nature portfolio

Peer Review File

Next-generation osteosarcoma models for precision medicine

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

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
While the manuscript addresses important aspects of osteosarcoma biology, modelling, and emerging therapeutics, several areas would benefit from clarification and restructuring to strengthen its scientific narrative and impact.
Overall, the review reads more like a thesis-style literature review, with many concepts presented sequentially but without a fully unified narrative. The manuscript attempts to discuss osteosarcoma biological features, in vitro models, novel therapeutic approaches and in silico approaches. These are substantial topics on their own, and combining them in a single review risk diluting the clarity of the sections.
I suggest the authors consider restructuring the review into two more coherent thematic sections—or even two separate reviews—depending on their intended scope:
1. A mechanistically grounded review of osteosarcoma biology and the models used to recapitulate its features, including 2D, 3D, animal models, and emerging platforms.
2. A distinct review focused on therapeutic development, integrating clinical data, drug-delivery advances, and novel systemic or cell-based interventions and the relevance of in silico approaches to explain results of clinical approaches or clinical data.

INTRODUCTION
The introduction provides useful background on osteosarcoma and bone biology; however, several areas would benefit from clarification to improve mechanistic coherence. At present, the text intermixes epidemiology, genetic predisposition, and bone homeostasis without clearly articulating how disruptions in normal osteoblast–osteoclast signalling contributes to osteosarcoma initiation and progression. As written, the transitions—from germline mutations to the RANK/RANKL/OPG axis, and subsequently to PTH/PTHrP-mediated osteolysis—feel disjointed. It is also unclear whether the described TGF-β/PTHrP “vicious cycle” refers specifically to intrinsic osteosarcoma biology or more broadly to metastatic bone disease mechanisms.
To strengthen the introduction, the authors should provide a more direct and integrated explanation of how impaired osteoblast differentiation, dysregulated bone remodelling, and key microenvironmental signalling pathways (e.g., RANKL, TGF-β, integrin αvβ3) contribute to osteosarcoma pathogenesis. Recent comprehensive reviews that outline these mechanisms clearly and may help refine the introduction include de Azevedo JWV, de Medeiros Fernandes TAA, Fernandes JV Jr, de Azevedo JCV, Lanza DCF, Bezerra CM, Andrade VS, de Araújo JMG, Fernandes JV. Biology and pathogenesis of human osteosarcoma. Oncol Lett. 2020.
If the intention is to keep these topics within a single review, the authors must more clearly explain how model systems (particularly 3D models) inform translational research, bridge mechanistic understanding to clinical trial design, and enable preclinical testing of candidate therapies. At present, the linkage between the biological/clinical narrative and the modelling section feels incomplete.

TABLE 2
It would benefit from a clinical-trial–oriented analysis. Specifically: Identify which therapeutic strategies are currently being evaluated in clinical trials and specify their trial phase; Provide context on how preclinical evidence has translated (or has not translated) into clinical investigation.Relevant references include: Kortam et al., Commun Mater 2024 — overview of drug-delivery strategies. Zhra et al., Pharmaceutics 2025 — detailed summary of ongoing/complete osteosarcoma clinical

trials. Kansara et al., Nat Rev Cancer 2014 — foundational translational biology insights. The authors may also consider including recent work on cell-based therapies, nanoparticle platforms, and hybrid approaches (e.g., Martella et al., Adv Therap 2023; Martella et al., Pharmaceutics 2022).
This additional clinical context would make Table 2 far more useful to readers and better integrated with the manuscript's translational emphasis.

3D IN VITRO MODELS
The manuscript would benefit from a clearer explanation of how 3D in vitro models (spheroids, organoids, scaffold-based systems) enable testing of innovative therapies. For example:
• How do 3D models recapitulate gradients of hypoxia, mineralization, and proliferative heterogeneity characteristic of osteosarcoma lesions?
• How might specific models be suited for evaluating immunotherapies, nanoparticles, or cell-based constructs?
• How can these systems streamline translation by better predicting clinical outcomes compared to 2D systems?
Explicitly articulating these links would unify the sections and highlight the translational relevance of the modelling platforms.

TERMINOLOGY
A glossary or legend would be beneficial, especially to clarify distinctions between:
• Spheroids: Simple, self-assembled 3D aggregates—typically tumour-cell–only—used to model gradients of oxygen, nutrients, and drug penetration.
• Organoids: More complex, stem-cell–derived or patient-derived 3D structures capable of partial self-organization and recapitulating some tissue architecture.
• Scaffold-based 3D models: Systems incorporating biomaterials (hydrogels, ceramics, collagen, synthetic scaffolds) to mimic extracellular matrix properties, stiffness, and mineralization.
These distinctions matter because each model type recapitulates different aspects of osteosarcoma biology:
• Spheroids may better reproduce proliferative gradients and hypoxic cores—thus relevant to modelling poorly mineralized, high-cellularity tumour regions.
• Scaffold-based systems can more effectively model mineralized components of osteosarcoma lesions.
• Organoids may capture tumour heterogeneity, patient-specific architecture, and stem-like subpopulations.

The manuscript should correlate each model type with the specific tumour characteristics described by the authors (e.g., line 269: "heterogeneous cellular distribution… highly mineralized vs poorly mineralized regions"). This would significantly strengthen the rationale for choosing and developing particular 3D approaches.

FIGURE 3
It requires improvement. At present, it is difficult to read due to small font size and appears visually inconsistent, as if composed from pasted images rather than a unified schematic. A clearer, purpose-built schematic would be more appropriate.

Reviewer #2

(Remarks to the Author)
This review addresses tissue engineering-based preclinical models and computational modelling strategies. The authors argue that integrating advanced in vitro systems with predictive in silico models can better capture tumour heterogeneity and enable patient-tailored therapies. A particular strength of the manuscript is its timely overview of mechanistic, data-driven, and hybrid computational approaches.

The topic is of high importance for the fields of osteosarcoma research, tissue engineering, and precision medicine in the context of oncology. However, the manuscript currently lacks a clear conceptual bridge between in vitro and in silico approaches, as well as structural coherence and clarity in figures and terminology. With stronger integration between experimental and computational frameworks, clearer figure design, and improved consistency and precision in presentation, the paper has the potential to make a significant contribution. The detailed suggestions are listed in the attached file.

Reviewer #3

(Remarks to the Author)
The present manuscript reviewed the in vitro and in silico models available for improvement of knowledge of the complex biology of osteosarcoma, its development and therapeutic advances to counteract osteosarcoma drug resistance.
First, authors revied last studies related to innovative therapies that have been recently developed for osteosarcoma including gene therapies, immune therapies (as CAR-T cells) and check point inhibitors. An important aspect during the development of these technologies is selection of the proper model that recapitulates the key features of the tumor microenvironment associated with tumor cells. In this sense, authors nicely described a section that is focused on the in vitro models available for osteosarcoma studies, from 2D culture to last developed 3D cultures and approaches, with a detailed description of associated pros and cons. The review also does a specific focus on the last in silico approaches that have been developed to elucidate the mechanisms that drive cancer progression by using mechanistic and data-driven methods, including the use of artificial intelligence.

This review manuscript is well structured with a comprehensive sequence of sections that allows the reader to improve his knowledge from last innovative therapies developed on the osteosarcoma field to the in vivo and in silico models available that may help to better understand the microenvironment and the complexity of osteosarcoma. This review offers a panel of last 3D culture strategies in osteosarcoma that could help the reader in the selection of the proper model of study. The review is supported by several figures that clearly summarize the content of the manuscript. The references used in this review are well annotated and compile the most recent works published in each section and discussed topic.
In summary, this manuscript constitutes an appealing review of potential in vitro and in silico models that can be used to better understand the complex heterogeneity of this disease. No major improvement is needed for this review. I recommend the acceptance of this work for publication.

Reviewer #5

(Remarks to the Author)
I have read the entire manuscript and carefully reviewed the computational section of the article “Precision Medicine in Osteosarcoma: From Tissue Engineering to Computational Modelling.” Osteosarcoma (OS) remains relatively poorly understood mechanistically, and therapeutically, when compared with other cancers, especially in light of its clinical relevance. The need to review both established knowledge and existing gaps in OS research is therefore important, and the authors clearly recognize this. From a computational perspective, the manuscript emphasizes translational goals; the introduction of white-, grey-, and black-box modeling concepts is clear and potentially educational. However, the execution of this section could be improved (see below).

Overall, the article reads as an exhaustive collection of OS-related topics, but it would benefit from several modifications. The sections appear sequentially stacked, with limited cross-talk between them and without clearly articulating the (clinical) decisions they are intended to support. I propose the following modifications:

- The computational section would strongly benefit from a concise table summarizing model inputs, outputs, and their clinical or other translational relevance.
- In its current form, the computational section represents more of a narrative perspective than a structured review; explicitly educating the reader on how the models connect, or fail to connect, would substantially strengthen this part.
- The concept of grey-box models is well motivated and represents a strong idea; however, its execution is not fully convincing. For example, ODE-based models are listed under white-box models in Figure 3, yet the grey-box article section (3.3) relies heavily on ODE models. This inconsistency should be addressed, and the borders and/or overlaps between these model categories should be more clearly defined.
- Introducing, early in the black-box section (after 1st paragraph), a brief rationale for why AI approaches are particularly suitable in this context would improve clarity.
- The manuscript briefly summarizes several AI approaches without sufficient explanation of why these tools were chosen or what specific problems they address; the proposed summary table would help resolve this issue.
- It is unclear why citation 147 (Rejniak et al.) appears under the “Challenges and Future Directions” subsection, as its placement with respect to the white/grey/black-box framework is not well defined.
- The literature search underlying the computational section appears selective rather than exhaustive; a more comprehensive literature search of relevant computational and hybrid (grey-box) modeling studies would strengthen the review and better reflect the state of the art around OS.
- Consider whether Section 3.3.1 should be renumbered as Section 3.4 for clarity.
- Finally, I suggest reorganizing Section 3 to follow the order presented in Figure 3, starting with white-box models, then grey-box, and finally black-box models, which would improve narrative flow and make the discussion more engaging.

Version 4:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I thank the authors for addressing the majority of my comments. However, I still have a concern regarding Figure 3. At present, it remains difficult to read because of the small font size, particularly in panels c and d. In addition, the figure appears visually inconsistent, giving the impression that it is composed of pasted images rather than presented as a unified schematic. A clearer, purpose-built schematic would be more appropriate.

Reviewer #2

(Remarks to the Author)
The quality of the manuscript has improved since the last revision. The comments and suggestions were addressed. The link between in vitro and in silico has been reflected in the text and illustrated in the graphical abstract. Figure 1 makes more sense now but still needs minor "polishing": standardizing letter case, aligning the text and icons in the legend, aligning the captions (this will make the figure tidier), and clarifying the caption starting with "RANKL is expressed..." (for now, the

wording of the caption is confusing). I believe, though, that minor edits do not need yet another round of revisions and can be addressed during the proofreading step.

Reviewer #5

(Remarks to the Author)
I have no further recommendations. From my computational perspective, the comments were addresses adequately and transitions between the models and/or applications now flow more smoothly.

17th November 2025

Dear Associate Editor, Dr. Laura Perez,

Thank you very much for your time and for the constructive feedback provided regarding our manuscript entitled "Multidisciplinary Strategies in Osteosarcoma Integrating Bioengineered Scaffolds to Predictive Models and Precision Medicine.". Regarding to your suggestions the manuscript is entitled "**Precision Medicine in Osteosarcoma: From Tissue Engineering to Computational Modeling**" for publication in the *Communications Biology.*

We appreciate your comments on the scope and length of the review. Following your guidance, we have thoroughly revised the manuscript to streamline the content and ensure closer alignment with the journal's requirements. In particular, we significantly reduced and focused the experimental section, as recommended, while maintaining and refining the computational and predictive modelling components. The revised version now complies with the suggested word limit and reflects the editorial suggestions for improved clarity and conciseness.

We are grateful for the opportunity to resubmit the manuscript, and we kindly confirm that all efforts were made to incorporate the recommended changes. We would be pleased to have the revised version considered for peer review.

We would like to inform you that we have added the name of a researcher that was also enrolled in the manuscript, Paula Faria, with the email, paula.faria@ipleiria.pt.

The authors declare no conflicts of interest and the manuscript or any part of it, is not and will not be submitted elsewhere for publication while under consideration by the *Communications Biology.*

Thank you again for your attention and guidance.

Looking forward to receiving feedback from you.

Kind regards,
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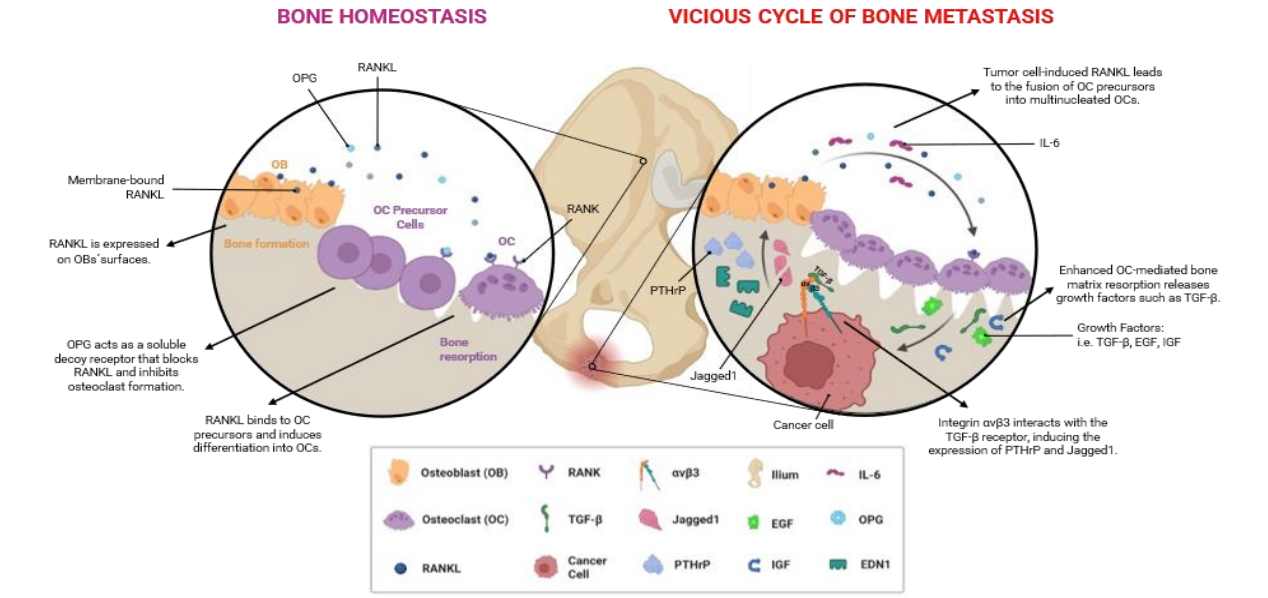
Manuscript COMMSBIO-25-9107A- Point-by-point response to the referees' comments

