

D-unused Constructing Dissemination Framework



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PDF

I am writing a summarising discussion of my PhD thesis. For context, I am uploading the introduction and a chapter of my thesis (I use latex, please learn the commands I used!).

Currently, I am working on a hypothetical framework that integrates both my results and that of my results. My notes are very fragmented, including raw paragraphs, bullet points and the literature sources being separated from the notes. I will need your help with putting all that information into cohesive and comprehensive sections and paragraphs that can span many pages.

But first, please understand the files I have uploaded and judge if my hypothetical framework makes sense. I fear that these hypotheses are actually already proven, or are too hypothetical to become untestable, hence delving into pseudo-science.

Here are my notes:

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% =====

`\newcommand{\footcadd}{%`

`\emph{\acf{CADD}}` (defined in this work): The observation and measurement of time-dependent changes in cell adhesion and detachment events.

`\ac{CADD}` characterizes the time cells spend attached, migrating or detached and associates these phases with molecular signatures, such as `\ac{CAM}` expression or cell signaling mediated by `\acp{CAM}` or the microenvironment. `\ac{CADD}` expands traditional `\emph{cell adhesion}` by a time component and implies an intention to predict attachment and detachment events.

`%`

`}`

`\unsubsection{Constructing a Hypothetical Framework of Dissemination}%`

`\label{sec:discussion_framework}%`

Until today, a mechanistic understanding of myeloma dissemination is still lacking. \cite{zeissigTumourDisseminationMultiple2020} has described dissemination as a multistep process along the lifetime of a myeloma cell. However, since evidence is collected mostly for individual steps \dashed{each step being very complex itself}, the connection of these steps remains unproven and therefore a hypothetical construct. A description of dissemination as a process hence becomes a rough patchwork of \emph{evidence fragments}. In that sense, this work adds further fragments, especially since its validity is limited to the \textit{INA} cell line. To regain understanding of dissemination as a process, the following sections attempt to integrate the findings of this work with available literature, formulating specific hypotheses to face future experiments. Given that direct observations of \emph{\textit{CADD}}\footnote{\footcadd\label{foot:cadd}} have proven insightful, this framework carries the name \emph{Dynamic Adhesion Hypothetical Framework for Myeloma Dissemination}.

\textbf{Generating Hypotheses from CADD:}

The following shows examples how hypotheses can be formed after \textit{CADD} observations:

- What predictions can be made from these results for steps of dissemination (retention, release, Extra-/Intravasation, colonization), assuming that \textit{INA} is representative for all myeloma?

- Retention:

Observation of CADD: \textit{INA} cells attach fast and strongly to \textit{hMSC} and grow into stable aggregates.

Hypothesis: Myeloma cells are retained through strong adhesion to the BM microenvironment and stable homotypic aggregation.

Experiment: Inject \textit{INA} into mice and make sections of Bone lesions, comparing the growth pattern to mice co-injected with an ICAM-1 or LFA-1 α /ITGB2 antibody, as that was shown to dissolve homotypic aggregates \textit{in vitro} and prevent \textit{INA} growth \textit{in vivo}\cite{kawanoHomotypicCellAggregations1991a, klauszNovelFcengineeredHuman2017}. If abrogating aggregation leads to a rather diffuse colonization of bone instead of fewer focal lesions, this would support that strong adhesion and aggregation are crucial for retention in the BMME.

- Release:

Observation: \textit{INA} cells detach from \textit{hMSC} through cell division, and external forces can detach single myeloma cells from aggregates.

Hypothesis: Myeloma cells detach from the BM microenvironment through cell division and external force after reaching a minimal aggregate size.

Experiment: This could be tested by injecting INA into mice and comparing the cell cycle profile of circulating cells vs. cells in the BM. If circulating cells are enriched with G1/G0 cells, this would support that detachment is more likely shortly after cell division.

- Intra-/Extravasation: CADD observations in this work did not make predictions, yet it could if MSCs were replaced by endothelial cells.

