# Increased percentage of multiple myeloma cells in bone marrow adipose tissue

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## Introduction

Multiple myeloma (MM) is characterized by the abnormal proliferation of plasma cells. Myeloma cells are primarily located in the bone marrow (BM) where they interact with the local microenvironment. Although one of the most common cells in the bone marrow are adipocytes, in situ data on how MM cells interact with adipocytes is scarce. Therefore, we analyzed human bone marrow biopsies and aspirates to examine interactions between adipocytes and MM cells.

## Methods

In our study, we analyzed bone marrow (BM) biopsy and aspirate samples from non-tumor controls, precursor cases (monoclonal gammopathy of undetermined significance, MGUS), newly diagnosed multiple myeloma (NDMM), and follow-up patients.

On microscopic sections of BM biopsies, we analyzed the distance between each myeloma cell and its nearest adipocyte. These distances were then compared to the distances of randomly distributed artificial myeloma cells generated by Monte Carlo simulations.

From BM aspirate samples, we isolated bone marrow adipose tissue (BMAT) using slow centrifugation followed by enzymatic digestion to obtain a single-cell suspension. We compared the cellular composition of BMAT and the aspirate using spectral flow cytometry.

## Results

We first investigated the spatial association between myeloma cells and adipose on bone marrow tissue sections using Qupath software and machine vision. Myeloma cells were identified via MUM1 staining, while bone marrow adipocytes were identified based on hematoxylin staining and morphology (**Figure 1**). We measured the surface-surface distance between each myeloma cell and its nearest adipocyte and compared these to distances generated by Monte Carlo simulations of randomly distributed myeloma cells.

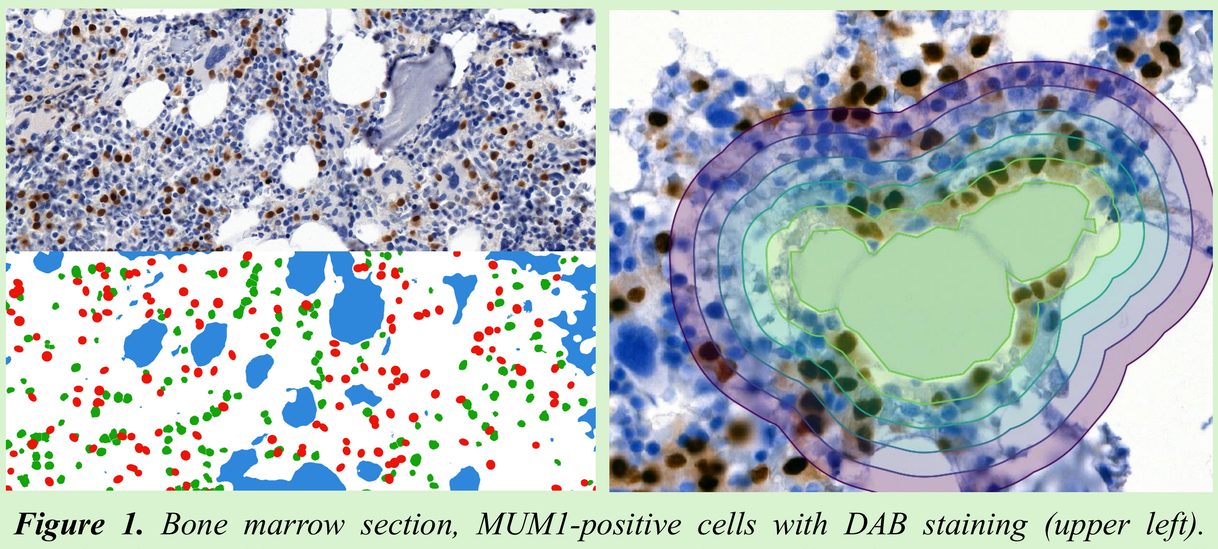
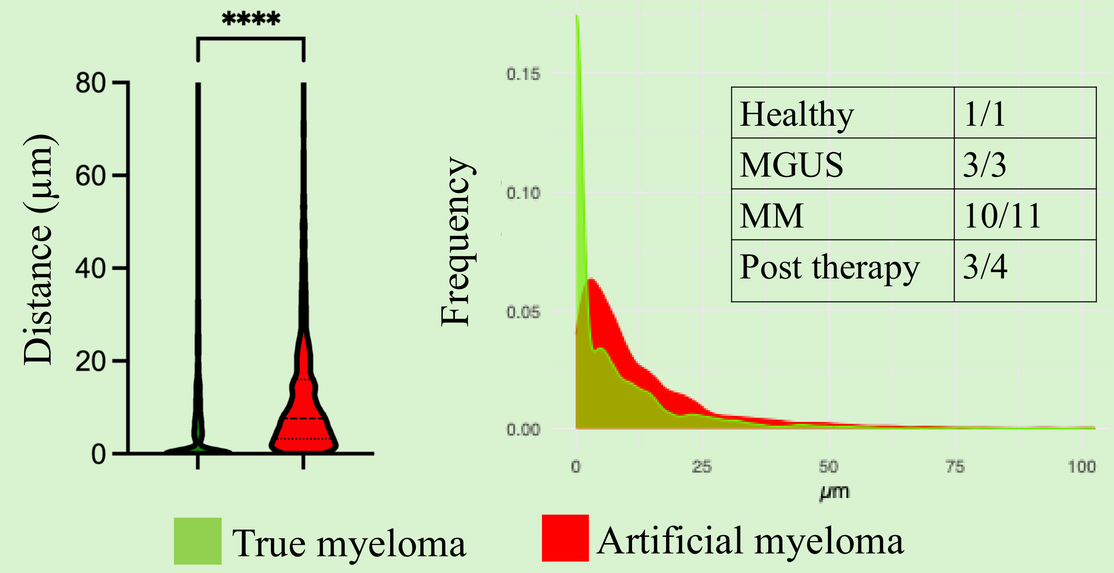