Referee comments	Response	Line number
1. Bridging in vitro and in silico This review lacks a connection between in vitro and in silico. A review by definition is meant to review the current state of art in the described field, but owing to the chosen topic, it can also help researchers from both sides, in vitro and in silico, by drawing a connection between these two approaches. What cells and cell-cell interactions are being modelled computationally, and what are being co-cultured in vitro? Is there a way of standardizing existing in vitro models in a way that would aid computational modelling and vice versa? If there are certain parameters modelled and tested in silico, can the authors propose, from their perspective, what components are current in vitro models lacking to become an instrument of validation for the computationally derived data? And what data derived computationally can be used in the design of in vitro models of osteosarcoma, and how? Answering these questions would be beneficial for the researchers in the field and would elevate the level of this manuscript.	We thank the reviewer for this insightful and constructive comment. We agree that strengthening the connection between the in vitro and in silico aspects of the review was important, and we have revised the manuscript accordingly. Specifically, we added a new section discussing which cells and cell–cell interactions are modelled computationally and which are co-cultured in vitro, as suggested by the reviewer (Lines 501–526). In addition, we included a discussion on the potential for standardization of in silico and in vitro models to better support computational modelling, which we also highlight as a current limitation in the field (Lines 665–693). Regarding the final point, rather than explicitly defining missing components in current in vitro models, we have expanded the discussion to focus on how in vitro and in silico approaches can be more effectively integrated, particularly in the context of iterative model refinement and data exchange (Lines 634–647). We believe this perspective provides a practical framework to bridge the two approaches and enhances the overall impact of the manuscript.	(501-526), (634-647), and (665-693)
Figure 1 - First of all, it might be beneficial for the reader to see the normal physiological cues happening in bone remodelling (left part of the figure, "Bone homeostasis" bubble). The illustration of those ques should not be too detailed – would be sufficient to at least represent interactions described in the Introduction ("Osteoblasts regulate osteoclast differentiation through the RANK/RANKL/OPG axis: RANKL expressed on their surface binds to RANK on osteoclast precursors to induce differentiation, while osteoprotegerin (OPG) acts as a soluble decoy receptor that blocks RANKL and inhibits osteoclast formation…")	We thank the reviewer for this helpful suggestion. In response, we have revised the left panel of the figure ("Bone homeostasis") to more clearly illustrate the key physiological cues involved in bone remodelling, while maintaining a simplified and accessible visual representation. The updated schematic now includes rounded osteoclast precursor cells, which remain in an undifferentiated state when RANK is free or bound by OPG, reflecting OPG´s inhibitory role. In contrast, when RANK binds to RANKL, these precursor cells are shown as having differentiated into more flattened, multinucleated osteoclasts, representing their mature and active form.	171
Figure 1 - The abbreviations for Osteoblast and Osteoclast (OB and OC) can be moved to the legend of the	We thank the reviewer for this suggestion. We have revised the figure accordingly by moving the definitions of the abbreviations	171

figure to not overcrowd the image, and the cells in the image can be renamed OB and OC instead of full names.	for osteoblast and osteoclast (OB and OC) to the figure legend. Additionally, the labels within the figure have been simplified to use the abbreviations OB and OC instead of the full terms. This change reduces visual clutter and improves the overall clarity of the illustration, as recommended.	
Figure 1 - The representation of the interaction of integrin with TGF-beta is misleading. It looks like both of them are sitting on the membrane of the cancer cell.	We thank the reviewer for pointing this out. We have revised the figure to improve the accuracy of this interaction. Specifically, TGF-β has been repositioned to the extracellular space outside the cancer cell membrane, while integrin remains embedded in the cell membrane. This adjustment clarifies their spatial relationship and avoids the misleading impression that both molecules are membrane-bound.	171
Figure 1 - Is it accurate to number the stages of the vicious cycle? When the stages are numbered, one may think that the cycle starts with interactions of integrin with TGFbeta, while the trigger point of the vicious cycle is still an "egg or chicken" question. One of the possible ways to avoid numbering is to bring the captions of the stages closer to the arrows. To give space for the captions, the naming of the proteins and factors can be omitted since they are present in the legend below.	We thank the reviewer for this insightful comment. In response, we have removed the explicit numbering of the stages in the figure to avoid suggesting a defined starting point in the vicious cycle. Instead, we have incorporated descriptive labels alongside arrows indicating where the different processes occur. Additionally, to reduce visual clutter and improve readability, the number of depicted proteins and growth factors has been reduced, as suggested. However, we have retained the captions for the different proteins and growth factors to ensure clarity and proper identification of the key elements within the figure.	171
Figure 1 -To correspond with the text in the introduction, the authors may consider illustrating both membrane-bound (on the OBs' surface) and soluble RANKL (in the present version of the manuscript, the text describes mebrane-bound RANKL, and the figure contains only soluble RANKL.	We thank the reviewer for this valuable suggestion. We have revised the figure accordingly to better align with the description provided in the Introduction by explicitly representing both forms of RANKL. In the updated figure, membrane-bound RANKL is depicted on the surface of osteoblasts (OBs), illustrating its cell-associated form, while soluble RANKL is shown diffusing freely within the extracellular environment. Both forms are also clearly distinguished through appropriate captions, ensuring accurate representation and improved clarity.	171
Figure 1 - Some captions should be rewritten if kept: "Metastatic cancer cells" can be rewritten as "Cancer cells", since only a single cell is depicted and	We thank the reviewer for this helpful suggestion. In response, we have revised the figure captions accordingly. The label "metastatic cancer cells" has been changed	171

there is no illustration of or connection to metastasis in the figure itself, even if metastasis is mentioned in the text. Arrow pointing at the growth factors has a mistake in caption: i.e., not ie. Caption for stage 3 of the cycle mentions "cytokines". It is better to use the same wording – either "growth factors" or "cytokines", not both.	to "cancer cell" to better reflect the single-cell representation and avoid implying metastatic processes within the figure. Additionally, the typographical correction from "ie." to "i.e." has been made. Furthermore, we have ensured consistency in terminology by replacing all references to "cytokines" with "growth factors" throughout the figure captions, in line with the terminology used in the manuscript.	
 Figure 1 (Updated) - Bone homeostasis and dysregulation of bone homeostasis		
Figure 2 - It is not correct to say "2D cell line". This should be changed to "2D cell culture" (because co-cultures and primary cells also can be cultured in 2D, and vice versa, cell lines can be present in spheroids and organoids)	We thank the reviewer for this helpful suggestion. We have revised the terminology accordingly by replacing "2D cell line" with "2D cell culture" throughout the figure/captions, as suggested, to more accurately reflect the experimental context and avoid ambiguity.	492
Figure 2 - To be consistent with the naming of classes of in vitro cultures, the second row, "Spheroids and organoids", can be renamed as "3D cell cultures" and then listed as "Spheroids, organoids, and tumoroids" (the term "tumoroids" has been used in the text of the manuscript).	We thank the reviewer for this suggestion. In response, we have revised the figure to improve consistency in terminology. Specifically, the label has been updated to "3D cell cultures (Spheroids, organoids, and tumoroids)", as shown in the revised figure, aligning with the nomenclature used throughout the manuscript.	492
Figure 2 - "Scaffold-based biomaterials" should sound as "Biomaterial-based scaffolds". But overall, this approach would be incorrect. To stay coherent with the text, the authors may use	We thank the reviewer for this suggestion. We have revised the figure label accordingly to improve coherence with the manuscript terminology. The caption has been updated to "Biomaterial scaffold-based cultures," as shown in the revised	492

"Biomaterial scaffold-based cultures/scaffold-based in vitro models".	figure, to better reflect the intended meaning and align with the text.	
Figure 2 - The color of the "Challenges" block should be changed to a lighter one to increase the contrast between the text and the background and therefore enhance readability for those who won't have access to color	We thank the reviewer for this valuable suggestion. We have updated the color of the "Challenges" block to a lighter shade of the same color, which improves the contrast between the text and the background and enhances readability, including for readers without access to color.	492
Figure 2 - For the same sake of readability, minor aesthetic details should not be overlooked: the indentation of the points in the lower bottom block differs from the other ones; there is an extra space before "3D cellular organization"; the number of colors should be minimized and picked thoughtfully to enhance the experience of color-blind readers.	We thank the reviewer for this thoughtful observation. We have corrected the indentation of the points in the lower block to ensure consistency with the other sections and removed the extra space before "3D cellular organization." Additionally, we have revised the color scheme by changing all icons to different tones, minimizing the number of colors used and improving accessibility for color-blind readers while enhancing overall readability.	492
Figure 2 - The content of the figure should better reflect the content of the manuscript text. For example, the authors mention several times that "compactness" of the cell mass is an advantage of working with 3D cultures. Thus, this advantage should be mentioned in the block with 3D cultures of the figure (and, maybe, in the block of challenges that are met in work with 2D cultures)	We thank the reviewer for this insightful suggestion. We have revised the figure to better align with the manuscript content by explicitly incorporating the concept of compactness. Specifically, we added "lack of compact 3D cell organization" to the challenges block and included "3D cellular distribution, organization, and compactness" in the advantages block for 3D cultures, thereby improving the consistency between the figure and the text.	492
Figure 2 - The authors are advised to check the wording (no need to use different words for saying the same, for example, "Useful for personalized medicine studies" and "Personalized medicine study's application") and possible writing mistakes (for example, "time-consuming handling are is required"	We thank the reviewer for this helpful suggestion. We carefully reviewed the wording throughout the manuscript to improve consistency and clarity, avoiding unnecessary variation in phrasing. In particular, we standardized the expression to "suitable for personalized medicine studies." We also corrected the indicated phrase to "time-consuming handling and specialized expertise required," along with other minor writing and grammatical issues identified during the revision.	492

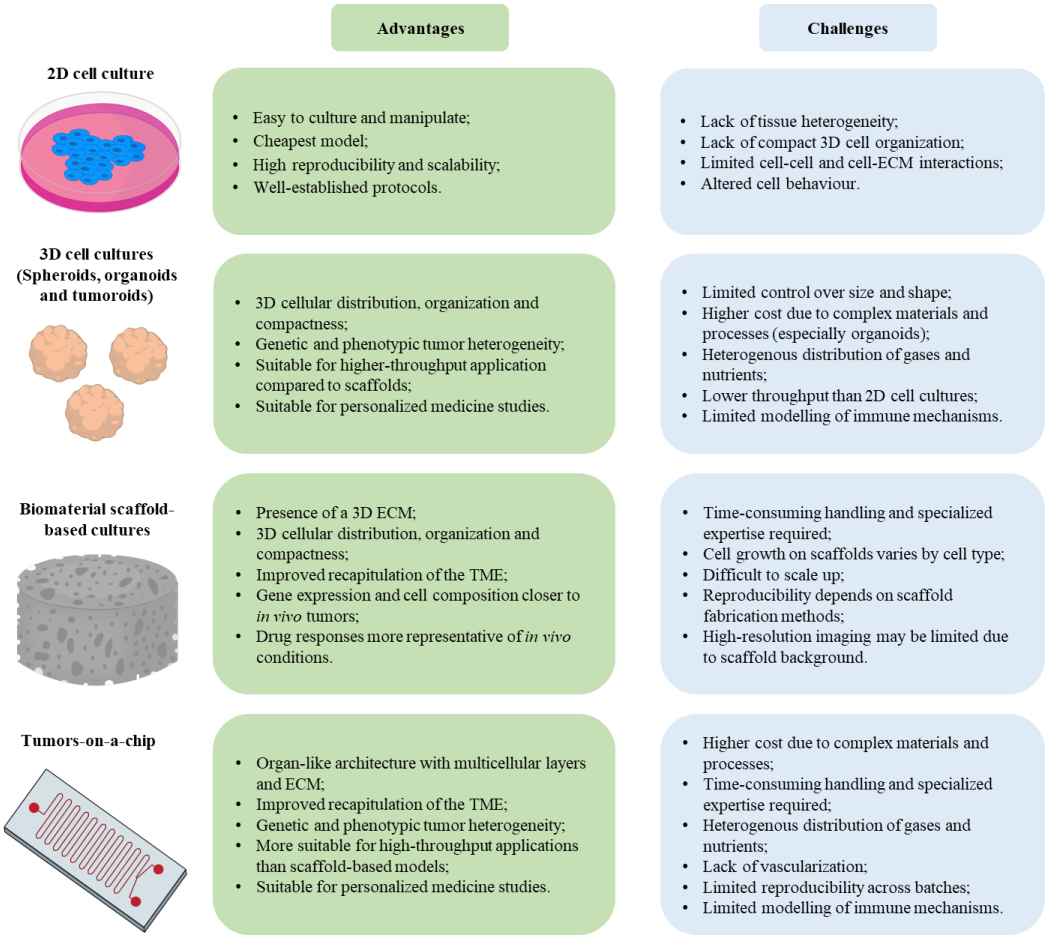


Figure 2 (Updated) - A summary of the advantages and the challenges of different in vitro models that used to study OS

Unclear/possibly incorrect statements; some examples: "Current OS treatments, …, but for metastatic cases, particularly lung or bone metastases, this type of therapy remains limited" – what is the percentage of OS undergoing lung metastasis and bone metastasis? How exactly does bone-to-bone metastasis occur?	We now state that OS metastasizes most commonly to the lungs, while bone and lymph node involvement are less frequent. We also included quantitative data indicating that metastatic cases consist of 74% lung-only metastases, 9% bone-only metastases, and 8% with both. Furthermore, we expanded the description of bone-to-bone metastasis to explain its progression via hematogenous spread, including tumor cell survival mechanisms (e.g., resistance to anoikis), chemokine-mediated homing to the bone microenvironment, and interactions with mesenchymal stem cells and osteoclasts that promote tumor progression.	173-183
Unclear/possibly incorrect statements; some examples: Line 274: organ-on-a-chip models, as well as	We thank the reviewer for this important observation. We agree that organ-on-a-chip systems and bioreactors may involve	285, and 434-436

bioreactors, can be characterized by chamber/channel/port dimensions exceeding microfluidics parameters. With this consideration, the authors may change the classification of OS in vitro models.	structural features that extend beyond the classical definition of microfluidic systems. To avoid potential ambiguity, we have revised the classification of in vitro osteosarcoma models to focus specifically on organ-on-a-chip platforms, rather than broadly grouping them under microfluidic systems line 285, and 434-436	
Unclear/possibly incorrect statements; some examples: Could the answer provide the dimension range of the spheroids enabling the development of a necrotic core? "This leads to the formation of distinct cellular zones, including a proliferative outer layer, a quiescent middle layer, and a necrotic core, thereby reflecting the heterogeneity of solid tumours."	We thank the reviewer for this helpful suggestion. We have revised the manuscript to include the typical size range of spheroids associated with the formation of a necrotic core (Line 335).	335
Unclear/possibly incorrect statements; some examples: "Physics-based models often focus on biophysical processes such as the diffusion of nutrients and chemicals, mechanical stresses on tumours and cells, and fluid flow in the circulatory system 121,122. Further mathematical models explore therapeutic interventions, drug delivery, and interactions between cancer and the immune system." – What is meant by "further mathematical models"? Are these separately developed models, or mathematical modelling performed on the basis of physics-based models?	We thank the reviewer for this clarification request. We have revised the wording to improve precision and avoid ambiguity. Specifically, we replaced "Further mathematical models" with "These mathematical models," making it clear that the same physics-based modelling frameworks are being extended to explore therapeutic interventions, drug delivery, and cancer–immune system interactions, rather than referring to a separate category of models.	540
Minor Concerns: 1- Typos, wording, and various writing mistakes; some examples: 1 • Introduction, line 195: "uses viral vectors to modify ~~a~~ patient's T-cells" 2 • Line 203: "toxin that kills the cells" 3 • Line 292: "using the chemical agent N-methyl-N-nitro-Nnitroguanidine (MNNG), the MNNG-HOS cell line was established" 4 • Line 302: "and efforts have been made to characterize them in terms of differentiation capacity" - - - - - - - - - 5 • Line 305: "First, the culture and expansion of 2D cell lines results in genetic homogeneity" 6 • Line 334: wording "OS cell spheroids"	We thank the reviewer for carefully identifying the typographical errors. All indicated typos have been corrected throughout the manuscript. For clarity, please find each comment number alongside the corresponding corrections line numbers. (1 line 204), (2 line 212), (3 line 304), (4 line 314), (5 line 317), (6 line 346), (7 line 354), (8 line 375), (9 line 379), (10 line 382), (11 line 420), (12 line 444), (13 figure 3).	204, 212, 304, 314, 317, 346, 354, 375, 379, 382, 420, 444, Figure 3