- Colonization:

Observation: Myeloma cells show quick attachment to MSCs within just 1 hour of contact and quick upregulation of a multitude of adhesion factors, including ECM factors.

Hypothesis: Quick attachment and fast expression of adhesion factors is a high potential to colonize new niches. This is reasonable especially since INA were isolated from the pleura [\cite{burgerGp130RasMediated2001c}](#), suggesting the capability to colonize extramedullary sites.

Experiment: Inject INA-6 into mice and see if they colonize extramedullary sites. Compare that to INA-6 with decreased adaptability,

This shows how CADD observations can lead to testable hypotheses using the results from this work alone. However, since these hypotheses are specialized on INA, a more generalized framework is favorable.

```
\newcommand{\caddadaptation}{ %
  \ac{CADD} is adapted in response to different microenvironments faced
  during dissemination %
}
\newcommand{\caddadaptationtitle}{ %
  \textit{Hypothesis 1}: \ac{CADD} is Adapted during Dissemination%
}%
```

```
\newcommand{\caddadaptability}{ %
  High adaptability of \ac{CADD} is a hallmark of aggressive myeloma %
}%
\newcommand{\caddadaptabilitytitle}{ %
  \textit{Hypothesis 2}: High Adaptability of \ac{CADD} is a Hallmark of
  Aggressive Myeloma %
}%
```

```
\newcommand{\cadddiversity}{%
  \ac{CADD} is highly diverse within both patients and cell lines %
}%
\newcommand{\cadddiversitytitle}{%
  \textit{Hypothesis 3}: \ac{CADD} is Highly Diverse Within both Patients
  and Cell Lines%
}%
```

```
\newcommand{\caddtrigger}{%
  Detachment is caused by multiple cues of varying nature, including
  external mechanical forces, cell division, loss of \ac{CAM} expression, or
  even pure chance.}%
\newcommand{\caddtriggertitle}{%
  \textit{Hypothesis 4}: Detachment is Caused by Multiple Cues of Varying
  Nature %
}%
```

\textbf{Key Hypotheses:}

The Dynamic Adhesion Hypothetical Framework is structured around four key hypotheses, each addressing fundamental aspects of myeloma cell dissemination based on both literature and the results of this work. These hypotheses are as follows:

```
\begin{enumerate}
  % \item \caddpredictions
  \item \caddadaptation
  \item \caddadaptability
  \item \cadddiversity
  \item \caddtrigger
\end{enumerate}
```

This framework sets the stage for a detailed exploration of each hypothesis, linking empirical data with theoretical constructs to provide a comprehensive framework of myeloma cell dissemination that can inform both future research to identify commonalities in dissemination and inform the development of targeted therapies.

```
% =====
\unsubsection{\caddadaptationtitle}%
\label{sec:discussion_caddadaptation}%
However, Chapter 1 shows that adhesion factors are
lost during MM progression. INA-6 are highly adhesive to hMSCs.
This is a contradiction that needs to be resolved.
```

One explanation is the dynamic change of adhesion factor expression.

However, INA-6 do not express adhesion factors. They do that only in hMSC presence
Hence MAINA-6 could be a smaller fraction of MM cells, specialized on preparing a new niche for the rest of the MM cells. This could be a reason why they are so adhesive.

- One has to consider that intravasation and/or intra-/extravasation would require a different set of adhesion factors than adhesion to BM or extramedullary environments.

Extravasation: Plasma cells are known to upregulate adhesion factors dynamically once they reach a target tissue (???).

This work showed that INA cells dynamically upregulate adhesion factors when in direct contact with hMSC. Such adhesion factors are not expressed by INA cells without contact to hMSC, or by INA cells emerging as daughter cells from MAINA cells. This implies that myeloma cells are capable of rapid changes in adhesion factor expression that are substantially dynamic.

Predicting when a myeloma cell starts regulating adhesion factors is a key question in understanding dissemination.

