorax 2021;76:1154-62.
17. Jongsma J, van Montfort E, Vooijs M, et al. A conditional mouse model for malignant mesothelioma. Cancer Cell 2008;13:261-71.
18. Jean D, Jaurand M-C. Mesotheliomas in Genetically Engineered Mice Unravel Mechanism of Mesothelial Carcinogenesis. International journal of molecular sciences 2018;19:2191.
19. Altomare DA, Vaslet CA, Skele KL, et al. A mouse model recapitulating molecular features of human mesothelioma. Cancer Res 2005;65:8090-5.
20. Fleury-Feith J, Lecomte C, Renier A, et al. Hemizygosity of Nf2 is associated with increased susceptibility to asbestos-induced peritoneal tumours. Oncogene 2003;22:3799-805.