7 • Line 339: unclear sentence “It was shown that 3D spheroids of U2-OS and SaOS-2, and 2D doxorubicin-resistant sublines, had higher levels of ABCB1 transporter, which is involved in doxorubicin efflux, compared to the parental cell lines, thus impairing chemoresistance to drugs.” What is meant by “parental cell lines”? U2-OS and SaOS 2 grown in standard 2D conditions? 8 • Line 361: wording in “Both natural and synthetic-based biomaterial scaffolds” 9 • Line 363: the last part of the sentence does not seem to correspond with the other parts and is unclear “Specifically, collagen and hydroxyapatite-based scaffolds are among the most used biomaterials for OS 3D cell cultures, given their natural presence in the in vivo tissue, and to embedded the in vivo tissue within a physiological like microenvironment.” 10 • Line 369: “The 3D matrix was supplemented with the bone-marrow proteins laminin and fibronectin, and NuOss® bone granules.” (or rephrase, not to have NuOss mixed with bone proteins) 11• Line 407: “Inkjet bioprinting, offers” 12• Line 430: “preferred tissue for bioengineering of highly hierarchical bioengineered constructs” 13• In Figure 3, there is mixed spelling (“tumor” and “tumour”, and “gray” while in the text the authors use “grey”). Extra parentheses in the caption.		
Missing abbreviation explanations/misused abbreviations; some examples: 1- Overall, the transcription of abbreviations is not standardized throughout the text. For example, the authors give explanations to OPG, PTH, and PTHrP at the beginning of the introduction, but omit those for PD-1, CTLA-4, etc. Abbreviations should be either set in the text or given as a list at the beginning of the manuscript. 2- The authors are kindly advised to stay consistent with the use of	We thank the reviewer for this important observation and fully agree that consistent and clear use of abbreviations is essential for readability. Accordingly, we have carefully revised the manuscript to standardize the use of abbreviations throughout. Specifically, we have (i) ensured that all abbreviations are properly defined upon first use, (ii) maintained consistent usage once introduced, and (iii) removed unnecessary or redundant abbreviations that are not used subsequently in the text. In addition, we have included a comprehensive list of abbreviations in the manuscript for clarity.	example: 248, 425, 466, and 495 751

abbreviations and to double-check that once an abbreviation is introduced, it is used (for example, “osteosarcoma” is not abbreviated in lines 412 and 481. 3- There is no need for creation abbreviations if they will no longer be used further in text (for example, “dOsEM” in line 450, “OOC” in line 453 – and the latter is even confusing because it repeats the OoC abbreviation but is used for a different term).	Given the extent of these revisions, it is not practical to indicate all individual line changes; however, all modifications have been highlighted in yellow in the revised manuscript. For transparency, we provide below a few representative examples of the corrections made. lines: 248, 425, 466, and 495	
Missing abbreviation explanations/misused abbreviations; some examples: Introduction, line 226: L-MTP-PE should be transcribed even if later on in the text the authors explain the function of the drug.	We thank the reviewer for this helpful suggestion and agree with this point. The abbreviation has now been fully transcribed at its first occurrence in the Introduction (Line 237), in accordance with standard practice.	236
Missing abbreviation explanations/misused abbreviations: some examples: In Figure 3, AI is abbreviated, while ML is not.	We thank the reviewer for this observation. In Figure 3, both AI and ML are now shown as abbreviations due to space limitations; however, both terms are fully transcribed and defined in the main text and in the abbreviations list to ensure clarity.	Figure 3

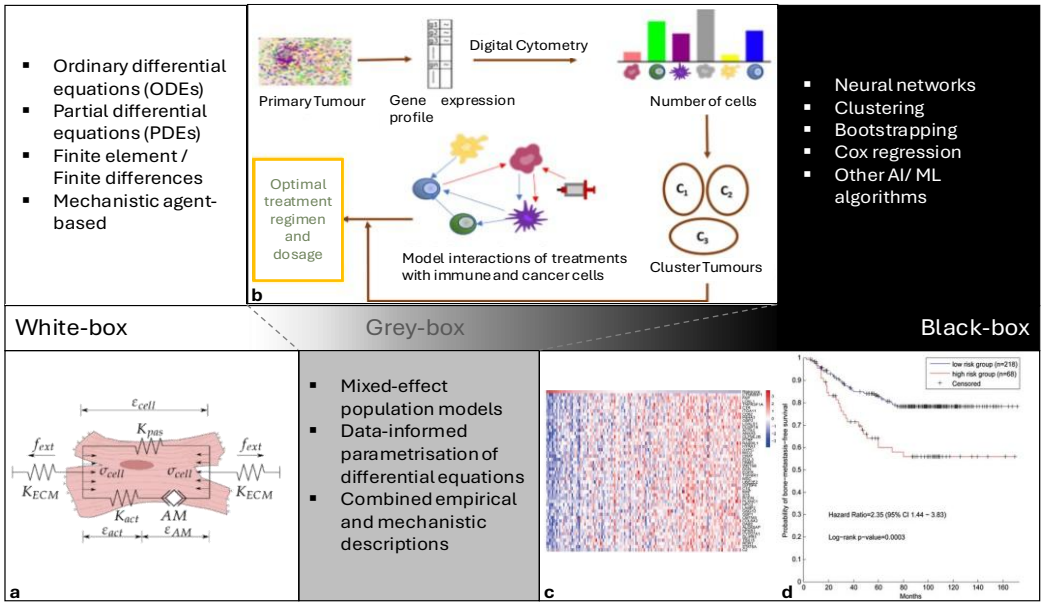
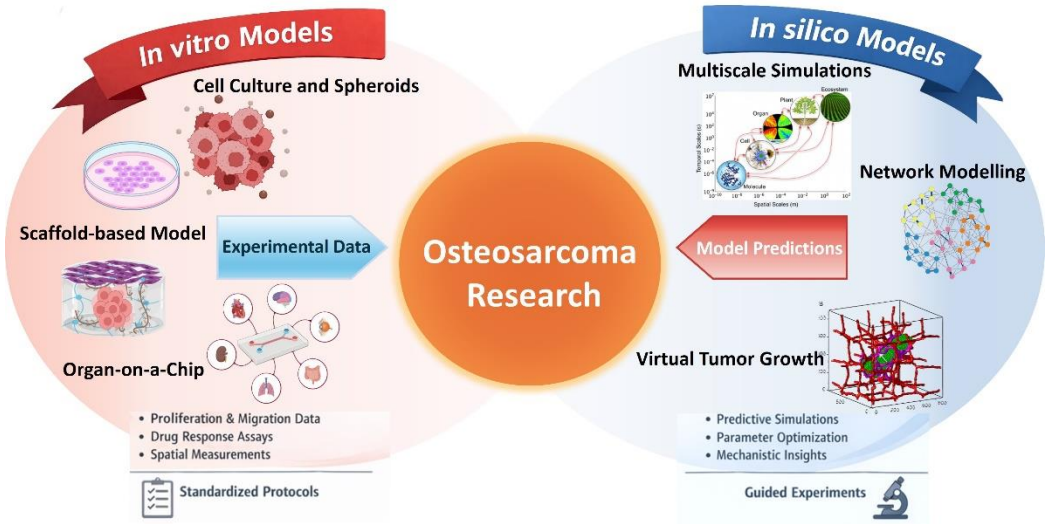


Figure 3 (Updated) - Collection of typical computational modelling approaches spanning the full white-to-black-box spectrum with illustrative applications in bone malignancies. a) Mechanical model of a single cell allowing computation of internal stress $\sigma cell$ due to contraction of actin–myosin filaments and cell membrane and cytoskeleton resistance. Used in the study of the interactions of myeloma cells with resident BM cells. b) Schematic pipeline used by Le and colleagues to simulate OS response to chemotherapy combining data-informed composition of tumour and ODE description of intercellular interactions. c) Heatmap of the top 50 negative correlation genes with the ML-derived prognostic signature risk score for OS outcome. d) Kaplan-

Meier curves of bone metastasis-free survival for defined risk groups amongst breast cancer patients		
Missing abbreviation explanations/misused abbreviations: Line 544: RSF (random survival forest)	We thank the reviewer for this suggestion. The term has been corrected to “random survival forest” at Line 584.	584

Point-by-point response to the Editor Comment of the Decision on manuscript COMMSBIO-25-9107B of the 15th April 2026 highlighted In blue in the manuscript

Editor comments	Response	Line number
the transitions between sections have not been modified, this was a major comment and we would require this to publish the manuscript.	We thank the reviewer for this insightful comment. To address this point, we have substantially expanded the manuscript to explicitly bridge in vitro and in silico approaches. In particular: - We have added a dedicated section at the end of Section 3.4 explicitly addressing the integration between in vitro and in silico approaches (line 689-725). - We have added a graphical abstract that shows a schematic overview of the bidirectional workflow between advanced in vitro models and computational approaches (line 92-93) -We discussed the correspondence between cellular components represented in computational models and those used in advanced in vitro systems.(line 692 – 702) - We outlined key requirements for standardizing in vitro models to enable their use for computational parameterization. (line 703-711) - We discussed current limitations of in vitro systems in serving as validation platforms for in silico predictions. (line 476-484) and (line 712-717) - We highlighted how computational modelling can guide the design of in vitro experiments. (line 718-725) - Additionally, two short paragraphs were added to highlight the need to integrate in vitro and in silico modeling, facilitating a smooth transition between sections.(line 126-133) and (line 174-180) These additions provide a clearer and more actionable framework for integrating experimental and computational approaches in osteosarcoma research.	line 689-725, line 92-93, line 692-702, line 703-711, line 476-484, line 712-717, line 718-725, line 126-133, and line 174-180)



Graphical abstract. Line 93

Please note Figure 1 is still identical and we cannot publish it because it would be plagiarism, please redraw.	Thank you for your comment. Figure 1 has been redrawn accordingly. The updated figure was created using elements from Servier Medical Art, which are licensed under CC BY 4.0, and has been modified to ensure originality and compliance with publication requirements. (line 156)	line 156

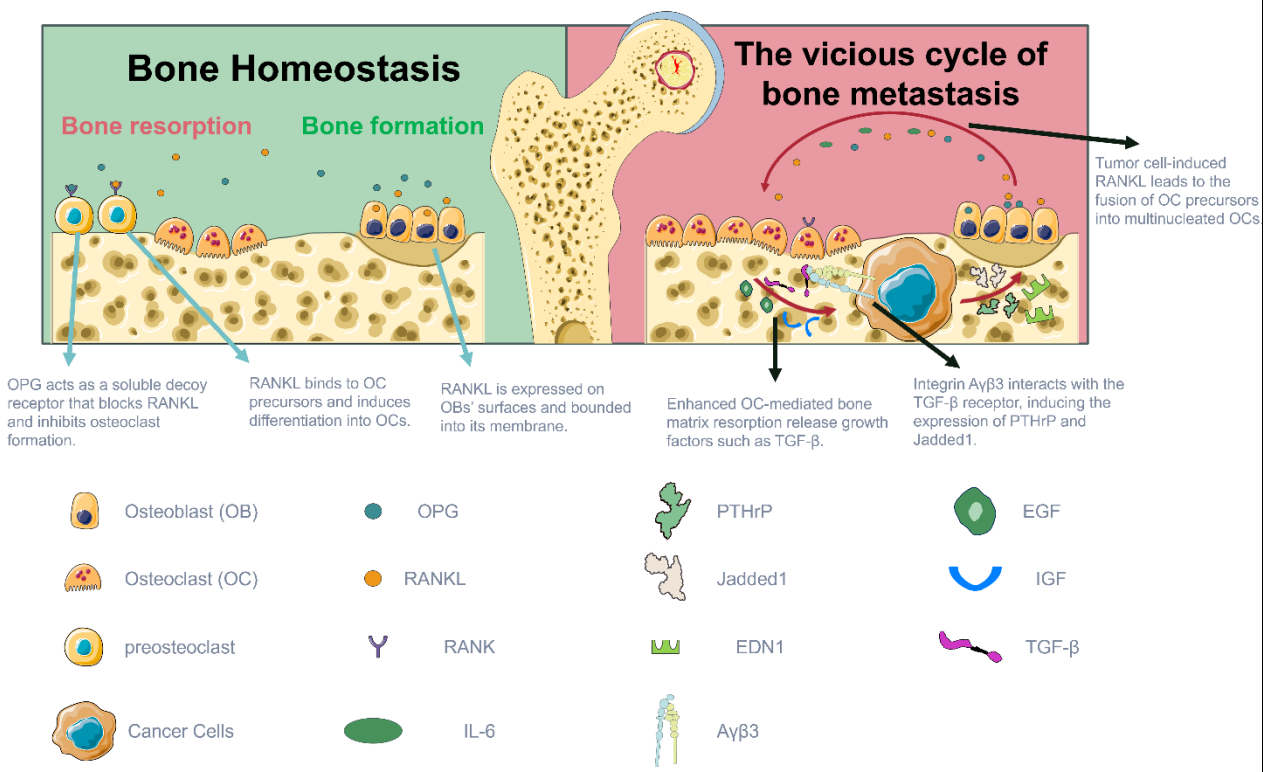
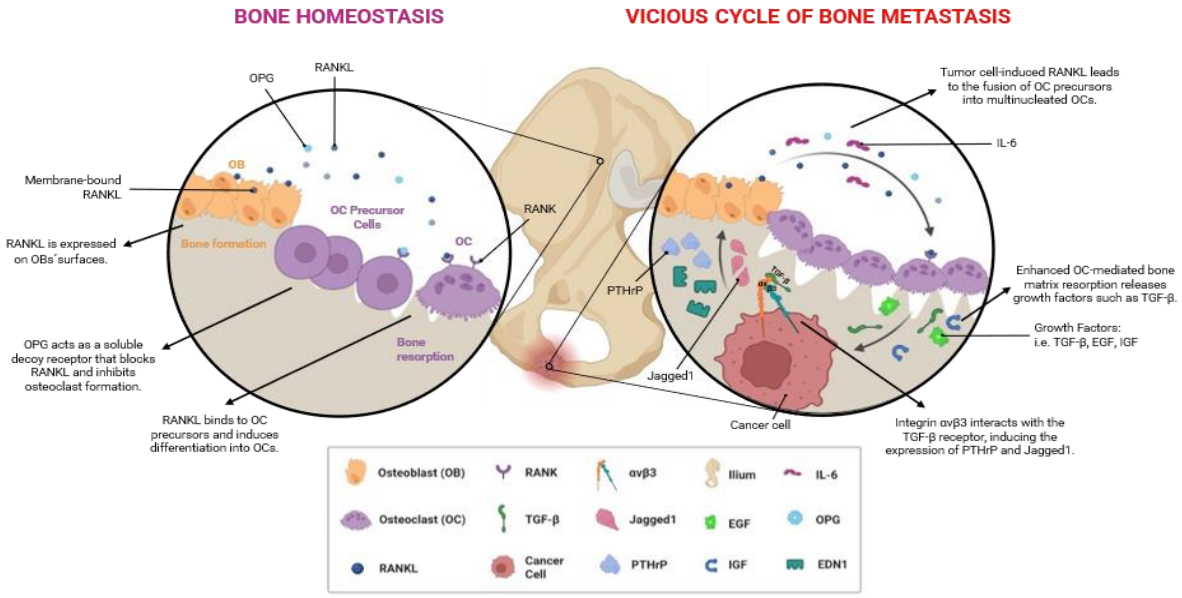


Figure 1 - Bone homeostasis and dysregulation of bone homeostasis. The figure was built using materials from Servier Medical Art.