The following paragraphs discuss how the idea of dynamic adhesion factor expression holds up against current knowledge.

What biological implications does CADD adaption have?

1 Location of Myeloma Cells:

- Different locations could require different adhesion factors:
- Circulating MM cells do not need adhesion, probably losing adhesion factors
- BM cells express adhesion factors to adhere to the Bone marrow microenvironment (MSCs, adipocytes, and osteoblasts)
- Extravasating/intravasating cells need adhesion factors for endothelium
- Extramedullary cells need adhesion factors for respective tissues

\cit{bouzerdanAdhesionMoleculesMultiple2022}: "Classically, the BMM has been divided into endosteal and vascular niches"

Overall, cell adhesion play a pivotal role in the attachment/detachment dynamics of myeloma, hence influencing the dissemination of myeloma cells. This is exemplified in this work, where INA cells dynamically upregulate adhesion factors in direct contact with hMSC. Predicting how and when myeloma cells regulate adhesion activity is a key question in understanding dissemination, since that

Myeloma cells are isolated from patients at a certain stage from a certain location. As summarized by \cit{zeissigTumourDisseminationMultiple2020}, dissemination could be a dynamic process during the lifetime of a myeloma cell that managed to exit the BMME into blood circulation. This implies that myeloma cells could change their adhesion factors during their course of dissemination to adapt to their current location for specialized tasks like exiting the BMME or intra-/extravasation.

why important?

Knowing how an MM cell can change their adhesive properties during its course of dissemination is crucial for understanding the process itself. These changes could be studied by tracking the expression of adhesion factors in MM cells at different locations in mouse models. For humans, designing studies that gather biopsies at different locations from the same patient, e.g. bone marrow and circulating myeloma cells could be a starting point.

How studied?

These changes could be studied by tracking the expression of adhesion factors in MM cells at different locations in mouse models. For humans, designing studies that gather biopsies at different locations from the same patient, e.g. bone marrow and circulating myeloma cells could be a starting point.

Literature:

1 Location of Myeloma Cells

Other Findings

The review by

Zeissig et al. (2020) could be

a starting point. She does not discuss adhesion factors, but seeing dissemination as a multistep process does imply different adhesion factors for different steps.

Malignant Plasma Cells express different adhesion factors than normal plasma cells (Cook et al., 1997; Bouzard et al., 2022).

Adhesion molecules have been a popular target for therapy for a decade (Nair et al., 2012).

In other cancers different adhesive subtypes are common and are molecularly clearly separated through EMT (Geng et al., 2014).

Extramedullary Involvement

Extramedullary involvement: HCAM dramatic upregulation of HCAM

CXCR4, the homing receptor, mediates production of adhesion factors in extramedullary MM cells

(Roccaro et al., 2015).

Intra-/Extravasation of Myeloma Cells

Blocking Endothelial Adhesion through JAM-A decreases progression:

(Solimando et al., 2020).

N-Cadherin is upregulated in MM compared to healthy plasma cells, and has been shown to be a potential target for therapy (Mrozik et al., 2015).

\item - NONE of Them were shown in Chapter 1 of this study, (except for JAM-B)
\end{itemize}

\item \textbf{Circulating Myeloma Cells}

\begin{itemize}

\item This work shows that \nMAina have increased survival during IL-6 deprivation, which could be a mechanism for surviving in circulation.

\item Circulating plasma cells are rare, but detectable in peripheral blood

\cite{witzigDetectionMyelomaCells1996}

\item studies demonstrate that circulating \ac{MM} cells exhibit reduced levels of integrin $\alpha4\beta1$, in contrast to those located in the \ac{BM}

\cite{paivaDetailedCharacterizationMultiple2013, paivaCompetitionClonalPlasma2011}

\item circulating MM cells were CD138/Syndecan-1 negative
\cite{akhmetzyanovaDynamicCD138Surface2020}

