

In-Depth Focus on INA-6



I am publishing a paper. It's under peer-review and a reviewer addresses the issue of utilizing just one cell line in my study. However, he is ok with some significant changes to my discussion:

"- Data presented throughout the manuscript are related to a single MM cell line (i.e., INA-6). The present reviewer acknowledges the amount of experiment properly performed. It would be of help to have the main assays performed using additional MM lines; alternatively, the discussion should be revised highlighting how all the results are devoted to a single MM cell line, thus representing a major limitation of the study."

So let's change my discussion! Here you see the paragraphs of my discussion, where I have already addressed some limitations of this model:

INA-6 cells serve as an excellent model system because they show: (1st) Fast and strong adherence to MSCs, (2nd) aggregation and growth beyond MSC saturation and (3rd) IL-6 dependency. Aggregation has been described for primary myeloma cell cultures (33,34) and histological samples, where "homogenous nodules and/or monotypic aggregation" correctly identified multiple myeloma (44,45). IL-6 dependency increases the similarity of myeloma cell lines with patient samples, and INA-6 ranks 13th among 66 cell lines (46).

We encourage independent validation with awareness of the limitations of this model: We recommend using primary hMSCs and not cell lines. Most importantly, understanding the adhesive properties of myeloma cell lines is crucial. Our protocols are optimized for strong adherence between myeloma cells and MSCs and would require adjustment for weak adherence. For primary myeloma cells, we highly recommend characterizing each donor in terms of hMSC adherence and homotypic aggregation behavior prior to any attachment/detachment analysis. We recognize variations in bone marrow-MSCs between MM and healthy states, but since INA-6 adheres strongly and hMSCs provide vital survival factors during long-term co-cultures, we don't expect changes in hMSCs post-contact with myeloma to compromise our results (57).

Now I want you to read it, understand it, and then implement the points that I will provide you below. When you re-writing my paragraph, Do NOT use Bullet points, but paragraphs of text. Please stay very concise and prevent repetitive arguments! Most importantly, please remember that we are arguing against a well established standard of using multiple cell lines. Hence, please don't sound too challenging but collaborative! Here are the points to implement into my discussion:

- We acknowledge the reviewer's suggestion of employing multiple cell lines to enhance the generalizability of our findings. While we understand that this is a common practice in the field, we would like to present our rationale for maintaining the focus on the INA-6 cell line in our study. Independence of Gene Associations: The gene associations we identified with patient survival stand independently of the specific cell line used. These findings provide valuable insights into the broader context of myeloma dissemination, transcending the limitations of a single cell line.

Robust Protocols for Adhesion Studies: The detailed protocols provided in the supplementary material offer a solid foundation for investigating adhesion dynamics. We believe this resource can serve as a valuable starting point for researchers studying similar processes with different cell lines. In fact, multiple groups have already asked for our adhesion assay protocols.

Optimization Challenges and Protocol Complexity: Our study optimized protocols for a highly adherent cell line, INA-6, as stated in the discussion. Expanding it with multiple cell lines or patient material would not only add considerable complexity to the paper (currently 29 pages in the supplementary material) but would also require distinct optimization for each cell line, diluting the clarity of our presented protocols.

Diversity in Adhesive Properties: The incredibly diverse adhesive properties of myeloma cell lines present a significant challenge, especially in the context of hMSC interactions and IL-6 dependency. For example U266's adheres weakly to hMSCs, MM.1S's adheres strongly to both hMSCs and plastic, INA-6 adheres only to hMSC but not plastic etc. Capturing this diversity within a single publication is highly impractical, if not impossible.

Limitations of In Vitro Approaches: We emphasize the inherent limitations of in vitro models in capturing the full complexity of myeloma behavior. Our focus on understanding the specific interactions of INA-6 with hMSCs allows for a more in-depth exploration of the phenomena observed, rather than attempting to generalize across a heterogeneous landscape. For example, INA-6 are unique in their aggregation capability, which greatly helped with describing in vitro dissemination behavior. Other cell lines don't provide that.

INA-6 as a Model: Our study intentionally delves into specific phenomena, aiming to derive generalizable conclusions by aligning in vitro findings with clinical survival data. The comparison highlights that the observed phenomena offer biologically consistent explanations and insights. To underscore this focus, we've implemented the following refinements:

1. We refined the title of our publication: "Describing Modeling Myeloma Dissemination in vitro with hMSC-Interacting Subpopulations of INA-6 Cells and their Aggregation/Detachment Dynamics"

2. We revised the first paragraph of our discussion, adding a sentence that emphasizes how our model reaches generalizability: "Third, we identified clinically relevant genes that were associated with either better or worse patient survival based on the adherence status of INA-6 cells to MSCs, demonstrating that our model can yield insights with broader applicability derived from in vitro data."

Concerns about Generalizability: Given the significant diversity and limitations in in vitro data, we are skeptical about achieving "generalizability" in any in vitro myeloma data. Making generalizations without a detailed understanding of the specific cell lines and patient sub-cohorts contributing to the observed diversity in adhesive capabilities can introduce inaccuracies or artifacts in the field of myeloma dissemination.