Manuscript COMMSBIO-25-9107A- Point-by-point response to the referees' comments
highlighted in Yellow in the manuscript

Referee comments	Response	Line number
1. Bridging in vitro and in silico This review lacks a connection between in vitro and in silico. A review by definition is meant to review the current state of art in the described field, but owing to the chosen topic, it can also help researchers from both sides, in vitro and in silico, by drawing a connection between these two approaches. What cells and cell-cell interactions are being modelled computationally, and what are being co-cultured in vitro? Is there a way of standardizing existing in vitro models in a way that would aid computational modelling and vice versa? If there are certain parameters modelled and tested in silico, can the authors propose, from their perspective, what components are current in vitro models lacking to become an instrument of validation for the computationally derived data? And what data derived computationally can be used in the design of in vitro models of osteosarcoma, and how? Answering these questions would be beneficial for the researchers in the field and would elevate the level of this manuscript.	We thank the reviewer for this insightful and constructive comment. We agree that strengthening the connection between the in vitro and in silico aspects of the review was important, and we have revised the manuscript accordingly. Specifically, we added a new section discussing which cells and cell–cell interactions are modelled computationally and which are co-cultured in vitro, as suggested by the reviewer (Lines 499–524). In addition, we included a discussion on the potential for standardization of in silico and in vitro models to better support computational modelling, which we also highlight as a current limitation in the field (Lines 663–690). Regarding the final point, rather than explicitly defining missing components in current in vitro models, we have expanded the discussion to focus on how in vitro and in silico approaches can be more effectively integrated, particularly in the context of iterative model refinement and data exchange (Lines 632–645). We believe this perspective provides a practical framework to bridge the two approaches and enhances the overall impact of the manuscript.	(499-524), (632-645), and (663-690)
Figure 1 - First of all, it might be beneficial for the reader to see the normal physiological cues happening in bone remodelling (left part of the figure, "Bone homeostasis" bubble). The illustration of those ques should not be too detailed – would be sufficient to at least represent interactions described in the Introduction ("Osteoblasts regulate osteoclast differentiation through the RANK/RANKL/OPG axis: RANKL expressed on their surface binds to RANK on osteoclast precursors to induce differentiation, while osteoprotegerin (OPG) acts as a soluble decoy receptor that blocks RANKL and inhibits osteoclast formation…")	We thank the reviewer for this helpful suggestion. In response, we have revised the left panel of the figure ("Bone homeostasis") to more clearly illustrate the key physiological cues involved in bone remodelling, while maintaining a simplified and accessible visual representation. The updated schematic now includes rounded osteoclast precursor cells, which remain in an undifferentiated state when RANK is free or bound by OPG, reflecting OPG´s inhibitory role. In contrast, when RANK binds to RANKL, these precursor cells are shown as having differentiated into more flattened, multinucleated osteoclasts, representing their mature and active form.	158

Figure 1 - The abbreviations for Osteoblast and Osteoclast (OB and OC) can be moved to the legend of the figure to not overcrowd the image, and the cells in the image can be renamed OB and OC instead of full names.	We thank the reviewer for this suggestion. We have revised the figure accordingly by moving the definitions of the abbreviations for osteoblast and osteoclast (OB and OC) to the figure legend. Additionally, the labels within the figure have been simplified to use the abbreviations OB and OC instead of the full terms. This change reduces visual clutter and improves the overall clarity of the illustration, as recommended.	158
Figure 1 - The representation of the interaction of integrin with TGF-beta is misleading. It looks like both of them are sitting on the membrane of the cancer cell.	We thank the reviewer for pointing this out. We have revised the figure to improve the accuracy of this interaction. Specifically, TGF-β has been repositioned to the extracellular space outside the cancer cell membrane, while integrin remains embedded in the cell membrane. This adjustment clarifies their spatial relationship and avoids the misleading impression that both molecules are membrane-bound.	158
Figure 1 - Is it accurate to number the stages of the vicious cycle? When the stages are numbered, one may think that the cycle starts with interactions of integrin with TGFbeta, while the trigger point of the vicious cycle is still an “egg or chicken” question. One of the possible ways to avoid numbering is to bring the captions of the stages closer to the arrows. To give space for the captions, the naming of the proteins and factors can be omitted since they are present in the legend below.	We thank the reviewer for this insightful comment. In response, we have removed the explicit numbering of the stages in the figure to avoid suggesting a defined starting point in the vicious cycle. Instead, we have incorporated descriptive labels alongside arrows indicating where the different processes occur. Additionally, to reduce visual clutter and improve readability, the number of depicted proteins and growth factors has been reduced, as suggested. However, we have retained the captions for the different proteins and growth factors to ensure clarity and proper identification of the key elements within the figure.	158
Figure 1 -To correspond with the text in the introduction, the authors may consider illustrating both membrane-bound (on the OBs’ surface) and soluble RANKL (in the present version of the manuscript, the text describes mebrane-bound RANKL, and the figure contains only soluble RANKL.	We thank the reviewer for this valuable suggestion. We have revised the figure accordingly to better align with the description provided in the Introduction by explicitly representing both forms of RANKL. In the updated figure, membrane-bound RANKL is depicted on the surface of osteoblasts (OBs), illustrating its cell-associated form, while soluble RANKL is shown diffusing freely within the extracellular environment. Both forms are also clearly distinguished through appropriate captions, ensuring accurate representation and improved clarity.	158

Figure 1 - Some captions should be rewritten if kept: “Metastatic cancer cells” can be rewritten as “Cancer cells”, since only a single cell is depicted and there is no illustration of or connection to metastasis in the figure itself, even if metastasis is mentioned in the text. Arrow pointing at the growth factors has a mistake in caption: i.e., not ie. Caption for stage 3 of the cycle mentions “cytokines”. It is better to use the same wording – either “growth factors” or “cytokines”, not both.	We thank the reviewer for this helpful suggestion. In response, we have revised the figure captions accordingly. The label “metastatic cancer cells” has been changed to “cancer cell” to better reflect the single-cell representation and avoid implying metastatic processes within the figure. Additionally, the typographical correction from “ie.” to “i.e.” has been made. Furthermore, we have ensured consistency in terminology by replacing all references to “cytokines” with “growth factors” throughout the figure captions, in line with the terminology used in the manuscript.	158
 Figure 1 (Updated) - Bone homeostasis and dysregulation of bone homeostasis		
Figure 2 - It is not correct to say “2D cell line”. This should be changed to “2D cell culture” (because co-cultures and primary cells also can be cultured in 2D, and vice versa, cell lines can be present in spheroids and organoids)	We thank the reviewer for this helpful suggestion. We have revised the terminology accordingly by replacing “2D cell line” with “2D cell culture” throughout the figure/captions, as suggested, to more accurately reflect the experimental context and avoid ambiguity.	488
Figure 2 - To be consistent with the naming of classes of in vitro cultures, the second row, “Spheroids and organoids”, can be renamed as “3D cell cultures” and then listed as “Spheroids, organoids, and tumoroids” (the term “tumoroids” has been used in the text of the manuscript).	We thank the reviewer for this suggestion. In response, we have revised the figure to improve consistency in terminology. Specifically, the label has been updated to “3D cell cultures (Spheroids, organoids, and tumoroids)”, as shown in the revised figure, aligning with the nomenclature used throughout the manuscript.	488
Figure 2 - “Scaffold-based biomaterials” should sound as “Biomaterial-based	We thank the reviewer for this suggestion. We have revised the figure label	488

scaffolds". But overall, this approach would be incorrect. To stay coherent with the text, the authors may use "Biomaterial scaffold-based cultures/scaffold-based in vitro models".	accordingly to improve coherence with the manuscript terminology. The caption has been updated to "Biomaterial scaffold-based cultures," as shown in the revised figure, to better reflect the intended meaning and align with the text.	
Figure 2 - The color of the "Challenges" block should be changed to a lighter one to increase the contrast between the text and the background and therefore enhance readability for those who won't have access to color	We thank the reviewer for this valuable suggestion. We have updated the color of the "Challenges" block to a lighter shade of the same color, which improves the contrast between the text and the background and enhances readability, including for readers without access to color.	488
Figure 2 - For the same sake of readability, minor aesthetic details should not be overlooked: the indentation of the points in the lower bottom block differs from the other ones; there is an extra space before "3D cellular organization"; the number of colors should be minimized and picked thoughtfully to enhance the experience of color-blind readers.	We thank the reviewer for this thoughtful observation. We have corrected the indentation of the points in the lower block to ensure consistency with the other sections and removed the extra space before "3D cellular organization." Additionally, we have revised the color scheme by changing all icons to different tones, minimizing the number of colors used and improving accessibility for color-blind readers while enhancing overall readability.	488
Figure 2 - The content of the figure should better reflect the content of the manuscript text. For example, the authors mention several times that "compactness" of the cell mass is an advantage of working with 3D cultures. Thus, this advantage should be mentioned in the block with 3D cultures of the figure (and, maybe, in the block of challenges that are met in work with 2D cultures)	We thank the reviewer for this insightful suggestion. We have revised the figure to better align with the manuscript content by explicitly incorporating the concept of compactness. Specifically, we added "lack of compact 3D cell organization" to the challenges block and included "3D cellular distribution, organization, and compactness" in the advantages block for 3D cultures, thereby improving the consistency between the figure and the text.	488
Figure 2 - The authors are advised to check the wording (no need to use different words for saying the same, for example, "Useful for personalized medicine studies" and "Personalized medicine study's application") and possible writing mistakes (for example, "time-consuming handling are is required"	We thank the reviewer for this helpful suggestion. We carefully reviewed the wording throughout the manuscript to improve consistency and clarity, avoiding unnecessary variation in phrasing. In particular, we standardized the expression to "suitable for personalized medicine studies." We also corrected the indicated phrase to "time-consuming handling and specialized expertise required," along with other minor writing and grammatical issues identified during the revision.	488

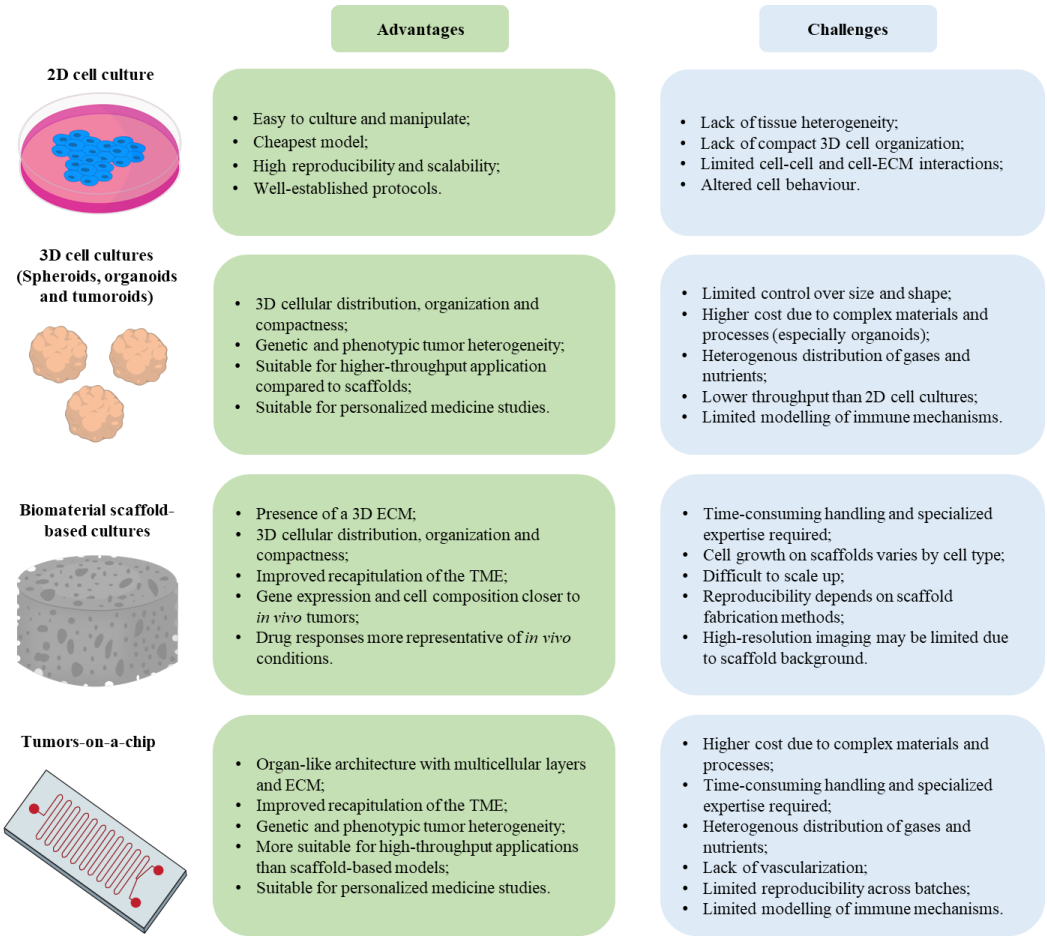


Figure 2 (Updated) - A summary of the advantages and the challenges of different in vitro models that used to study OS

Unclear/possibly incorrect statements; some examples: "Current OS treatments, …, but for metastatic cases, particularly lung or bone metastases, this type of therapy remains limited" – what is the percentage of OS undergoing lung metastasis and bone metastasis? How exactly does bone-to-bone metastasis occur?	We now state that OS metastasizes most commonly to the lungs, while bone and lymph node involvement are less frequent. We also included quantitative data indicating that metastatic cases consist of 74% lung-only metastases, 9% bone-only metastases, and 8% with both. Furthermore, we expanded the description of bone-to-bone metastasis to explain its progression via hematogenous spread, including tumor cell survival mechanisms (e.g., resistance to anoikis), chemokine-mediated homing to the bone microenvironment, and interactions with mesenchymal stem cells and osteoclasts that promote tumor progression.	162-174

Unclear/possibly incorrect statements; some examples: Line 274: organ-on-a-chip models, as well as bioreactors, can be characterized by chamber/channel/port dimensions exceeding microfluidics parameters. With this consideration, the authors may change the classification of OS in vitro models.	We thank the reviewer for this important observation. We agree that organ-on-a-chip systems and bioreactors may involve structural features that extend beyond the classical definition of microfluidic systems. To avoid potential ambiguity, we have revised the classification of in vitro osteosarcoma models to focus specifically on organ-on-a-chip platforms, rather than broadly grouping them under microfluidic systems line 273, and 422-424	273, and 422-424
Unclear/possibly incorrect statements; some examples: Could the answer provide the dimension range of the spheroids enabling the development of a necrotic core? "This leads to the formation of distinct cellular zones, including a proliferative outer layer, a quiescent middle layer, and a necrotic core, thereby reflecting the heterogeneity of solid tumours."	We thank the reviewer for this helpful suggestion. We have revised the manuscript to include the typical size range of spheroids associated with the formation of a necrotic core (Line 335).	335
Unclear/possibly incorrect statements; some examples: "Physics-based models often focus on biophysical processes such as the diffusion of nutrients and chemicals, mechanical stresses on tumours and cells, and fluid flow in the circulatory system 121,122. Further mathematical models explore therapeutic interventions, drug delivery, and interactions between cancer and the immune system." – What is meant by "further mathematical models"? Are these separately developed models, or mathematical modelling performed on the basis of physics-based models?	We thank the reviewer for this clarification request. We have revised the wording to improve precision and avoid ambiguity. Specifically, we replaced "Further mathematical models" with "These mathematical models," making it clear that the same physics-based modelling frameworks are being extended to explore therapeutic interventions, drug delivery, and cancer–immune system interactions, rather than referring to a separate category of models.	538
Minor Concerns: 1- Typos, wording, and various writing mistakes; some examples: 1 • Introduction, line 195: "uses viral vectors to modify ~~a~~ patient's T-cells" 2 • Line 203: "toxin that kills the cells" 3 • Line 292: "using the chemical agent N-methyl-N-nitro-Nnitroguanidine (MNNG), the MNNG-HOS cell line was established" 4 • Line 302: "and efforts have been made to characterize them in terms of differentiation capacity" - - - - - - - - - 5 • Line 305: "First, the culture and expansion of 2D cell lines results in	We thank the reviewer for carefully identifying the typographical errors. All indicated typos have been corrected throughout the manuscript. For clarity, please find each comment number alongside the corresponding corrections line numbers. (1 line 192), (2 line 200), (3 line 292), (4 line 302), (5 line 305), (6 line 334), (7 line 342), (8 line 363), (9 line 367), (10 line 371), (11 line 408), (12 line 432), (13 figure 3).	192, 200, 292, 302, 305, 334, 342, 363, 367, 371, 408, 432, Figure 3

genetic homogeneity” 6 • Line 334: wording “OS cell spheroids” 7 • Line 339: unclear sentence “It was shown that 3D spheroids of U2-OS and SaOS-2, and 2D doxorubicin-resistant sublines, had higher levels of ABCB1 transporter, which is involved in doxorubicin efflux, compared to the parental cell lines, thus impairing chemoresistance to drugs.” What is meant by “parental cell lines”? U2-OS and SaOS 2 grown in standard 2D conditions? 8 • Line 361: wording in “Both natural and synthetic-based biomaterial scaffolds” 9 • Line 363: the last part of the sentence does not seem to correspond with the other parts and is unclear “Specifically, collagen and hydroxyapatite-based scaffolds are among the most used biomaterials for OS 3D cell cultures, given their natural presence in the in vivo tissue, and to embedded the in vivo tissue within a physiological like microenvironment.” 10 • Line 369: “The 3D matrix was supplemented with the bone-marrow proteins laminin and fibronectin, and NuOss® bone granules.” (or rephrase, not to have NuOss mixed with bone proteins) 11• Line 407: “Inkjet bioprinting, offers” 12• Line 430: “preferred tissue for bioengineering of highly hierarchical bioengineered constructs” 13• In Figure 3, there is mixed spelling (“tumor” and “tumour”, and “gray” while in the text the authors use “grey”). Extra parentheses in the caption.		
Missing abbreviation explanations/misused abbreviations; some examples: 1- Overall, the transcription of abbreviations is not standardized throughout the text. For example, the authors give explanations to OPG, PTH, and PTHrP at the beginning of the introduction, but omit those for PD-1, CTLA-4, etc. Abbreviations should be	We thank the reviewer for this important observation and fully agree that consistent and clear use of abbreviations is essential for readability. Accordingly, we have carefully revised the manuscript to standardize the use of abbreviations throughout. Specifically, we have (i) ensured that all abbreviations are properly defined upon first use, (ii) maintained consistent usage once introduced, and (iii) removed unnecessary or redundant	example: 238, 413, 455, and 493 768

either set in the text or given as a list at the beginning of the manuscript. 2- The authors are kindly advised to stay consistent with the use of abbreviations and to double-check that once an abbreviation is introduced, it is used (for example, “osteosarcoma” is not abbreviated in lines 412 and 481. 3- There is no need for creation abbreviations if they will no longer be used further in text (for example, “dOsEM” in line 450, “OOC” in line 453 – and the latter is even confusing because it repeats the OoC abbreviation but is used for a different term).	abbreviations that are not used subsequently in the text. In addition, we have included a comprehensive list of abbreviations in the manuscript for clarity. Given the extent of these revisions, it is not practical to indicate all individual line changes; however, all modifications have been highlighted in yellow in the revised manuscript. For transparency, we provide below a few representative examples of the corrections made. lines: 238, 413, 455, and 493	
Missing abbreviation explanations/misused abbreviations; some examples: Introduction, line 226: L-MTP-PE should be transcribed even if later on in the text the authors explain the function of the drug.	We thank the reviewer for this helpful suggestion and agree with this point. The abbreviation has now been fully transcribed at its first occurrence in the Introduction (Line 237), in accordance with standard practice.	224
Missing abbreviation explanations/misused abbreviations: some examples: In Figure 3, AI is abbreviated, while ML is not.	We thank the reviewer for this observation. In Figure 3, both AI and ML are now shown as abbreviations due to space limitations; however, both terms are fully transcribed and defined in the main text and in the abbreviations list to ensure clarity.	Figure 3