\end{itemize}

\item \textbf{BM-Resident Myeloma Cells}

\begin{itemize}

\item The role of CXCL12 \dashed{which is highly expressed by MSCs} in inducing adhesion factors in MM is well established
\cite{ullahRoleCXCR4Multiple2019}

\item THIS WORK: INA-6 cells are highly adhesive to hMSCs, dynamically upregulating adhesion factors when in direct contact with hMSCs, and subsequently losing adhesion factor expression after cell division

\item BM-resident MM cells maintain high levels of adhesion molecules to interact with MSCs, adipocytes, and osteoblasts within the BM niche \cite{bouzerdanAdhesionMoleculesMultiple2022, burgerGp130RasMediated2001, chatterjeePresenceBoneMarrow2002}.

\end{itemize}

\end{itemize}

According to this, this thesis predicts a low expression of adhesion factors in circulating myeloma cells, but a high expression in adhesive cells, e.g. non-circulating, or rather those

This has huge implications for studying adhesion factors in MM \textit{in vitro}. Given that some factors are not present in MM cells, but are potentially rapidly expressed with the right signal. Hence, further studies focusing on adhesion factor expression \textit{in vitro} should provide one specific microenvironmental context, and not generalize to all available niches.

This has great implications for targeting adhesion factors for therapy, as it suggests that different adhesion factors should either be antagonized or agonized depending on the function of the adhesion factor. According to this assumption, adhesion factors involved in intra- and extravasation adhesion should be antagonized, while adhesion factors involved in BM adhesion \dashed{as identified in Chapter 2} should be agonized. Indeed, Adhesion factors for endothelium were shown to decrease tumour burden in mouse models \cite{asosinghUniquePathwayHoming2001a,mrozikTherapeuticTargetingNcadherin2015}

Together, a detailed mapping of the niches available in the bone marrow is required to understand the adhesion factors required for each niche. This is a highly complex task, as the bone marrow is a highly complex organ.

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\unnssubsection{\caddadaptabilitytitle}%
\label{sec:discussion_caddadaptability}%

biological implications:

Disease Stage:

- Higher disease stages imply changes in adhesion factors that favor aggressiveness.
- Aggressiveness includes:
 - Better Colonization of new niches, including extramedullary ones
 - This implies a more diverse set of available adhesion factors
 - Faster regulation to adapt to new niches
 - Better survival in circulation

This assumption dictates that aggressive myeloma cells gain the ability to dynamically express adhesion factors.

It could be that INA-6 has gained the capability to express adhesion factors fast in order to colonize new niches, such as pleura from which they were isolated.

indeed, 3 temporal subtypes have been identified, associating higher risk with faster changes over time \cite{keatsClonalCompetitionAlternating2012}.

Is Disease stage a proxy for tumor aggressiveness?

yes, adhesion has prognostic value: A recent study by \citet{huDevelopmentCellAdhesionbased2024} developed a cell adhesion-based prognostic model for MM, calculating an adhesion-related risk score (ARRS) based on expression of only twelve adhesion related genes.

Supporting Literature:

