

Myeloma Model Limitations Clarified



I am revising the discussion of my research paper where I discuss the limitations of my model:

In this study, we developed an in vitro model to characterize the attachment/detachment dynamics of hMSC-myeloma interactions and isolate the attached and detached intermediates of INA-6 cells (nMA- and MA-INA6). Second, this model proposes that myeloma cell division is linked to a direct switch from hMSC adhesion to detachment, which starts a cycle of (re)attachment, division, and detachment (Fig. 7). 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.

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).

I like these paragraphs, and I would like you to read the following points, understand them, compress their meaning together to few most important points, and then find a way how to merge them into my discussion!

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.

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.

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. 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.

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.

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:

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.

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 this study, we developed an in vitro model using INA-6 cells to investigate hMSC-myeloma interactions, identifying key genes associated with patient survival regardless of the specific cell line used. Our detailed protocols, optimized for strong adherence, offer a valuable resource for studying adhesion dynamics in similar processes. While we acknowledge the diverse adhesive properties of myeloma cell lines, attempting to capture this complexity within a single publication is impractical. Despite the limitations of in vitro models, our focus on INA-6 interactions with hMSCs allows for a detailed exploration of observed phenomena, such as unique aggregation capabilities. We intentionally delve into specific aspects, aligning in vitro findings with clinical data to derive biologically consistent conclusions. However, achieving "generalizability" in in vitro myeloma data is challenging, and we caution against making broad generalizations without understanding the specific cell lines and patient sub-cohorts contributing to the observed diversity.

To enhance the robustness of future studies, we recommend independent validation with primary hMSCs and awareness of myeloma cell line adhesive properties. While acknowledging the potential complexity of incorporating multiple cell lines, our study's protocols can serve as a starting point for researchers. We encourage dedicating separate inquiries to other myeloma cell lines or patient samples to yield a more comprehensive understanding rather than attempting to publish an average of inherently diverse phenomena.



ok, using your input, I have written the first two paragraphs. Can you give me feedback?

INA-6 cells are an excellent choice for modelling dissemination due to fast and strong adherence, aggregation and growth beyond MSC saturation. Their IL-6 dependency increases the similarity of myeloma cell lines with patient samples, with INA-6 ranking 13th among 66 cell lines (46). Despite variations in bone marrow-MSCs between multiple myeloma (MM) and healthy states, we expect results remain robust, as MSCs maintain strong adherence and growth signaling to INA-6 during long-term co-cultures (57).

We acknowledge that a single cell line can not describe the full complexity of myeloma behavior. However, the diverse adhesive properties of myeloma cell lines are a challenge, so we reasoned that attempting to capture this complexity within a single publication is impractical (if possible at all). Despite the limitations of in vitro models, our focus on INA-6 interactions with hMSCs allows for a detailed exploration of observed phenomena, such as unique aggregation capabilities that enable easy detection of detaching cells in vitro. Still, a major portion of our confidence is derived from aligning our in vitro findings with clinical data. Their independence from the specific cell line used allowed us to derive biologically consistent and generalizable conclusions.



Your revised paragraphs are well-constructed and effectively convey the key points. Here are some suggestions to enhance clarity and flow:

Original:

INA-6 cells are an excellent choice for modelling dissemination due to fast and strong adherence, aggregation and growth beyond MSC saturation. Their IL-6 dependency increases the similarity of myeloma cell lines with patient samples, with INA-6 ranking 13th among 66 cell lines (46). Despite variations in bone marrow-MSCs between multiple myeloma (MM) and healthy states, we expect results remain robust, as MSCs maintain strong adherence and growth signaling to INA-6 during long-term co-cultures (57).

Revised:

INA-6 cells emerge as a robust model for studying myeloma dissemination, showcasing rapid and strong adherence, as well as aggregation exceeding MSC saturation. The IL-6 dependency of INA-6 enhances the resemblance of myeloma cell lines to patient samples, with INA-6 ranking 13th among 66 cell lines (46). Despite variations in bone marrow-MSCs between multiple myeloma (MM) and healthy states, we anticipate the robustness of our results, given the persistent strong adherence and growth signaling from MSCs to INA-6 during long-term co-cultures (57).

Original:

We acknowledge that a single cell line can not describe the full complexity of myeloma behavior. However, the diverse adhesive properties of myeloma cell lines are a challenge, so we reasoned that attempting to capture this complexity within a single publication is impractical (if possible at all). Despite the limitations of in vitro models, our focus on INA-6 interactions with hMSCs allows for a detailed exploration of observed phenomena, such as unique aggregation capabilities that enable easy detection of detaching cells in vitro. Still, a major portion of our confidence is derived from aligning our in vitro findings with clinical data. Their independence from the specific cell line used allowed us to derive biologically consistent and generalizable conclusions.

Revised:

While recognizing the inherent limitations of using a single cell line to fully represent the complexity of myeloma behavior, we acknowledge the challenge posed by the diverse adhesive properties of myeloma cell lines. Attempting to encapsulate this diversity within a single publication is deemed impractical, if not impossible. Despite the acknowledged limitations of in vitro models, our deliberate

focus on studying INA-6 interactions with hMSCs enables a meticulous exploration of observed phenomena, such as the unique aggregation capabilities facilitating the easy detection of detaching cells in vitro. A substantial portion of our confidence stems from aligning these in vitro findings with clinical data, where their independence from the specific cell line used empowers us to derive biologically consistent and generalizable conclusions.



ok I like it! However the last sentence seems convoluted. Can it be rewritten to be more concise without losing meaning?



Certainly! Here's a more concise version of the last sentence:

"Our confidence is bolstered by aligning in vitro findings with clinical data, ensuring biologically consistent and generalizable conclusions due to their independence from the specific cell line used."



I also don't like the term "confidence", it kinda seems un-scientific, or am I mistaken..?



You're correct in noting that the term "confidence" might introduce a more subjective tone. In scientific writing, it's often preferable to use terms that emphasize objectivity and precision. Here's a revised version:

"The robustness of our conclusions is reinforced by aligning in vitro findings with clinical data, ensuring biological consistency and generalizability, independent of the specific cell line employed."



I have over-used "robust" is there something else?



Certainly! Here's an alternative phrasing to avoid repetition:

"The validity of our conclusions is substantiated by aligning in vitro findings with clinical data, ensuring biological consistency and generalizability, irrespective of the specific cell line employed."



ok thanks!

I've used your input to write the last paragraph, can you help improving that as well?

The protocols presented in this study are cost-efficient, convenient. They could be a valuable resource to study cell interactions in general, but extensive adaptations are necessary to accommodate different samples needs, such as overall adhesion strength and homotypic aggregation behavior. We discourage averaging over inherently diverse phenomena without prior understanding what constitutes these differences. This experience might prove helpful when facing the diversity of myeloma patient samples.



Certainly! Here's a refined version of your last paragraph:

"The protocols outlined in this study offer a cost-effective and convenient framework. While they serve as a valuable resource for studying cell interactions in a broader context, it's crucial to recognize that substantial adaptations are required to address diverse sample needs, including variations in overall adhesion strength and homotypic aggregation behavior. We caution against averaging results across inherently diverse phenomena without a thorough understanding of the underlying differences. This insight may prove invaluable when confronted with the diversity inherent in myeloma patient samples."

ChatGPT kann Fehler machen. Überprüfe wichtige Informationen.