***Figure 1.*** *Bone marrow section, MUM1-positive cells with DAB staining (upper left). Localized areas: blue – adipocyte, green – true myeloma cell, red – artificial myeloma cell (lower left). Myeloma cells in the adipocyte microenvironment, boundary line width 10 µm (right).*

Our analysis demonstrated that a significant proportion of myeloma cells are located in close proximity to bone marrow adipocytes, and the average distance between myeloma cells and adipocytes was significantly smaller than a random distribution would imply (**Figure 2**).




| | |
|---|---|
| Healthy | 1/1 |
| MGUS | 3/3 |
| MM | 10/11 |
| Post therapy | 3/4 |

***Figure 2.*** *Distance of true (green) and artificial (red) myeloma cells from the nearest adipocyte per patient (representative result, left). Distribution of MM – adipocyte distances (representative histogram, middle). Summary of analyzed samples (right) showing number of cases where MM cells are closer to BMATs than a random placement would imply.*

## Results continued

Our flow cytometric analysis revealed a significantly higher proportion of myeloma cells in the BMAT throughout the disease. In non-tumor controls, BMAT contained a higher the frequency of plasma cells compared to the aspirate (**Figure 3, top**).

While the percentage of CD16$^{hi}$ cytotoxic NK cells was similar between the aspirate and BMAT in non-tumor controls, the increase in the frequency of cytotoxic NK cells observed in the aspirate in MM patients was absent from BMAT **(Figure 3, bottom).**

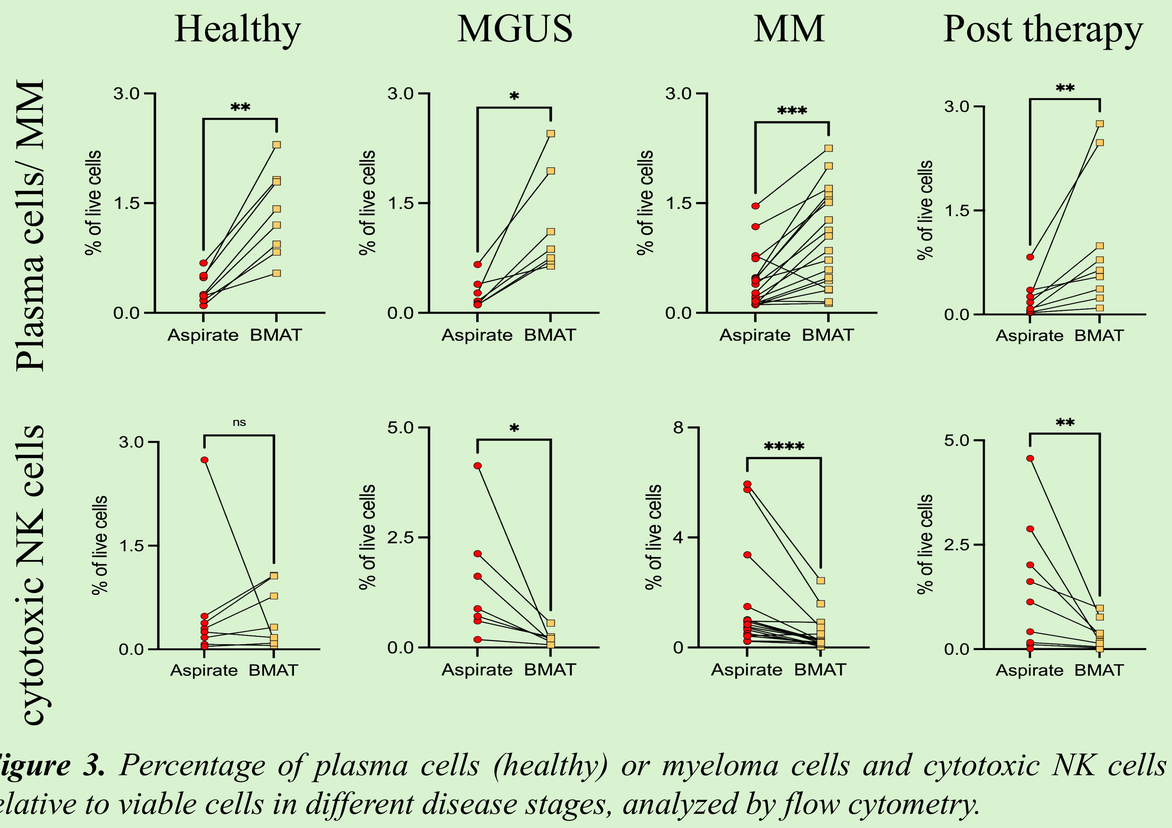



***Figure 3.*** *Percentage of plasma cells (healthy) or myeloma cells and cytotoxic NK cells relative to viable cells in different disease stages, analyzed by flow cytometry.*

## Conclusions

Our findings suggest that BMAT might serve as a pro-plasma cell niche, regardless of clonality. Furthermore, the significantly lower frequency of cytotoxic NK cells suggests that BMAT might suppress anti-myeloma immune responses. The exact significance of these fat-associated MM cells and their contribution to disease burden warrants further investigation.

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