\begin{enumerate}


```

\item \textbf{Disease Stage}
\begin{itemize}
\item THIS WORK: Expression decreases during progression from
\ac{MGUS} to \ac{MMR} of adhesion factors involved in hMSC
adhesion.
\item The idea that MM pathogenesis involves transformative
processes has been around for decades
\cite{hallekMultipleMyelomaIncreasing1998}, but a
detailed understanding of changing adhesive properties
is still lacking, especially during the progression of
MM.
\item It is discussed that myeloma cell lines derived from
advanced stages show different expression than newly
diagnosed patients, discussing that they come from
multiply relapsed patients
\cite{sarinEvaluatingEfficacyMultiple2020}. This work
also shows that Myeloma cell lines have the lowest
expression of adhesion factors compared to all stages of
\ac{MM} and \ac{MGUS}.
\item For B-Cell Chronic Lymphocytic Leukemia, adhesion
molecule expression patterns define distinct phenotypes in
disease subsets \cite{derossiAdhesionMoleculeExpression1993}.
\item \cite{terposIncreasedCirculatingVCAM12016} reported an
increase in adhesion molecule expression of ICAM-1 and
VCAM-1 in patients with \ac{MM} compared to those with
\ac{MGUS} and \ac{aMM}.
\item However, \cite{perez-andresClonalPlasmaCells2005}
reported that CD40 is downregulated in \ac{PCL}
patients. Hence, different \ac{CAM} could serve
ambiguous roles in \ac{MM} progression.
\end{itemize}

\end{enumerate}

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How could this be studied?

Databases of expression from Myeloma cells gathered from bone marrow \ac{MGUS}, \ac{aMM}, \ac{MM}, \ac{MMR} already exist \cite{akhmetzyanovaDynamicCD138Surface2020, seckingerCD38ImmunotherapeuticTarget2018}. Going through such databases gives a good overview. One could categorize genes using curated databases, get lists associated with extravasation, intravasation, Bone marrow adhesion. For every gene of these gene lists, they could be filtered for significant differences between the stages. Further categorizations of pairwise comparisons of stages are required. but overall, these gene lists could be a starting point for This approach is similar to the gene lists published in chapter 1, with the difference that the gene list was further filtered by the RNAseq results of \textit{in vitro} experiments.

What new implications do these dimensions have on targeting adhesion factors for therapy?

- Specialized treatment for each stage?
- Aggressive MM cells have potential improved control over adhesion factor expression, regulating a more diverse set of adhesion factors faster. This poses further challenges to targeting.

It could be smarter to not target effector-molecules, but rather upstream regulators of adhesion. This work shows that NF-kappaB signaling, which by itself is not treatable, but regulators downstream of NF-kappaB were shown to be effective

\cite{adamikEZH2HDAC1Inhibition2017,adamikXRK3F2InhibitionP62ZZ2018}

% =====

\unsubsection{\cadddiversitytitle}%

\label{sec:discussion_cadddiversity}%

- Describe different cell lines: MM1.S being plastic adhering non-aggregating and moderately MSC-adhering, INA-6 being non adhering aggregate forming and MSC-adhering, U266 being plastic adhering, non-aggregating and non MSC-adhering.
- Results from this work: CXCL12 expresion varies from QM between QM

One important dimension that is missing here is the genetic background of the myeloma cells. These are based on recurrent patterns of chromosomal aberrations or mutational signatures, defining structural and single nucleotide variants

\cite{kumarMultipleMyelomasCurrent2018a,

hoangMutationalProcessesContributing2019}. The prognostic value of genetic variants in MM is well established \cite{sharmaPrognosticRoleMYC2021}, and their identification is becoming precise and cost-effective using \emph{optical genome mapping}, making progress towards personalized therapies

\cite{zouComprehensiveApproachEvaluate2024,

budurleanIntegratingOpticalGenome2024}. The prognostic value of adhesion factor expression is nowhere nearly as advanced, with establishing cell adhesion as a reliable prognostic factor only recently

\cite{huDevelopmentCellAdhesionbased2024}.

% =====

\unsubsection{\caddtriggertitle}%

\label{sec:discussion_caddtrigger}%

biological implications:

- Different cues could trigger different adhesional changes
- Soluble signals?
- Loss of CD138 \cite{akhmetzyanovaDynamicCD138Surface2020}
- Detachment through intercellular effects: cell division, Saturation of hMSC adhesion surface

- Detachment with mechanical influence: External forces and instability after aggregate size

-

why is this important?

The cues that trigger the detachment of MM cells are not well understood. It could be that MM cells detach due to a combination of factors, such as loss of adhesion factors, changes in the BM microenvironment, or cell division or even completely random. Knowing specific dissemination signals helps preventing dissemination.

Papers like \cite{akhmetzyanovaDynamicCD138Surface2020} make it seem as if there is one molecule that decides if a myeloma cell is circulating or not.

It's less about one clear (molecular) mechanism that decides that a myeloma cell decides to become a disseminating cell, but rather a indirect consequence of a combination of many processes.

These processes are:

- Loss of adhesion factors or dynamic expression of adhesion factors
- Loss of dependency from bone marrow microenvironment
- asdf

Our thesis postulates that there is no big switch that decides if a myeloma cell detaches from the bone marrow, but rather a prolonged process of continuously downregulating adhesion factors, a dynamic upregulation of adhesion factors when they're needed, but the ultimate event that triggers release is better explained by external mechanical forces intercellular effects (cell division, saturation of adhesive surface and rising instability of aggregates after reaching a minimum size).

Detachment is triggered by external mechanical forces on cell conglomerates previously sensitized by changes in cell adhesion behaviour

Supporting Literature:

\begin{enumerate}

\item \textbf{Cues or Processes}

\begin{itemize}

- \item This work showed that detachment happened mostly mechanically and cell biologically through cell division. - Detachment through intercellular effects: cell division, Saturation of hMSC adhesion surface - Detachment with mechanical influence: External forces and instability after aggregate size.

- \item Soluble signals within the BM microenvironment, such as cytokines and chemokines, play significant roles in modulating adhesion factor expression in MM cells

\cite{aggarwalChemokinesMultipleMyeloma2006, alsayedMechanismsRegulationCXCR42007}.