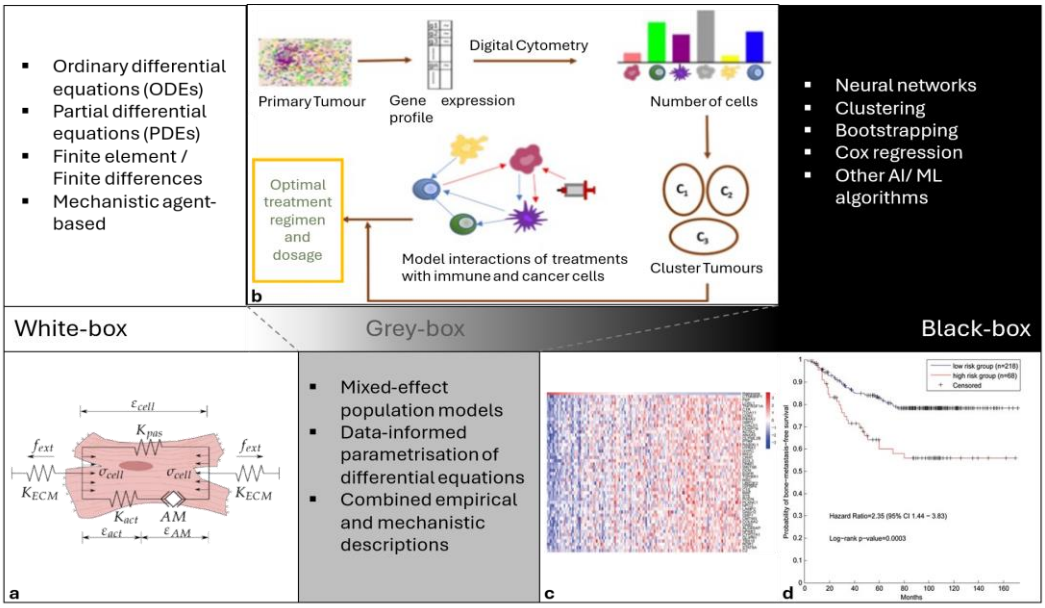


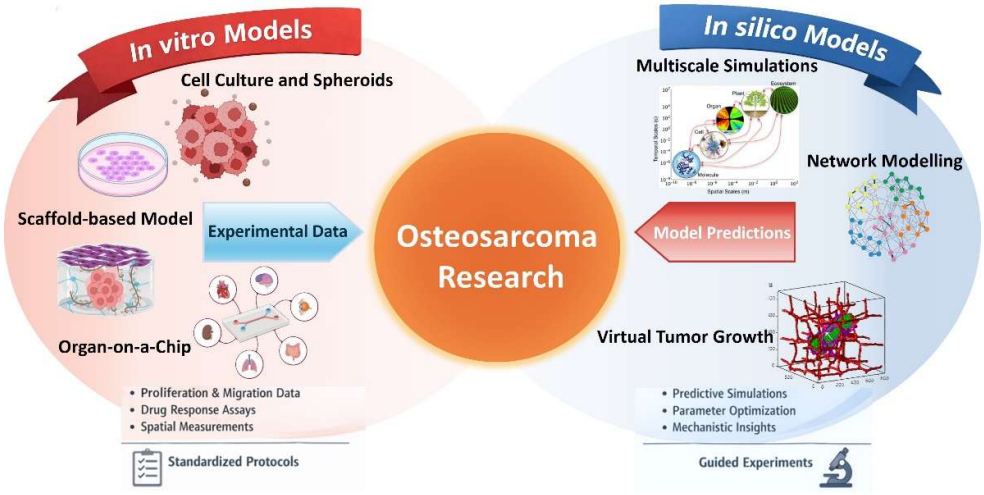
Figure 3 (Updated) - Collection of typical computational modelling approaches spanning the full white-to-black-box spectrum with illustrative applications in bone malignancies. a) Mechanical model of a single cell allowing computation of internal stress $\sigma cell$ due to contraction of actin–myosin filaments and cell membrane and cytoskeleton resistance. Used in the study of the interactions of myeloma cells with resident BM cells. b) Schematic pipeline used by Le and

colleagues to simulate OS response to chemotherapy combining data-informed composition of tumour and ODE description of intercellular interactions. c) Heatmap of the top 50 negative correlation genes with the ML-derived prognostic signature risk score for OS outcome. d) Kaplan-Meier curves of bone metastasis-free survival for defined risk groups amongst breast cancer patients		
Missing abbreviation explanations/misused abbreviations: Line 544: RSF (random survival forest)	We thank the reviewer for this suggestion. The term has been corrected to “random survival forest” at Line 582.	582

Rebuttal Letter

Point-by-point response to the Editor Comment highlighted In blue in the manuscript

Editor comments	Response	Line number
the transitions between sections have not been modified, this was a major comment and we would require this to publish the manuscript.	We thank the reviewer for this insightful comment. To address this point, we have substantially expanded the manuscript to explicitly bridge in vitro and in silico approaches. In particular: • We have added a dedicated section at the end of Section 3.4 explicitly addressing the integration between in vitro and in silico approaches (line 790–826). • We have added a graphical abstract that shows a schematic overview of the bidirectional workflow between advanced in vitro models and computational approaches (line 92–93) • We discussed the correspondence between cellular components represented in computational models and those used in advanced in vitro systems (line 793–803). • We outlined key requirements for standardizing in vitro models to enable their use for computational parameterization (line 804–812). • We discussed current limitations of in vitro systems in serving as validation platforms for in silico predictions (line 571–579) and (line 813–818). • We highlighted how computational modelling can guide the design of in vitro experiments (line 819–826). • Additionally, two short paragraphs were added to highlight the need to integrate in vitro and in silico modeling, facilitating a smooth transition between sections (line 126–133) and (line 201–207). These additions provide a clearer and more actionable framework for integrating experimental and computational approaches in osteosarcoma research.	716–752, 92–93, 719–729, 730–738, 503–511, 739–744, 745–752, 126–133, 201–207



Graphical abstract. Line 93

Please note Figure 1 is still identical and we cannot publish it because it would be plagiarism, please redraw.	Thank you for your comment. Figure 1 has been redrawn accordingly. The updated figure was created using elements from Servier Medical Art, which are licensed under CC BY 4.0, and has been modified to ensure originality and compliance with publication requirements. (line 162)	line 162

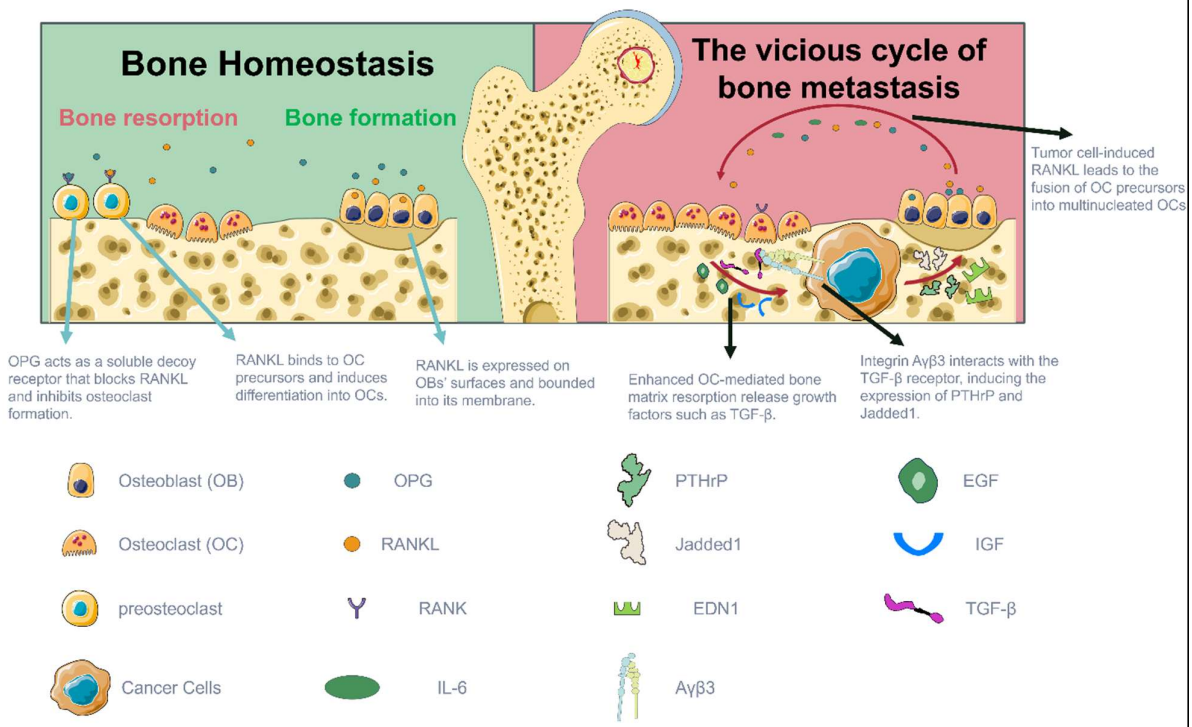


Figure 1 - Bone homeostasis and dysregulation of bone homeostasis. The figure was built using materials from Servier Medical Art.

Point-by-point response to the referees 1 comments highlighted in Green in the manuscript

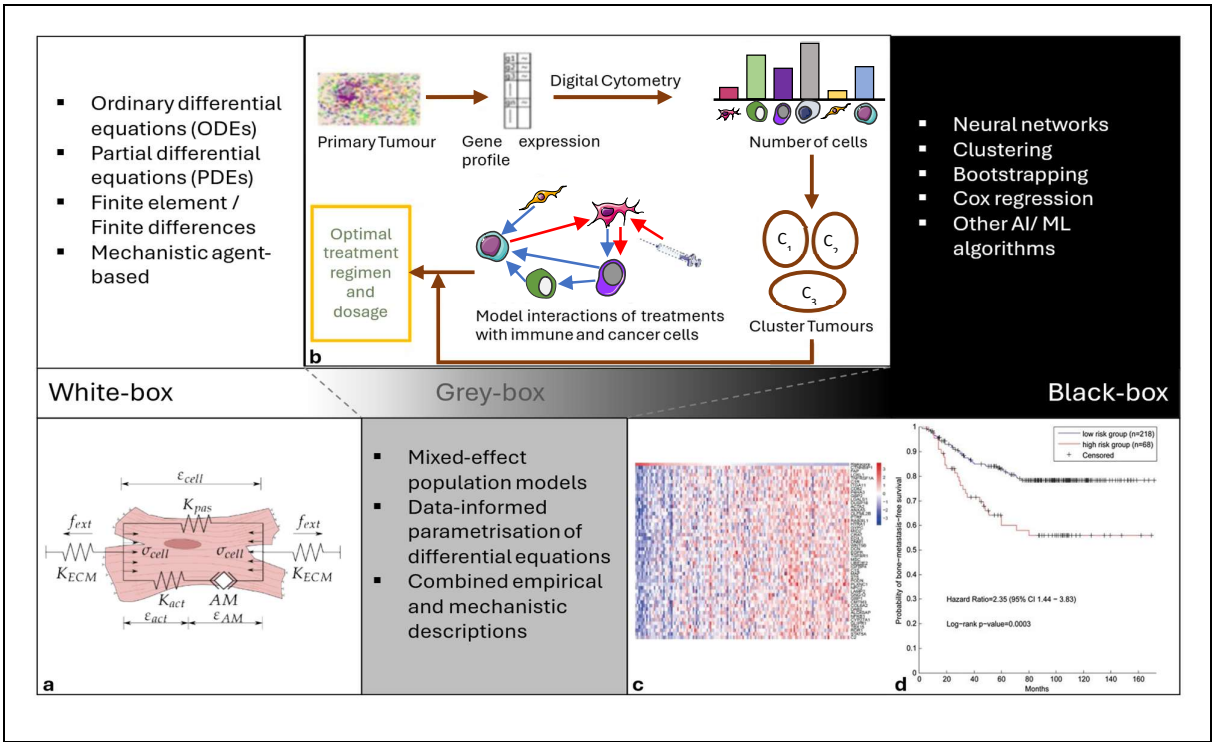
Referee comments	Response	Line number
I suggest the authors consider restructuring the review into two more coherent thematic sections—or even two separate reviews—depending on their intended scope: 1. A mechanistically grounded review of osteosarcoma biology and the models used to recapitulate its features, including 2D, 3D, animal models, and emerging platforms. 2. A distinct review focused on therapeutic development, integrating clinical data, drug-delivery advances, and novel systemic or cell-based interventions and the relevance of in silico approaches to explain results of clinical approaches or clinical data.	We thank the reviewer for this helpful suggestion. We agree that the manuscript is best structured as a mechanistically grounded review of osteosarcoma biology and the models used to recapitulate it, rather than as a separate therapeutic development review. In the revised manuscript, we have therefore strengthened the biological background (following your 2nd and 3rd comment), expanded the discussion of in vitro modelling, and improved the connection between model systems and translational/clinical relevance, including examples linked to clinical trial and preclinical contexts in response to you 4th, 6th, and 7th comments.	
INTRODUCTION The introduction provides useful background on osteosarcoma and bone biology; however, several areas would benefit from clarification to improve mechanistic coherence. At present, the text intermixes epidemiology, genetic predisposition, and bone homeostasis without clearly articulating how disruptions in normal osteoblast–osteoclast signalling contributes to osteosarcoma initiation and progression. As written, the transitions—from germline mutations to the RANK/RANKL/OPG axis, and subsequently to PTH/PTHrP-mediated osteolysis—feel disjointed. It is also unclear whether the described TGF-β/PTHrP "vicious cycle" refers specifically to intrinsic osteosarcoma biology or more broadly to metastatic bone disease mechanisms.	We thank the reviewer for this insightful comment and for highlighting the need for improved mechanistic coherence in the introduction. In the revised manuscript, we have carefully restructured and clarified this section to better articulate how disruptions in osteoblast–osteoclast signalling and bone homeostasis contribute to osteosarcoma initiation and progression. Specifically, we have strengthened the transitions between bone remodelling pathways by clarifying that, in addition to the canonical RANK/RANKL/OPG axis, tumour-associated cytokines and signalling cues can further amplify osteoclast activation and reinforce a tumour-supportive bone remodelling environment. Line 151-153 We have also expanded the description of osteosarcoma initiation mechanisms by explicitly linking malignant transformation to disrupted osteogenic differentiation of osteoblast-lineage cells, and by highlighting the role of key regulators such as p53 and RUNX2 in this process. Furthermore, we have improved the explanation of how the bone microenvironment contributes to disease progression through reciprocal crosstalk between tumour cells, MSCs, osteoblasts, osteoclasts, and immune cells, mediated	151-153 163-184 154-155 182-184