Recommendation for Future Publications: We are convinced that it would be far more productive to dedicate other myeloma cell lines or patient samples to further publications. We suggest that focusing on a single line of inquiry will yield a more comprehensive understanding than attempting to publish an average of phenomena that may be inherently diverse.

- In conclusion, while we acknowledge the significance of employing multiple cell lines in research (such as the intriguing comparison of CMA-03 and CMA-03/06 cells), we maintain our conviction that, the in-depth understanding gained with the INA-6 cell line surpasses the challenges associated with introducing additional cell lines.



We appreciate the thoughtful suggestion of the reviewer regarding the inclusion of multiple myeloma (MM) cell lines in our study. While we recognize the standard practice of using diverse cell lines for

generalizability, we advocate for the intentional focus on the INA-6 cell line for several compelling reasons.

Firstly, the gene associations with patient survival identified in our study transcend the limitations of a single cell line. These findings offer valuable insights into the broader context of myeloma dissemination, emphasizing the independence of our identified associations from the specific cell line used.

Our provided adhesion study protocols serve as a robust foundation for researchers exploring similar processes with different cell lines. Notably, multiple groups have expressed interest in adopting our protocols, attesting to their utility beyond the scope of our chosen cell line.

Optimizing protocols for a highly adherent cell line like INA-6, as detailed in our supplementary material, is no small feat. Introducing multiple cell lines or patient material would not only complicate the paper but also necessitate distinct optimizations for each, potentially diluting the clarity of our presented protocols.

The diverse adhesive properties of myeloma cell lines, including variations in hMSC interactions and IL-6 dependency, present a formidable challenge. Attempting to encapsulate this diversity within a single publication is impractical, if not impossible, due to the inherent complexities involved.

We acknowledge the limitations of in vitro models in capturing the full complexity of myeloma behavior. Our deliberate focus on understanding specific interactions with the INA-6 cell line allows for a more in-depth exploration of observed phenomena, as opposed to a generalized approach across a heterogeneous landscape.

To underscore the generalizability of our study, we have refined the title and added a sentence to the first paragraph of our discussion. Additionally, we express skepticism about achieving full "generalizability" in any in vitro myeloma data, considering the significant diversity and limitations inherent in such studies.

We recommend dedicating future publications to exploring other myeloma cell lines or patient samples to achieve a more comprehensive understanding. Focusing on a single line of inquiry can yield a deeper and more nuanced exploration of specific phenomena, contributing to the overall understanding of myeloma dissemination.

In conclusion, while we acknowledge the importance of employing multiple cell lines in research, we maintain that the in-depth understanding gained with the INA-6 cell line outweighs the challenges associated with introducing additional cell lines. Our study contributes valuable insights that extend beyond the limitations of a single cell line, providing a nuanced perspective on myeloma dissemination.



you completely omitted the points addressed in the original discussion. Please merge both original discussion and the points presented here into one cohesive and concise discussion



The focus on the INA-6 cell line in our study was intentional, as it serves as an excellent model system with unique characteristics such as fast adherence to mesenchymal stem cells (MSCs), aggregation beyond MSC saturation, and IL-6 dependency. These features make INA-6 a valuable tool for studying myeloma dissemination, aligning with our specific research objectives.

While we acknowledge the reviewer's suggestion to use multiple myeloma (MM) cell lines, we want to emphasize the rationale behind maintaining our focus on INA-6. The gene associations we identified with patient survival are independent of the specific cell line used, providing insights into the broader context of myeloma dissemination.

Our detailed protocols for adhesion studies, outlined in the supplementary material, offer a solid foundation for investigating adhesion dynamics. Notably, researchers have expressed interest in adopting our protocols, demonstrating their applicability beyond the context of the INA-6 cell line. Optimizing protocols for highly adherent cell lines, such as INA-6, is a non-trivial task. Introducing multiple cell lines or patient material would add considerable complexity to the paper, currently 29 pages in the supplementary material, and require distinct optimization for each cell line, potentially diluting the clarity of our presented protocols.

The diverse adhesive properties of myeloma cell lines, with variations in hMSC interactions and IL-6 dependency, pose a significant challenge. Attempting to capture this diversity within a single publication is impractical, given the complexities involved.

We recognize the limitations of in vitro models and emphasize the inherent challenges in achieving "generalizability" in any in vitro myeloma data. Our study intentionally delves into specific phenomena observed with INA-6, allowing for a more in-depth exploration of the interactions between myeloma cells and MSCs.

To underscore the generalizability of our study, we refined the title and added a sentence to the first paragraph of our discussion. We also express skepticism about achieving full "generalizability" in any in vitro myeloma data, considering the significant diversity and limitations inherent in such studies. We recommend dedicating future publications to exploring other myeloma cell lines or patient samples to achieve a more comprehensive understanding. Focusing on a single line of inquiry allows for a deeper exploration of specific phenomena, contributing to the overall understanding of myeloma dissemination.

In conclusion, while we appreciate the suggestion to use multiple cell lines, we maintain that the in-depth understanding gained with the INA-6 cell line is valuable for achieving our research objectives. Our study contributes unique insights that extend beyond the limitations of a single cell line, providing a nuanced perspective on myeloma dissemination.

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