

# In vitro Myeloma Study



I have used chatGPT to revise my discussion. Can you tell me, which of the following paragraphs I used chatGPT?

In this study, we developed an in vitro model to investigate the attachment/detachment dynamics of INA-6 cells to/from hMSCs and established methods to isolate the attached and detached intermediates (nMA- and MA-INA6). Second, this model suggests a cycle of (re)attachment, division, and detachment, linking cell division to the switch that makes myeloma cells detach from hMSC adhesion. (Fig. 7). Third, we identified clinically relevant genes associated with patient survival, where better or worse survival was based on the adherence status of INA6 to hMSCs. INA-6 cells emerge as a robust choice for studying myeloma dissemination in vitro, 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).

We acknowledge that INA-6 cells alone cannot fully represent the complexity of myeloma behavior. However, the diverse adhesive properties of myeloma cell lines pose a challenge. We reasoned that attempting to capture this complexity within a single publication is impractical, if not possible. Our focus on INA-6 interactions with hMSCs allows for a detailed exploration of observed phenomena, such as unique aggregation capabilities facilitating the easy detection of detaching cells in vitro. The validity of our conclusions stems from aligning in vitro findings with clinical data, ensuring biological consistency and generalizability, independent of the specific cell line employed.

The protocols presented in this study offer a cost-efficient and convenient solution, making them potentially valuable for the broader study of cell interactions. We encourage optimizations to meet the varied adhesive properties of samples, like decreasing the washing steps if the adhesive strength is weak. We caution against averaging over inherently diverse phenomena without prior understanding what constitutes these differences, e.g. side-effects caused by homotypic aggregation. These insights might prove instrumental when facing the diversity of myeloma patient samples across different disease stages (33, 34).

The intermediates nMA- and MA-INA6 were distinct and shared only few similarities in response to cell stress, intrinsic apoptosis, and regulation by p53. Unique regulatory patterns for nMA-INA6 were related to E2F1 and for MA-INA6 to NF- $\kappa$ B (with RELA/p65), SRF, and JUN. This distinction might have been established through antagonism between p53 and RELA/p65 (37,38). Similar regulatory patterns were found in transwell experiments with RPMI1-8226 myeloma cells, where direct contact with an MSC cell line led to NF- $\kappa$ B signaling and soluble factors to E2F signaling (47). The first subpopulation, nMA-INA6, represented proliferative and disseminative cells: nMA-INA6 drove detachment through cell division, which was regulated by E2F, p53, and likely their crosstalk (48). They upregulated cell cycle progression genes associated with worse prognosis, because proliferation is a general risk factor (49). Additionally, they survived IL-6 withdrawal better than CM-INA6 and MA-INA6, implying their ability to proliferate independently of the bone marrow (2). Indeed, xenografted INA-6 cells develop autocrine IL-6 signaling but remain IL-6-dependent after explantation (21). The increased autonomy of nMA-INA-6 can be explained by

them upregulating IGF-1 (50). Other reports have characterized disseminating cells differently: Unlike nMA-INA6, circulating myeloma tumor cells were reported to be non-proliferative and bone marrow retentive (51). We explain this with nMA-INA6 not representing other proposed steps of dissemination (e.g., intravasation, circulation, intravascular arrest, etc.) (3), since nMA-INA6 was isolated shortly after detachment. Furthermore, Brandl et al. described proliferative and disseminative myeloma cells as separate entities, depending on the surface expression of CD138 or JAM-C (4,52). While CD138 was not differentially regulated in nMA- or MA-INA6, both subpopulations upregulated JAM-C, indicating disease progression (52).

Furthermore, nMA-INA6 showed that cell division directly contributes to dissemination. This was because INA-6 daughter cells emerged from the mother cell at a location that is disconnected from the hMSC plane in a 2D setup. A similar mechanism was described in an intravasation model in which tumor cells disrupt the vessel endothelium through cell division and detach into blood circulation (53). Overall, cell division offers key mechanistic insights into dissemination and metastasis.

The other subpopulation, MA-INA6, represented cells retained in the bone marrow: MA-INA6 strongly adhered to MSCs, showed NF- $\kappa$ B signaling, and upregulated several retention, adhesion, and ECM factors. The production of ECM-associated factors was recently described in MM.1S and RPMI-8226 cells (54). Another report did not identify upregulation of such factors after direct contact with the MSC cell line HS5; hence, primary hMSCs might be crucial for studying myeloma-MSC interactions (47). Moreover, MA-INA6 upregulates adhesion genes associated with prolonged patient survival, and shows decreased expression in relapsed myeloma. Since myeloma progression implies independence of myeloma cells from the bone marrow (46), we interpret these adhesion genes as mediators of bone marrow retention, thereby decreasing the risk of dissemination and potentially prolonging patient survival. The overall impact of cell adhesion or ECM on patient survival remains unclear. Multiple adhesion factors have been proposed as potential therapeutic targets (52,55). Recent studies have described the prognostic value of multiple ECM genes, such as those driven by NOTCH (54). Another study focused on ECM gene families, of which only six of 26 genes overlapped with our gene set (Tab. S2) (56). The expression of only one gene (COL4A1) showed a different association with overall survival than that in our cohort. The lack of overlap and differences can be explained by dissimilar definitions of gene sets (homology vs. gene ontology), methodological discrepancies, and cohort composition.

In summary, our in vitro model is a starting point for understanding the initiation of dissemination and its implications for patient survival, providing preliminary methods, mechanistic insights into attachment/detachment, and a set of clinically relevant genes that imply a role in bone marrow retention. This might prove useful when facing heterogeneity of disseminative behaviors among myeloma cell lines or primary materials, thereby contributing to the development of therapeutic strategies against this devastating disease.



It's challenging to definitively determine whether you used ChatGPT for revising the discussion based on the provided paragraphs alone. The text appears to be well-written and focused on a scientific study, making it challenging to identify specific markers of AI-generated content.

If you made substantial revisions or added content with the help of ChatGPT, it would be helpful to know which sections or ideas specifically were influenced by the AI. If you integrated the suggestions seamlessly, it may not be apparent from the text alone.



ok, I wrote the following paragraphs together with chatGPT. Do you think it is safe to claim that the author "worked alone" using chatGPT, or can I be accused of plagiarism?

INA-6 cells emerge as a robust choice for studying myeloma dissemination in vitro, 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).

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The paragraphs you provided appear to be well-written and focused on the scientific study of myeloma dissemination, discussing the properties of INA-6 cells and the experimental approach. However, it's important to note that the question of whether it is safe to claim that you "worked alone" using ChatGPT depends on the extent of the AI's contribution and the nature of the collaboration. If you actively collaborated with ChatGPT in generating ideas, structure, or specific wording, it would be more accurate and ethical to acknowledge the AI's assistance in your work. Failing to disclose such assistance could be considered misleading, and there may be concerns about potential plagiarism or lack of transparency.

To avoid any issues, it's recommended to be transparent about your collaboration with AI, especially if it significantly contributed to the content. You can mention that you used AI assistance for generating and refining certain parts of the text. This approach aligns with ethical writing practices and ensures proper attribution.

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