	by signalling factors including SDF-1, MIF, TGF-β, and the RANKL/M-CSF axis. Line 163-181 In addition, we have clarified that the TGF-β/PTHrP "vicious cycle" is not limited to osteosarcoma alone but represents a broader mechanism involved in tumour–bone microenvironment interactions that are relevant in metastatic bone disease contexts. Line 154-155 Finally, we added a scope statement indicating that the biological mechanisms presented are selected to support the rationale for the treatment and modelling approaches discussed in this review, while a more comprehensive overview of osteosarcoma biology is available in the existing literature.line 182-184	
To strengthen the introduction, the authors should provide a more direct and integrated explanation of how impaired osteoblast differentiation, dysregulated bone remodelling, and key microenvironmental signalling pathways (e.g., RANKL, TGF-β, integrin αvβ3) contribute to osteosarcoma pathogenesis. Recent comprehensive reviews that outline these mechanisms clearly and may help refine the introduction include de Azevedo JWV, de Medeiros Fernandes TAA, Fernandes JV Jr, de Azevedo JCV, Lanza DCF, Bezerra CM, Andrade VS, de Araújo JMG, Fernandes JV. Biology and pathogenesis of human osteosarcoma. Oncol Lett. 2020.	We thank the reviewer for this useful suggestion and for highlighting the review by de Azevedo et al. (Oncol Lett., 2020), which we agree provides a clear and comprehensive overview of osteosarcoma biology and pathogenesis. We have incorporated this reference in the revised manuscript at different occasions: line 165, 171, and 181.	**171-181**
If the intention is to keep these topics within a single review, the authors must more clearly explain how model systems (particularly 3D models) inform translational research, bridge mechanistic understanding to clinical trial design, and enable preclinical testing of candidate therapies. At present, the linkage between the biological/clinical narrative and the modelling section feels incomplete.	We thank the reviewer for this important suggestion. In the revised manuscript, we have strengthened the translational link between the biological/clinical narrative and the modelling section. First, we added an introductory statement to emphasize that these model systems are not only useful for understanding osteosarcoma biology, but are also increasingly applied in clinically relevant settings (Lines 296–301). In addition, for each 3D in vitro model discussed in the revised manuscript, we included at least one representative example showing its translational or clinical	296–301 380–387 425–433 451–460 462–476 527–534 535–549

	relevance. Specifically, we highlighted examples of clinical use (clinical trials only available for organoids) or preclinical studies demonstrating how these models can better reproduce treatment responses and support therapeutic evaluation. This was done for spheroids (Lines 380–387), organoids (Lines 425–433), scaffold-based models (Lines 451–460 and 462–476), and organ-on-a-chip systems (Lines 527–534 and line 535–549). Overall, these additions were made to better underscore the relevance of 3D models for preclinical testing, mechanistic understanding, and potential clinical application.	
It would benefit from a clinical-trial–oriented analysis. Specifically: Identify which therapeutic strategies are currently being evaluated in clinical trials and specify their trial phase; Provide context on how preclinical evidence has translated (or has not translated) into clinical investigation.Relevant references include: Kortam et al., Commun Mater 2024 — overview of drug-delivery strategies. Zhra et al., Pharmaceutics 2025 — detailed summary of ongoing/complete osteosarcoma clinical trials. Kansara et al., Nat Rev Cancer 2014 — foundational translational biology insights. The authors may also consider including recent work on cell-based therapies, nanoparticle platforms, and hybrid approaches (e.g., Martella et al., Adv Therap 2023; Martella et al., Pharmaceutics 2022). This additional clinical context would make Table 2 far more useful to readers and better integrated with the manuscript's translational emphasis.	Related to this, the point is indeed interesting; however, the purpose of this table is to highlight the limitations of the currently available treatments and why they remain insufficient to adequately address the disease. The advanced therapeutic approaches are subsequently discussed in Section 1.1. The main objective of this review is to identify the existing challenges and the current gaps in reproducing and understanding the disease microenvironment, thereby supporting the development of more physiologically relevant models and, ultimately, more effective and targeted treatment strategies.	
3D IN VITRO MODELS - The manuscript would benefit from a clearer explanation of how 3D in vitro models (spheroids, organoids, scaffold-based systems) enable testing of innovative therapies. For example: • How do 3D models recapitulate gradients of hypoxia, mineralization, and proliferative heterogeneity characteristic of osteosarcoma lesions?	We thank the reviewer for this important suggestion. To better highlight the translational relevance of the modelling platforms, we have strengthened the revised manuscript in several ways. First, the ability of spheroids and organoids to recapitulate hypoxic gradients and proliferative heterogeneity is now more explicitly discussed in the introductory paragraphs of the corresponding sections (Lines 351–361 and 398–406).	351–361 398–406 437–438 462 453 413–424 535–549 380–387 425–433 451–460 462–476 527–534

• How might specific models be suited for evaluating immunotherapies, nanoparticles, or cell-based constructs? • How can these systems streamline translation by better predicting clinical outcomes compared to 2D systems? Explicitly articulating these links would unify the sections and highlight the translational relevance of the modelling platforms.	In addition, we expanded the scaffold-based model section to clarify how these systems can be used to mimic osteosarcoma mineralization (Line 437–438), including examples where the scaffold itself is composed of a mineral phase such as β-TCP (Line 462) and where biomineralization was used to mineralize collagen-based scaffolds (Line 453). Regarding immunotherapy screening, we agree this is an important point and have therefore added two recent examples of osteosarcoma 3D models incorporating immune components for evaluating immunotherapeutic approaches: one organoid-based model (Lines 413–424) and one scaffold-based organ-on-a-chip model (Lines 535–549). We also note that nanoparticle testing has been reported across several 3D model types, and therefore we did not emphasize this as a feature unique to any single platform. Finally, to better address the translational aspect, we included at least one representative examples for each 3D model type showing how these systems more closely reproduce osteosarcoma tissue biology than 2D culture and may serve as screening platforms for candidate therapies. This includes spheroids (Lines 380–387), organoids (Lines 425–433), scaffold-based models (Lines 451–460 and 462–476), and organ-on-a-chip systems (Lines 527–534 and 535–549). Collectively, these additions were made to better underscore the value of 3D models for mechanistic studies, preclinical testing, and potential clinical translation.	535–549
TERMINOLOGY – A glossary or legend would be beneficial, especially to clarify distinctions between: • Spheroids: Simple, self-assembled 3D aggregates—typically tumour-cell–only—used to model gradients of oxygen, nutrients, and drug penetration. • Organoids: More complex, stem-cell–derived or patient-derived 3D structures capable of partial self-organization and recapitulating some tissue architecture.	We thank the reviewer for this helpful suggestion. In response, we have added a dedicated glossary in the revised manuscript (line 308), providing clear definitions of spheroids, organoids, and scaffold-based 3D in vitro models. In addition, we have indicated that due to the complexity of the osteosarcoma it's challenging, and each model can replicate an aspect of the tumour (line 293–296). In the revised manuscript, we have further clarified within the main text the specific	307 293–296 356–360 407–411 489–491

• Scaffold-based 3D models: Systems incorporating biomaterials (hydrogels, ceramics, collagen, synthetic scaffolds) to mimic extracellular matrix properties, stiffness, and mineralization. These distinctions matter because each model type recapitulates different aspects of osteosarcoma biology: • Spheroids may better reproduce proliferative gradients and hypoxic cores—thus relevant to modelling poorly mineralized, high-cellularity tumour regions. • Scaffold-based systems can more effectively model mineralized components of osteosarcoma lesions. • Organoids may capture tumour heterogeneity, patient-specific architecture, and stem-like subpopulations.	biological aspects recapitulated by each model type. In particular, the features reproduced by spheroids are described in line 356–360, those of organoids in line 407–411, and those of scaffold-based systems in line 489–491. These additions aim to improve clarity and ensure consistent terminology throughout the manuscript.	
The manuscript should correlate each model type with the specific tumour characteristics described by the authors (e.g., line 269: “heterogeneous cellular distribution… highly mineralized vs poorly mineralized regions”). This would significantly strengthen the rationale for choosing and developing particular 3D approaches. -	We appreciate this valuable suggestion. However, in this section, our intention was to discuss osteosarcoma as a heterogeneous disease entity rather than focusing on specific osteosarcoma subtypes. The characteristics mentioned, such as heterogeneous cellular distribution and differences in mineralisation, are general hallmarks commonly observed within the osteosarcoma tumor microenvironment. Therefore, the objective was to highlight the overall complexity and heterogeneity of the disease that current models must recapitulate, rather than to directly associate individual features with specific tumour subtypes. Nevertheless, we agree that establishing a clearer connection between these pathological characteristics and the capabilities of different 3D model approaches could further strengthen the discussion, and this aspect has been clarified in the revised manuscript.	
FIGURE 3 It requires improvement. At present, it is difficult to read due to small font size and appears visually inconsistent, as if composed from pasted images rather than a unified schematic. A clearer, purpose-built schematic would be more appropriate.	We thank the reviewer for this valuable comment. The quality of the graphics has been improved to ensure better clarity and readability.	Figure 3

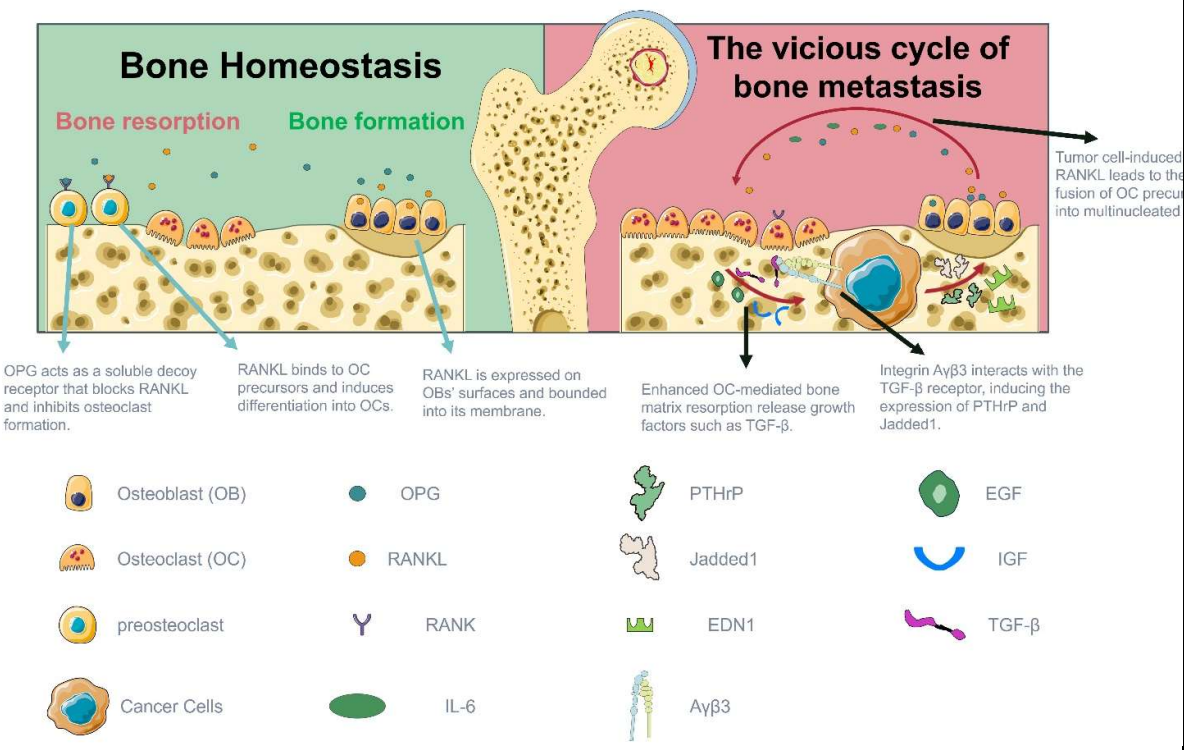


Point-by-point response to the <u>referees 2</u> comments highlighted in Yellow in the manuscript

Referee comments	Response	Line number
1. Bridging in vitro and in silico This review lacks a connection between in vitro and in silico. A review by definition is meant to review the current state of art in the described field, but owing to the chosen topic, it can also help researchers from both sides, in vitro and in silico, by drawing a connection between these two approaches. What cells and cell-cell interactions are being modelled computationally, and what are being co-cultured in vitro? Is there a way of standardizing existing in vitro models in a way that would aid computational modelling and vice versa? If there are certain parameters modelled and tested in silico, can the authors propose, from their perspective, what components are current in vitro models lacking to become an instrument of validation for the computationally derived data? And what data derived computationally can be used in the design of in vitro models of osteosarcoma, and how? Answering	We thank the reviewer for this insightful and constructive comment. We agree that strengthening the connection between the in vitro and in silico aspects of the review was important, and we have revised the manuscript accordingly. Specifically, we added a new section discussing which cells and cell–cell interactions are modelled computationally and which are co-cultured in vitro, as suggested by the reviewer (Lines 592–617). In addition, we included a discussion on the potential for standardization of in silico and in vitro models to better support computational modelling, which we also highlight as a current limitation in the field (Lines 762–789). In addition to highlighting key requirements for standardizing in vitro models to enable their use for	592–617 762–789 804–812 731–744 790–826

these questions would be beneficial for the researchers in the field and would elevate the level of this manuscript.	computational parameterization (Lines 804–812). Regarding the final point, rather than explicitly defining missing components in current in vitro models, we have expanded the discussion to focus on how in vitro and in silico approaches can be more effectively integrated, particularly in the context of iterative model refinement and data exchange (Lines 731–744). Additionally, we have added a dedicated section at the end of Section 3.4 explicitly addressing the integration between in vitro and in silico approaches (Lines 790–826). We believe this perspective provides a practical framework to bridge the two approaches and enhances the overall impact of the manuscript.	
Figure 1 - First of all, it might be beneficial for the reader to see the normal physiological cues happening in bone remodelling (left part of the figure, “Bone homeostasis” bubble). The illustration of those ques should not be too detailed – would be sufficient to at least represent interactions described in the Introduction (“Osteoblasts regulate osteoclast differentiation through the RANK/RANKL/OPG axis: RANKL expressed on their surface binds to RANK on osteoclast precursors to induce differentiation, while osteoprotegerin (OPG) acts as a soluble decoy receptor that blocks RANKL and inhibits osteoclast formation…”)	We thank the reviewer for this helpful suggestion. In response, we have revised the left panel of the figure (“Bone homeostasis”) to more clearly illustrate the key physiological cues involved in bone remodelling, while maintaining a simplified and accessible visual representation. The updated schematic now includes rounded osteoclast precursor cells, which remain in an undifferentiated state when RANK is free or bound by OPG, reflecting OPG´s inhibitory role. In contrast, when RANK binds to RANKL, these precursor cells are shown as having differentiated into more flattened, multinucleated osteoclasts, representing their mature and active form. Additionally, we have redesigned the figure keeping the same scientific content following the suggestion of the editor comments.	162
Figure 1 - The abbreviations for Osteoblast and Osteoclast (OB and OC) can be moved to the legend of the figure to not overcrowd the image, and the cells in the image can be renamed OB and OC instead of full names.	We thank the reviewer for this suggestion. We have revised the figure accordingly by moving the definitions of the abbreviations for osteoblast and osteoclast (OB and OC) to the figure legend. Additionally, the labels within the figure have been simplified to use the abbreviations OB and OC instead	162

	of the full terms. This change reduces visual clutter and improves the overall clarity of the illustration, as recommended.	
Figure 1 - The representation of the interaction of integrin with TGF-beta is misleading. It looks like both of them are sitting on the membrane of the cancer cell.	We thank the reviewer for pointing this out. We have revised the figure to improve the accuracy of this interaction. Specifically, TGF-β has been repositioned to the extracellular space outside the cancer cell membrane, while integrin remains embedded in the cell membrane. This adjustment clarifies their spatial relationship and avoids the misleading impression that both molecules are membrane-bound.	162
Figure 1 - Is it accurate to number the stages of the vicious cycle? When the stages are numbered, one may think that the cycle starts with interactions of integrin with TGFbeta, while the trigger point of the vicious cycle is still an "egg or chicken" question. One of the possible ways to avoid numbering is to bring the captions of the stages closer to the arrows. To give space for the captions, the naming of the proteins and factors can be omitted since they are present in the legend below.	We thank the reviewer for this insightful comment. In response, we have removed the explicit numbering of the stages in the figure to avoid suggesting a defined starting point in the vicious cycle. Instead, we have incorporated descriptive labels alongside arrows indicating where the different processes occur. Additionally, to reduce visual clutter and improve readability, the number of depicted proteins and growth factors has been reduced, as suggested.	162
Figure 1 -To correspond with the text in the introduction, the authors may consider illustrating both membrane-bound (on the OBs' surface) and soluble RANKL (in the present version of the manuscript, the text describes mebrane-bound RANKL, and the figure contains only soluble RANKL.	We thank the reviewer for this valuable suggestion. We have revised the figure accordingly to better align with the description provided in the Introduction by explicitly representing both forms of RANKL. In the updated figure, membrane-bound RANKL is depicted on the surface of osteoblasts (OBs), illustrating its cell-associated form, while soluble RANKL is shown diffusing freely within the extracellular environment. Both forms are also clearly distinguished through appropriate captions, ensuring accurate representation and improved clarity.	162
Figure 1 - Some captions should be rewritten if kept: "Metastatic cancer cells" can be rewritten as "Cancer cells", since only a single cell is depicted and there is no illustration of or connection to metastasis in the figure itself, even if metastasis is mentioned in the text. Arrow pointing at the growth factors has a mistake in caption: i.e., not ie.	We thank the reviewer for this helpful suggestion. In response, we have revised the figure captions accordingly. The label "metastatic cancer cells" has been changed to "cancer cell" to better reflect the single-cell representation and avoid implying metastatic processes within the figure.	162