```

\item CD138 was proposed as a switch between adhesion and
migration in MM cells, its blockage triggering migration
and intravasation
\cite{akhmetzyanovaDynamicCD138Surface2020}.
\end{itemize}
\end{enumerate}

```

How can this be studied?

3 Cues: Identifying such signals might be challenging without having understood the other two hypotheses about adaptability first.

What new implications do these dimensions have on targeting adhesion factors for therapy?

3 Cues or associated processes:

- It could represent a valid strategy to strengthen myeloma adhesion, provided that targeted adhesion molecule is proven to not be involved in other steps of dissemination, such as extravasation. Stimulating adhesion factor expression or activity is harder than inhibition, yet not impossible. For instance, the short polypeptide SP16 can activate the receptor LRP1 \dashed{its high expression being associated with improved survival of MM patients in this work}, showing promising results during phase I clinical trial \cite{wohlfordPhaseClinicalTrial2021}, but could potentially increase survival of MM through PI3K/Akt signaling \cite{potereDevelopingLRP1Agonists2019, heinemannInhibitingPI3KAKT2022} -
- One could also accept that many cues are simply not controllable, and hope for systemic therapies like CAR- T Cells



To construct a comprehensive discussion that integrates your findings with existing literature, it's crucial to ensure that your hypothetical framework and hypotheses are grounded in current scientific knowledge and are testable. Here's a step-by-step approach to help you create a cohesive and scientifically sound discussion:

Step 1: Review Current Understanding and Identify Gaps

Literature Review Summary:

- **Dissemination Mechanism:** Current understanding of myeloma dissemination involves invasion, intravasation, intravascular arrest, extravasation, and colonization.
- **Adhesion Factors:** Adhesion factors like CXCL12 and CD138 play crucial roles in retention and dissemination.
- **Dynamic Regulation:** Myeloma cells exhibit dynamic regulation of adhesion factors, crucial for their interaction with the bone marrow microenvironment (BMM) and for dissemination.

Identified Gaps:

- **Mechanistic Understanding:** While individual steps of dissemination are well-studied, their integration into a coherent mechanistic model remains incomplete.
- **Temporal Dynamics:** The role of time-dependent changes in adhesion and detachment (CADD) during dissemination is underexplored.
- **Predictive Models:** There is a lack of predictive models linking CADD observations with dissemination stages.

Step 2: Formulate Hypotheses Based on Your Findings

1. Hypothesis 1: Adaptation of CADD During Dissemination

- **Observation:** Adhesion factors are lost during MM progression, but INA-6 cells upregulate adhesion factors in response to hMSC contact.
- **Implication:** This suggests that myeloma cells dynamically adapt their adhesion properties based on the microenvironment.
- **Testable Prediction:** Tracking adhesion factor expression in mouse models at different dissemination stages can validate this dynamic adaptation.

2. Hypothesis 2: High Adaptability as a Hallmark of Aggressive Myeloma

- **Observation:** INA-6 cells rapidly adapt adhesion factor expression, indicating a potential mechanism for aggressive colonization.
- **Implication:** High adaptability might correlate with more aggressive disease phenotypes.
- **Testable Prediction:** Comparing adhesion factor variability and disease progression in patient samples can assess this hypothesis.

