Author's Response to Peer Reviews

Authors' Response to Peer Reviews of "Localized Immune Cascade Programming in Desmoplastic Tumors: In Silico Modeling and Validation Study"

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This is the authors' response to peer-review reports for "Localized Immune Cascade Programming in Desmoplastic Tumors: In Silico Modeling and Validation Study."

Round 1 Review

Reviewer AH [1]

General Comments

This paper [2] presents a conceptual framework for a sequential, localized immune cascade ("Second Breath") aimed at reprogramming "immune-cold" desmoplastic tumors to enhance immunotherapy responsiveness. It is positioned as a preclinical concept paper, relying entirely on literature synthesis and in silico modeling without generating new experimental data. While the integration of immunology, biochemistry, and systems biology concepts is ambitious and potentially innovative, the work has significant limitations.

Specific Comments

Major Comments

1. *The abstract does not require reference citations. Please remove the citations in the abstract. If reference citations are deemed really necessary, please start with 1 instead of 4. Also, please go on in ascending order instead of skipping references, like [4,5,6,7,32,33].*
2. *The main text instead should be backed up by reference citations to increase its value. However, the same principle also applies to starting from 1 and continuing in ascending order, but not starting in the middle and skipping references. Also, the references need to be cited in a uniform format. The following needs to be revised in the Introduction: "...(ECM) [6,7,32,33].... (ICIs) [13,14,15]....exposure [1,2,4,5,9,10,11].....Therapy for a Sustainable Outcome' (M. Novruzov, 2025),..." The same format of reference citations should be used, thus "(M. Novruzov, 2025)" should be cited as per others: "[n]." Please revise.*

3. *The references are drawn from reputable sources, but the synthesis appears biased toward supportive evidence. For example, citations for bacterial priming (eg, Clostridium novyi-NT) highlight antitumor responses but downplay failures in clinical trials, such as high toxicity or limited efficacy in advanced tumors. Similarly, extracellular matrix (ECM) modulation via collagenase/hyaluronidase is presented positively but ignores biochemical drawbacks like enzyme instability in vivo, nonspecific proteolysis leading to tissue damage, or rebound ECM deposition. It is unclear how the authors reconciled conflicting literature. For example, the Wnt pathway enrichment is noted as dominant, but its protumorigenic role in desmoplastic tumors (eg, promoting fibrosis via β-catenin) is only briefly mentioned in the Discussion without quantitative risk assessment. Please add in a more balanced view and references.*
4. *The framework proposes a 10-stage sequence, gated by biomarkers like interferon (IFN)–γ signature or interstitial fluid pressure reduction. However, this complexity introduces numerous failure points without clear prioritization. Biochemically, the "IL-12 → IFN-γ → TNF-α axis" assumes linear signaling, ignoring feedback loops (eg, tumor necrosis factor α [TNF-α]–induced apoptosis resistance via NF-κB) or crosstalk with immunosuppressive pathways (eg, transforming growth factor β in desmoplastic stroma). The "Warmth Readiness Index (WRI)" is mentioned but undefined quantitatively. How is it calculated? What thresholds were derived from it? This vagueness makes the model hard to falsify, as the "prediction matrix" relies on qualitative outcomes rather than measurable biochemical end points. Please revise and supplement.*
5. *The go/no-go criteria (eg, "≥2-fold fold increase in CXCL9/10/11") are arbitrary; please justify them from literature or simulations.*

Reviewer E [3]

General Comments

In this paper, overall, there is a clear scientific narrative defining "second breath" as a testable conceptual framework for converting immune-cold tumors to a more responsive state. The graphical representation of the model and the concise logical sequence make the manuscript more appealing to read. The Introduction highlights key biological barriers like low T-cell infiltration, dense ECM, and stromal resistance that justify the need for a localized immune therapy. The overall study framework is clearly articulated, well-structured, and presented in a concise and accessible manner. The citations are well integrated and support the key arguments effectively. The abstract is informative, but the functional role of β-catenin/Wnt–linked programs in the cascade requires more clarity. The Results section highlighting Wnt signaling dominance is contradictory to the existing literature and the Discussion section of the manuscript, which requires further supporting evidence with human-specific models.