Caption for stage 3 of the cycle mentions “cytokines”. It is better to use the same wording – either “growth factors” or “cytokines”, not both.	Additionally, the “ie.” mistake has been removed. Furthermore, we have ensured consistency in terminology by replacing all references to “cytokines” with “growth factors” throughout the figure captions, in line with the terminology used in the manuscript.	
 Figure 1 (Updated) - Bone homeostasis and dysregulation of bone homeostasis		
Figure 2 - It is not correct to say “2D cell line”. This should be changed to “2D cell culture” (because co-cultures and primary cells also can be cultured in 2D, and vice versa, cell lines can be present in spheroids and organoids)	We thank the reviewer for this helpful suggestion. We have revised the terminology accordingly by replacing “2D cell line” with “2D cell culture” throughout the figure/captions, as suggested, to more accurately reflect the experimental context and avoid ambiguity.	582
Figure 2 - To be consistent with the naming of classes of in vitro cultures, the second row, “Spheroids and organoids”, can be renamed as “3D cell cultures” and then listed as “Spheroids, organoids, and tumoroids” (the term “tumoroids” has been used in the text of the manuscript).	We thank the reviewer for this suggestion. In response, we have revised the figure to improve consistency in terminology. Specifically, the label has been updated to “3D cell cultures (Spheroids, organoids, and tumoroids)”, as shown in the revised	582

	figure, aligning with the nomenclature used throughout the manuscript.	
Figure 2 - "Scaffold-based biomaterials" should sound as "Biomaterial-based scaffolds". But overall, this approach would be incorrect. To stay coherent with the text, the authors may use "Biomaterial scaffold-based cultures/scaffold-based in vitro models".	We thank the reviewer for this suggestion. We have revised the figure label accordingly to improve coherence with the manuscript terminology. The caption has been updated to "Biomaterial scaffold-based cultures," as shown in the revised figure, to better reflect the intended meaning and align with the text.	582
Figure 2 - The color of the "Challenges" block should be changed to a lighter one to increase the contrast between the text and the background and therefore enhance readability for those who won't have access to color	We thank the reviewer for this valuable suggestion. We have updated the color of the "Challenges" block to a lighter shade of the same color, which improves the contrast between the text and the background and enhances readability, including for readers without access to color.	582
Figure 2 - For the same sake of readability, minor aesthetic details should not be overlooked: the indentation of the points in the lower bottom block differs from the other ones; there is an extra space before "3D cellular organization"; the number of colors should be minimized and picked thoughtfully to enhance the experience of color-blind readers.	We thank the reviewer for this thoughtful observation. We have corrected the indentation of the points in the lower block to ensure consistency with the other sections and removed the extra space before "3D cellular organization." Additionally, we have revised the color scheme by changing all icons to different tones, minimizing the number of colors used and improving accessibility for color-blind readers while enhancing overall readability.	582
Figure 2 - The content of the figure should better reflect the content of the manuscript text. For example, the authors mention several times that "compactness" of the cell mass is an advantage of working with 3D cultures. Thus, this advantage should be mentioned in the block with 3D cultures of the figure (and, maybe, in the block of challenges that are met in work with 2D cultures)	We thank the reviewer for this insightful suggestion. We have revised the figure to better align with the manuscript content by explicitly incorporating the concept of compactness. Specifically, we added "lack of compact 3D cell organization" to the challenges block and included "3D cellular distribution, organization, and compactness" in the advantages block for 3D cultures, thereby improving the consistency between the figure and the text.	582
Figure 2 - The authors are advised to check the wording (no need to use different words for saying the same, for example, "Useful for personalized medicine studies" and "Personalized medicine study's application") and possible writing mistakes (for example,	We thank the reviewer for this helpful suggestion. We carefully reviewed the wording throughout the manuscript to improve consistency and clarity, avoiding unnecessary variation in phrasing. In particular, we standardized the expression to "suitable for	582

"time-consuming handling are is required"	personalized medicine studies." We also corrected the indicated phrase to "time-consuming handling and specialized expertise required," along with other minor writing and grammatical issues identified during the revision.	

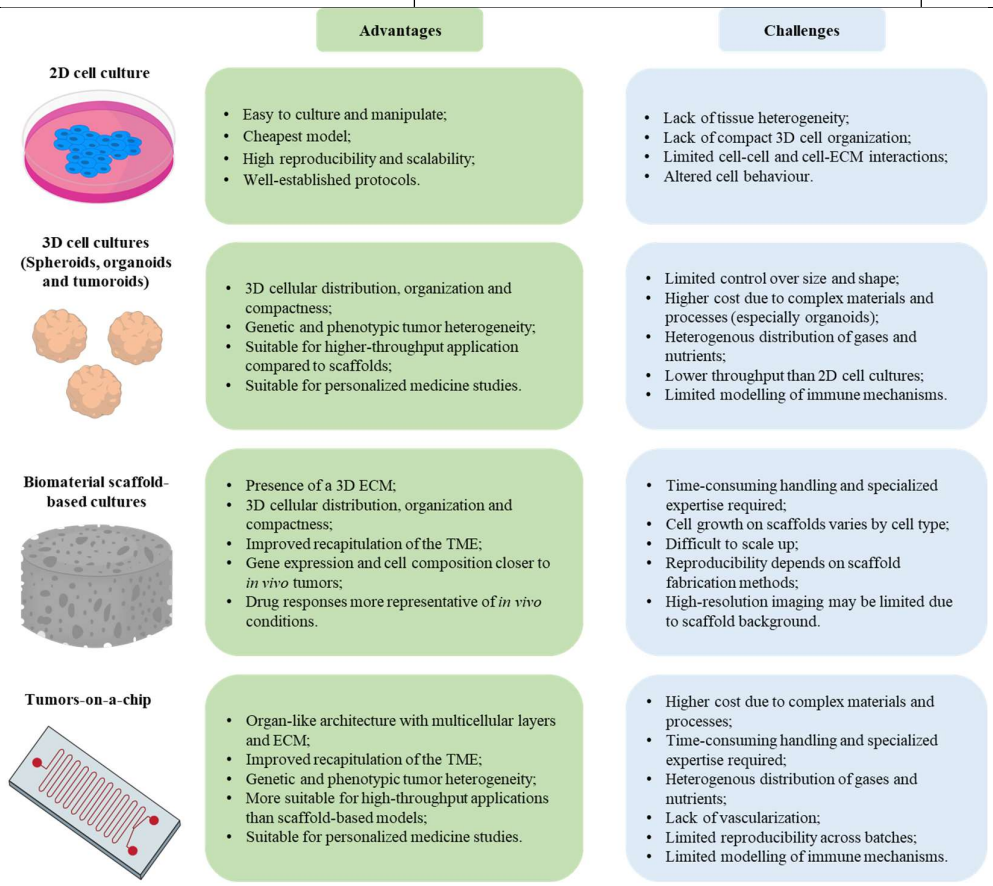


Figure 2 (Updated) - A summary of the advantages and the challenges of different in vitro models that used to study OS

Unclear/possibly incorrect statements; some examples: "Current OS treatments, …, but for metastatic cases, particularly lung or bone metastases, this type of therapy remains limited" – what is the percentage of OS undergoing lung metastasis and bone metastasis? How exactly does bone-to-bone metastasis occur?	We now state that OS metastasizes most commonly to the lungs, while bone and lymph node involvement are less frequent. We also included quantitative data indicating that metastatic cases consist of 74% lung-only metastases, 9% bone-only metastases, and 8% with both. Furthermore, we expanded the description of bone-to-bone metastasis to explain its progression via hematogenous spread, including tumor cell survival mechanisms (e.g., resistance to anoikis), chemokine-mediated homing to the bone microenvironment, and interactions with mesenchymal stem cells and	187-199

	osteoclasts that promote tumor progression.	
Unclear/possibly incorrect statements; some examples: Line 274: organ-on-a-chip models, as well as bioreactors, can be characterized by chamber/channel/port dimensions exceeding microfluidics parameters. With this consideration, the authors may change the classification of OS in vitro models.	We thank the reviewer for this important observation. We agree that organ-on-a-chip systems and bioreactors may involve structural features that extend beyond the classical definition of microfluidic systems. To avoid potential ambiguity, we have revised the classification of in vitro osteosarcoma models to focus specifically on organ-on-a-chip platforms, rather than broadly grouping them under microfluidic systems line 304, and 499-501.	304 499-501
Unclear/possibly incorrect statements; some examples: Could the answer provide the dimension range of the spheroids enabling the development of a necrotic core? "This leads to the formation of distinct cellular zones, including a proliferative outer layer, a quiescent middle layer, and a necrotic core, thereby reflecting the heterogeneity of solid tumours."	We thank the reviewer for this helpful suggestion. We have revised the manuscript to include the typical size range of spheroids associated with the formation of a necrotic core (Line 357).	357
Unclear/possibly incorrect statements; some examples: "Physics-based models often focus on biophysical processes such as the diffusion of nutrients and chemicals, mechanical stresses on tumours and cells, and fluid flow in the circulatory system 121,122. Further mathematical models explore therapeutic interventions, drug delivery, and interactions between cancer and the immune system." – What is meant by "further mathematical models"? Are these separately developed models, or mathematical modelling performed on the basis of physics-based models?	We thank the reviewer for this clarification request. We have revised the wording to improve precision and avoid ambiguity. Specifically, we replaced "Further mathematical models" with "These mathematical models," making it clear that the same physics-based modelling frameworks are being extended to explore therapeutic interventions, drug delivery, and cancer–immune system interactions, rather than referring to a separate category of models.	631
Minor Concerns: 1- Typos, wording, and various writing mistakes; some examples: 1 • Introduction, line 195: "uses viral vectors to modify a patient's T-cells" 2 • Line 203: "toxin that kills the cells" 3 • Line 292: "using the chemical agent N-methyl-N-nitro-Nnitroguanidine (MNNG), the MNNG-HOS cell line was established" 4 • Line 302: "and efforts have been made to characterize them in terms of differentiation capacity" - - - - - - - - -	We thank the reviewer for carefully identifying the typographical errors. All indicated typos have been corrected throughout the manuscript. For clarity, please find each comment number alongside the corresponding corrections line numbers. (1 line 217), (2 line 225), (3 line 324), (4 line 334), (5 line 337), (6 line 370), (7 line 378), (8 line 438), (9 line 442), (10 line 446), (11 line 484), (12 line 509), (13 figure 3)	217 225 324 334 337 370 378 438 442 446 484 509 figure 3

5 • Line 305: “First, the culture and expansion of 2D cell lines results in genetic homogeneity” 6 • Line 334: wording “OS cell spheroids” 7 • Line 339: unclear sentence “It was shown that 3D spheroids of U2-OS and SaOS-2, and 2D doxorubicin-resistant sublines, had higher levels of ABCB1 transporter, which is involved in doxorubicin efflux, compared to the parental cell lines, thus impairing chemoresistance to drugs.” What is meant by “parental cell lines”? U2-OS and SaOS 2 grown in standard 2D conditions? 8 • Line 361: wording in “Both natural and synthetic-based biomaterial scaffolds” 9 • Line 363: the last part of the sentence does not seem to correspond with the other parts and is unclear “Specifically, collagen and hydroxyapatite-based scaffolds are among the most used biomaterials for OS 3D cell cultures, given their natural presence in the in vivo tissue, and to embedded the in vivo tissue within a physiological like microenvironment.” 10 • Line 369: “The 3D matrix was supplemented with the bone-marrow proteins laminin and fibronectin, and NuOss® bone granules.” (or rephrase, not to have NuOss mixed with bone proteins) 11• Line 407: “Inkjet bioprinting, offers” 12• Line 430: “preferred tissue for bioengineering of highly hierarchical bioengineered constructs” 13• In Figure 3, there is mixed spelling (“tumor” and “tumour”, and “gray” while in the text the authors use “grey”). Extra parentheses in the caption.		
Missing abbreviation explanations/misused abbreviations; some examples: 1- Overall, the transcription of abbreviations is not standardized throughout the text. For example, the authors give explanations to OPG, PTH, and PTHrP at the beginning of the introduction, but omit those for PD-1,	We thank the reviewer for this important observation and fully agree that consistent and clear use of abbreviations is essential for readability. Accordingly, we have carefully revised the manuscript to standardize the use of abbreviations throughout. Specifically, we have (i) ensured that all abbreviations are properly defined upon first use, (ii)	example: 265 443 489 520

CTLA-4, etc. Abbreviations should be either set in the text or given as a list at the beginning of the manuscript. 2- The authors are kindly advised to stay consistent with the use of abbreviations and to double-check that once an abbreviation is introduced, it is used (for example, “osteosarcoma” is not abbreviated in lines 412 and 481. 3- There is no need for creation abbreviations if they will no longer be used further in text (for example, “dOsEM” in line 450, “OOC” in line 453 – and the latter is even confusing because it repeats the OoC abbreviation but is used for a different term).	maintained consistent usage once introduced, and (iii) removed unnecessary or redundant abbreviations that are not used subsequently in the text. In addition, we have included a comprehensive list of abbreviations in the manuscript for clarity. Given the extent of these revisions, it is not practical to indicate all individual line changes; however, all modifications have been highlighted in yellow in the revised manuscript. For transparency, we provide below a few representative examples of the corrections made. lines: 265, 443,489, and 520	
Missing abbreviation explanations/misused abbreviations; some examples: Introduction, line 226: L-MTP-PE should be transcribed even if later on in the text the authors explain the function of the drug.	We thank the reviewer for this helpful suggestion and agree with this point. The abbreviation has now been fully transcribed at its first occurrence in the Introduction (Line 249), in accordance with standard practice.	249
Missing abbreviation explanations/misused abbreviations: some examples: In Figure 3, AI is abbreviated, while ML is not.	We thank the reviewer for this observation. In Figure 3, both AI and ML are now shown as abbreviations due to space limitations; however, both terms are fully transcribed and defined in the main text and in the abbreviations list to ensure clarity. Additionally we improved the resolution of the used graphics to improve the clarity.	Figure 3