3. Hypothesis 3: Diversity of CADD in Patients and Cell Lines

- **Observation:** Different cell lines exhibit varied adhesion and detachment behaviors.
- **Implication:** The diversity of adhesion behaviors might reflect different genetic backgrounds and disease stages.
- **Testable Prediction:** Analyzing genetic and adhesion profiles across multiple myeloma cell lines and patient samples can reveal correlations.

4. Hypothesis 4: Multiple Cues Trigger Detachment

- **Observation:** Detachment occurs due to cell division and external forces.
- **Implication:** Detachment is likely influenced by multiple, possibly stochastic, factors.
- **Testable Prediction:** Experimental setups applying mechanical forces and tracking cell cycle phases can elucidate detachment triggers.

Step 3: Integrate Findings into a Coherent Framework

Framework: Dynamic Adhesion Hypothetical Framework for Myeloma Dissemination

Introduction:

- Myeloma dissemination remains poorly understood at a mechanistic level.
- This framework integrates CADD observations to propose testable hypotheses on dissemination dynamics.

Retention Phase:

- **Observation:** INA-6 cells attach strongly to hMSCs.
- **Hypothesis:** Strong adhesion to the BMM and stable aggregation are crucial for retention.
- **Experiment:** Inject INA-6 cells into mice with and without ICAM-1 or LFA-1 antibodies to test the role of aggregation in retention.

Release Phase:

- **Observation:** Detachment occurs during cell division.
- **Hypothesis:** Myeloma cells detach after reaching a minimal aggregate size or due to external forces.
- **Experiment:** Compare cell cycle profiles of circulating and BMM-resident cells in mice.

Intra-/Extravasation Phase:

- **Observation:** No direct CADD predictions, but endothelial interaction is crucial.
- **Hypothesis:** Adhesion factor expression changes dynamically to facilitate endothelial interactions.
- **Experiment:** Replace hMSCs with endothelial cells in vitro to study adhesion dynamics.

Colonization Phase:

- **Observation:** Quick attachment and adhesion factor upregulation.
- **Hypothesis:** Rapid adaptation to new niches is crucial for colonization.
- **Experiment:** Compare colonization efficiency of INA-6 with and without decreased adaptability in mice.

Key Hypotheses:

1. **Adaptation of CADD During Dissemination:** Dynamic regulation of adhesion factors.
2. **High Adaptability as a Hallmark of Aggressive Myeloma:** Correlation with disease aggressiveness.
3. **Diversity of CADD:** Reflects genetic and phenotypic diversity.
4. **Multiple Cues Trigger Detachment:** Combined influence of cell division and external forces.

Step 4: Addressing Potential Issues and Validation

Avoiding Pseudo-Science:

- **Grounding in Literature:** Ensure each hypothesis is supported by existing evidence or fills a recognized gap.
- **Testability:** Clearly define experiments to validate each hypothesis.
- **Peer Review:** Seek feedback from colleagues and mentors to refine hypotheses and experimental designs.

Conclusion

The proposed Dynamic Adhesion Hypothetical Framework provides a structured approach to understanding myeloma dissemination. By integrating CADD observations and formulating testable hypotheses, this framework aims to bridge the gap between individual findings and a comprehensive mechanistic model.

If you need further assistance in expanding or refining specific sections, please let me know!

ChatGPT kann Fehler machen. Überprüfe wichtige Informationen.