Specific Comments

Major Comments

1. *Wnt-signaling dominance in the Results section: It is known that APC, CTNNB1, and AXIN1 gene mutations of the canonical Wnt/β-catenin pathway give rise to cancers. Inappropriate activation of the Wnt/β-catenin pathway is believed to be involved in carcinogenesis. Specifically, there are multiple genetic abnormalities involved in the activation of the Wnt/β-catenin pathway; nonetheless, the CTNNB1 mutation is a typical driver mutation that is found in approximately 30% of hepatocellular carcinoma cases. In 2015, Spranger et al [4] reported that the Wnt/β-catenin pathway activation inhibits cytotoxic T-cell infiltration in the immune microenvironment of malignant melanoma, resulting in resistance to immune checkpoint inhibitors. Wnt/β-catenin signaling was shown to inhibit this dendritic cell invasion into the tumor. However, the manuscript has reported dominance of Wnt signaling in the pathway enrichment analysis, which is contradictory. Provide more clarity by offering more predictive insights into human-specific responses. The Discussion raises important safety concerns, and so to strengthen practical relevance, propose any mitigation strategies or alternative approaches.*

Minor Comments

1. *Although it is stated in the manuscript that "Second breath" represents a novel preclinical approach for antitumor immunity, kindly clarify the novelty by elaborating on how the model is better than existing intratumoral immunotherapy strategies (like immunostimulatory antibodies, gene therapy, and combination therapy) while both are aimed at limiting systemic toxicities. A strong statement is needed emphasizing how the model can be translated into a therapeutic approach.*
2. *While the sequential representation in the Materials section is clear and logically structured, the overall writing tends to be overly repetitive and unnecessarily elaborate, which makes it difficult to follow and detracts from readability. I'd suggest streamlining the content of the Materials and Methods section.*

Response: Dear reviewers, thank you for your detailed and valuable comments. The amendments have been made in accordance with your recommendations and, I hope, fully meet your expectations. We have taken all points into account, with the exception of rearranging the list of references; such an adjustment would require rewriting the article from scratch, which is beyond the scope of the current revision. We are open to discussing an alternative option for formatting references that would preserve the structure of the work.

Round 2 Review

We appreciate the opportunity to revise our manuscript and extend our sincere gratitude to both reviewers for their rigorous evaluation and constructive recommendations, which have substantially enhanced the scientific rigor and translational relevance of our work.

Reviewer AH

General Comments

This paper has undergone revisions and looks much better.

Specific Comments

Major Comments

1. *The references do not appear in ascending order upon a citation in the main text. The first citation that showed up was [6-7,32-33], followed by [13-15], and then [1-2,4-5,9-11].*

The authors also addressed this in their rebuttal letter, showing their difficulties in rearranging the list of references, because such an adjustment would require rewriting the article from scratch, which is beyond the scope of the current revision. They are open to discussion on an alternative option for formatting references that would preserve the structure of the work. I think the editorial office or I can help rearrange the reference numbers.

Response: We are grateful for reviewer AH's acknowledgment of the reference formatting challenge. All 53 citations have been manually reordered to ensure a strict ascending numerical sequence corresponding to their first appearance in the main text, from the Introduction section to the Discussion section.

Reviewer E

General Comments

Overall, the authors have made commendable efforts to address the majority of the primary comments in the revised submission. Nonetheless, the Discussion section remains problematic due to conflicting interpretations of the literature and insufficient data to address potential safety concerns.

Specific Comments

Major Comments

1. *Add more references to paragraph 3 of the Discussion section: As the proposed model is entirely in silico and derived from literature synthesis without experimental validation, the Discussion requires greater precision and depth. To enhance its rigor, the author should incorporate additional references that present experimental data or propose concrete mitigation strategies to address the model's limitations and potential toxicities. Moreover, the Discussion would benefit from supporting evidence derived from human-specific models to strengthen the translational relevance of the findings. Provide more clarity by offering more predictive insights into human-specific responses. The Discussion raises important safety concerns and needs to strengthen its practical relevance. The paradox of Wnt enrichment versus fibrosis/immunosuppression is noted but left unresolved.*
2. *The Introduction could be confusing for readers. Earlier in the Introduction, the conceptual framework is proposed to consist of five modules, but later in the text, it expands into a 7-stage framework. This discrepancy could confuse readers. Consider harmonizing the framework (define stages clearly and keep it consistent).*

Response: We have strengthened the Discussion section by incorporating eight additional peer-reviewed references [5-12] that provide experimental validation from patient-derived organoid co-culture systems, murine desmoplastic tumor models, and mechanistic studies of focal adhesion kinase signaling. The apparent paradox of Wnt pathway enrichment has been addressed through a biomarker-stratified adaptive strategy wherein focal adhesion kinase inhibition substitutes for collagenase-based ECM modulation in tumors exhibiting elevated β-catenin nuclear localization. Additionally, we have reconciled the framework nomenclature to consistently describe 10 sequential intervention stages organized within five functional modules throughout the manuscript.

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Abbreviations

ECM: extracellular matrix
INF: interferon
TNF-α: tumor necrosis factor α

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