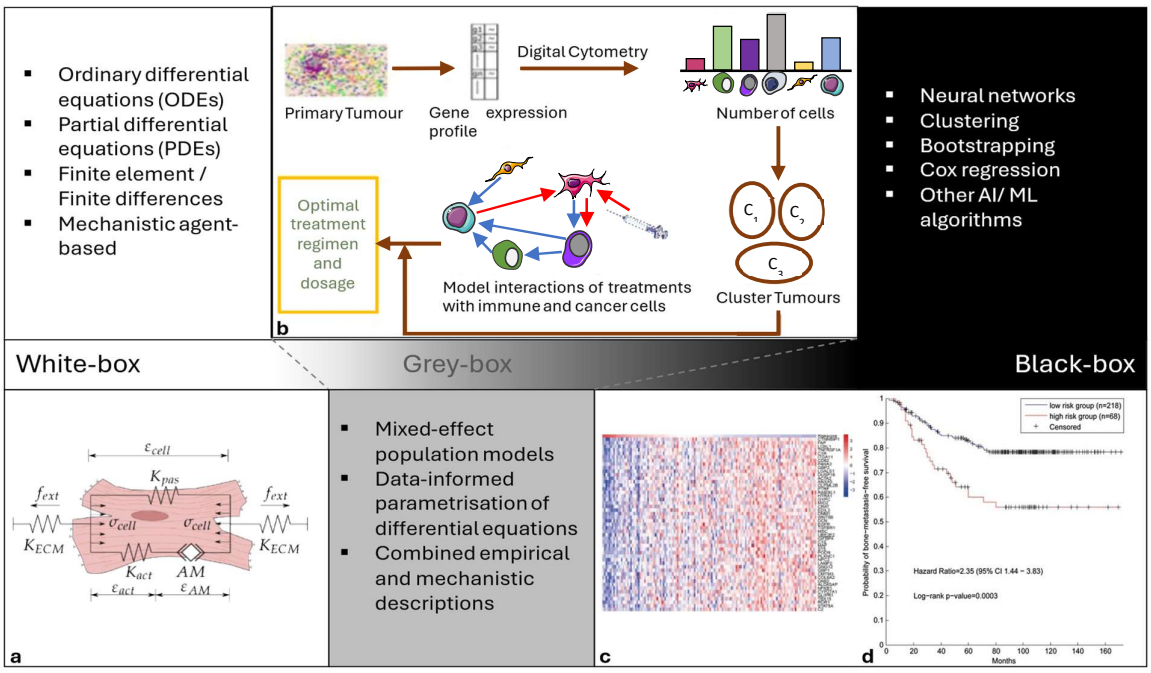


Figure 3 (Updated) - Collection of typical computational modelling approaches spanning the full white-to-black-box spectrum with illustrative applications in bone malignancies. a) Mechanical model of a single cell allowing computation of internal stress $\sigma cell$ due to contraction of actin–myosin filaments and cell membrane and cytoskeleton resistance. Used in the study of the interactions of myeloma cells with resident BM cells. b) Schematic pipeline used by Le and colleagues to simulate OS response to chemotherapy combining data-informed composition of tumour and ODE description of intercellular interactions. c) Heatmap of the top 50 negative correlation genes with the ML-derived prognostic signature risk score for OS outcome. d) Kaplan-Meier curves of bone metastasis-free survival for defined risk groups amongst breast cancer patients

Missing abbreviation explanations/misused abbreviations: Line 544: RSF (random survival forest)	We thank the reviewer for this suggestion. The term has been corrected to “random survival forest”.	675

Point-by-point response to the referees 4 comments highlighted in Green in the manuscript

- The computational section would strongly benefit from a concise table summarizing model inputs, outputs, and their clinical or other translational relevance.	We completely agree. We have therefore added Table 3 (line 716), which concisely summarizes all in silico models discussed in the manuscript, including their classification, main inputs, main outputs, and translational relevance.	716
- In its current form, the computational section represents more of a narrative perspective than a structured review; explicitly educating the reader on how the models connect, or fail to connect, would substantially strengthen this part.	We appreciate this suggestion. To improve the structure and readability of this section, we expanded the discussion to clarify how in silico models can be integrated with in vitro systems (lines 731–744 (in yellow) and 790–826 (in blue)).	731–744 790–826 762–789

	In addition, we added a discussion of standardization efforts and how they may facilitate interoperability and integration among in silico models themselves (lines 762–789) (In yellow).	
- The concept of grey-box models is well motivated and represents a strong idea; however, its execution is not fully convincing. For example, ODE-based models are listed under white-box models in Figure 3, yet the grey-box article section (3.3) relies heavily on ODE models. This inconsistency should be addressed, and the borders and/or overlaps between these model categories should be more clearly defined.	We thank the reviewer for this important observation. We have revised the text to clarify that grey-box models are hybrid frameworks that combine mechanistic, white-box formulations with data-driven components. Accordingly, ODEs may appear in both categories depending on how they are used: as purely mechanistic models in the white-box section, or as part of hybrid frameworks in the grey-box section when they are calibrated or parameterized using empirical data. We also clarified the boundaries and overlaps among the three categories in the revised manuscript (Line 688-692).	688-692
- Introducing, early in the black-box section (after 1st paragraph), a brief rationale for why AI approaches are particularly suitable in this context would improve clarity.	We strongly agree. To improve clarity, we first added a short introductory paragraph at the end of the in vitro section to explain the need for in silico modelling (lines 571–578) in blue. We then added an introductory paragraph at the beginning of the in silico section describing what can be captured by these models and why they are relevant in this context (lines 592–617).	571–578 592-617
- The manuscript briefly summarizes several AI approaches without sufficient explanation of why these tools were chosen or what specific problems they address; the proposed summary table would help resolve this issues in the challenges section e.g. standardation .	We agree. We addressed this by adding Table 3 (line 716), which summarizes the role of the different in silico approaches in a concise and structured manner. We also expanded the challenges section to discuss key limitations, including standardization, in greater detail (lines 762–826).	716 762-826
- It is unclear why citation 147 (Rejniak et al.) appears under the “Challenges and Future Directions” subsection, as its placement with respect to the white/grey/black-box framework is not well defined.	We thank the reviewer for highlighting this point. We added this citation as an example of a multi-modal grey-box framework that bridges molecular, cellular, and tissue-level processes and may support therapeutic decision-making in osteosarcoma.	745
- The literature search underlying the computational section appears selective rather than exhaustive; a more comprehensive literature search of relevant computational and hybrid (grey-box) modeling studies would strengthen the review and better reflect the state of the art around OS.	We appreciate this comment. In revising the manuscript, we expanded the computational section to better cover different aspects of the topic, including the classification of in silico models (lines 592–617), the integration between in silico and in vitro modelling (lines 731–744 and 790–826), and ongoing standardization efforts (lines 762–789). We believe these additions improve the breadth and balance of the section.	592–617 731–744 and 790–826 762–789

- Consider whether Section 3.3.1 should be renumbered as Section 3.4 for clarity.	Done.	620
- Finally, I suggest reorganizing Section 3 to follow the order presented in Figure 3, starting with white-box models, then grey-box, and finally black-box models, which would improve narrative flow and make the discussion more engaging.	We appreciate this suggestion. However, we believe that introducing the framework by first defining white-box and black-box models, and then presenting grey-box models as a hybrid of the two, provides a clearer conceptual progression. For this reason, we retained this order while revising the text to better explain the relationships and overlaps among the three categories.	

Summary

This review addresses tissue engineering-based preclinical models and computational modelling strategies. The authors argue that integrating advanced *in vitro* systems with predictive *in silico* models can better capture tumour heterogeneity and enable patient-tailored therapies. A particular strength of the manuscript is its timely overview of mechanistic, data-driven, and hybrid computational approaches.

The topic is of high importance for the fields of osteosarcoma research, tissue engineering, and precision medicine in the context of oncology. However, the manuscript currently lacks a clear conceptual bridge between *in vitro* and *in silico* approaches, as well as structural coherence and clarity in figures and terminology. With stronger integration between experimental and computational frameworks, clearer figure design, and improved consistency and precision in presentation, the paper has the potential to make a significant contribution. The detailed suggestions are listed below.

Major Concerns

1. *Bridging in vitro and in silico*

This review lacks a connection between *in vitro* and *in silico*. A review by definition is meant to review the current state of art in the described field, but owing to the chosen topic, it can also help researchers from both sides, *in vitro* and *in silico*, by drawing a connection between these two approaches. What cells and cell-cell interactions are being modelled computationally, and what are being co-cultured *in vitro*? Is there a way of standardizing existing *in vitro* models in a way that would aid computational modelling and vice versa? If there are certain parameters modelled and tested *in silico*, can the authors propose, from their perspective, what components are current *in vitro* models lacking to become an instrument of validation for the computationally derived data? And what data derived computationally can be used in the design of in vitro models of osteosarcoma, and how? Answering these questions would be beneficial for the researchers in the field and would elevate the level of this manuscript.

2. *Figure 1*

Figure 1 is reprinted from another manuscript; thus, it may be difficult to adapt it by introducing some details or redrawing it from scratch. However, it can be heavily enhanced by addressing the following points:

- First of all, it might be beneficial for the reader to see the normal physiological cues happening in bone remodelling (left part of the figure, “Bone homeostasis” bubble). The illustration of those ques should not be too detailed – would be sufficient to at least represent interactions described in the Introduction (“Osteoblasts regulate osteoclast differentiation through the RANK/RANKL/OPG axis: RANKL expressed on their surface binds to RANK on osteoclast precursors to induce differentiation, while osteoprotegerin (OPG) acts as a soluble decoy receptor that blocks RANKL and inhibits osteoclast formation…”)
- The abbreviations for Osteoblast and Osteoclast (OB and OC) can be moved to the legend of the figure to not overcrowd the image, and the cells in the image can be renamed OB and OC instead of full names.
- The representation of the interaction of integrin with TGF-beta is misleading. It looks like both of them are sitting on the membrane of the cancer cell.

- Is it accurate to number the stages of the vicious cycle? When the stages are numbered, one may think that the cycle starts with interactions of integrin with TGF-beta, while the trigger point of the vicious cycle is still an “egg or chicken” question. One of the possible ways to avoid numbering is to bring the captions of the stages closer to the arrows. To give space for the captions, the naming of the proteins and factors can be omitted since they are present in the legend below.
- To correspond with the text in the introduction, the authors may consider illustrating both membrane-bound (on the OBs’ surface) and soluble RANKL (in the present version of the manuscript, the text describes mebrane-bound RANKL, and the figure contains only soluble RANKL.
- Some captions should be rewritten if kept: “Metastatic cancer cells” can be rewritten as “Cancer cells”, since only a single cell is depicted and there is no illustration of or connection to metastasis in the figure itself, even if metastasis is mentioned in the text. Arrow pointing at the growth factors has a mistake in caption: i.e., not ie. Caption for stage 3 of the cycle mentions “cytokines”. It is better to use the same wording – either “growth factors” or “cytokines”, not both.

3. *Figure 2*

- It is not correct to say “2D cell line”. This should be changed to “2D cell culture” (because co-cultures and primary cells also can be cultured in 2D, and vice versa, cell lines can be present in spheroids and organoids).
- To be consistent with the naming of classes of in vitro cultures, the second row, “Spheroids and organoids”, can be renamed as “3D cell cultures” and then listed as “Spheroids, organoids, and tumoroids” (the term “tumoroids” has been used in the text of the manuscript).
- “Scaffold-based biomaterials” should sound as “Biomaterial-based scaffolds”. But overall, this approach would be incorrect. To stay coherent with the text, the authors may use “Biomaterial scaffold-based cultures/scaffold-based *in vitro* models”.
- The color of the “Challenges” block should be changed to a lighter one to increase the contrast between the text and the background and therefore enhance readability for those who won’t have access to color.
- For the same sake of readability, minor aesthetic details should not be overlooked: the indentation of the points in the lower bottom block differs from the other ones; there is an extra space before “3D cellular organization”; the number of colors should be minimized and picked thoughtfully to enhance the experience of color-blind readers.
- The content of the figure should better reflect the content of the manuscript text. For example, the authors mention several times that “compactness” of the cell mass is an advantage of working with 3D cultures. Thus, this advantage should be mentioned in the block with 3D cultures of the figure (and, maybe, in the block of challenges that are met in work with 2D cultures).
- The authors are advised to check the wording (no need to use different words for saying the same, for example, “Useful for personalized medicine studies” and “Personalized medicine study’s application”) and possible writing mistakes (for example, “time-consuming handling ~~are~~ is required”)

4. *Unclear/possibly incorrect statements; some examples:*

- Line 173: “Current OS treatments, …, but for metastatic cases, particularly lung or bone metastases, this type of therapy remains limited” – what is the percentage of OS

undergoing lung metastasis and bone metastasis? How exactly does bone-to-bone metastasis occur?

- Line 274: organ-on-a-chip models, as well as bioreactors, can be characterized by chamber/channel/port dimensions exceeding microfluidics parameters. With this consideration, the authors may change the classification of OS *in vitro* models.
- Line 324: Could the answer provide the dimension range of the spheroids enabling the development of a necrotic core? "This leads to the formation of distinct cellular zones, including a proliferative outer layer, a quiescent middle layer, and a necrotic core, thereby reflecting the heterogeneity of solid tumours."
- Line 498: "Physics-based models often focus on biophysical processes such as the diffusion of nutrients and chemicals, mechanical stresses on tumours and cells, and fluid flow in the circulatory system 121,122. Further mathematical models explore therapeutic interventions, drug delivery, and interactions between cancer and the immune system." – What is meant by "further mathematical models"? Are these separately developed models, or mathematical modelling performed on the basis of physics-based models?

Minor Concerns

1. *Typos, wording, and various writing mistakes; some examples:*

- Introduction, line 195: "uses viral vectors to modify ~~a~~ patient's T-cells"
- Line 203: "toxin that kills the cells"
- Line 292: "using the chemical agent N-methyl-N-nitro-Nnitroguanidine (MNNG), the MNNG-HOS cell line was established"
- Line 302: "and efforts have been made to characterize them in terms of differentiation capacity"
- Line 305: "First, the culture and expansion of 2D cell lines results in genetic homogeneity"
- Line 334: wording "OS cell spheroids"
- Line 339: unclear sentence "It was shown that 3D spheroids of U2-OS and SaOS-2, and 2D doxorubicin-resistant sublines, had higher levels of ABCB1 transporter, which is involved in doxorubicin efflux, compared to the parental cell lines, thus impairing chemoresistance to drugs." What is meant by "parental cell lines"? U2-OS and SaOS-2 grown in standard 2D conditions?
- Line 361: wording in "Both natural and synthetic-based biomaterial scaffolds"
- Line 363: the last part of the sentence does not seem to correspond with the other parts and is unclear "Specifically, collagen and hydroxyapatite-based scaffolds are among the most used biomaterials for OS 3D cell cultures, given their natural presence in the in vivo tissue, and to embedded the in vivo tissue within a physiological like-microenvironment."
- Line 369: "The 3D matrix was supplemented with the bone-marrow proteins laminin and fibronectin, and NuOss® bone granules." (or rephrase, not to have NuOss mixed with bone proteins)
- Line 407: "Inkjet bioprinting~~,~~ offers"
- Line 430: "preferred tissue for bioengineering of highly hierarchical bioengineered constructs"
- In Figure 3, there is mixed spelling ("tumor" and "tumour", and "gray" while in the text the authors use "grey"). Extra parentheses in the caption.

2. *Missing abbreviation explanations/misused abbreviations; some examples:*

- Overall, the transcription of abbreviations is not standardized throughout the text. For example, the authors give explanations to OPG, PTH, and PTHrP at the beginning of the introduction, but omit those for PD-1, CTLA-4, etc. Abbreviations should be either set in the text or given as a list at the beginning of the manuscript.
- Introduction, line 226: L-MTP-PE should be transcribed even if later on in the text the authors explain the function of the drug.
- The authors are kindly advised to stay consistent with the use of abbreviations and to double-check that once an abbreviation is introduced, it is used (for example, “osteosarcoma” is not abbreviated in lines 412 and 481.
- There is no need for creation abbreviations if they will no longer be used further in text (for example, “dOsEM” in line 450, “OOC” in line 453 – and the latter is even confusing because it repeats the OoC abbreviation but is used for a different term).
- In Figure 3, AI is abbreviated, while ML is not.
- Line 544: RSF (random survival forest)