XIX CONGRESSO NAZIONALE SIES 2026

SPATIAL IMAGING UNLOCKS THE POTENTIAL OF CHARTING MULTIPLE MYELOMA AND EXTRAMEDULLARY DISEASE

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Palazzo degli Affari

Disclosures

In the last two years I have had the following relationships, including financing ones, with entities with commercial interests in the healthcare field:

Pfizer - Sanofi - BMS - Takeda - J & J – AstraZeneca – Csl B - Amgen - Menarini - GSK

These potential conflicts of interest do not imply bias or influence on Prof. Solimando's opinions or actions. Prof. Solimando is aware of the importance of transparency in the scientific field and is committed to defending his integrity and the trust placed in him within the scientific community.

Multiple myeloma: Extramedullary Disease (EMD)

Malignant plasma cells grow at anatomic sites distant from the bone marrow:

•soft tissue, spleen, lymph nodes, malignant effusion, CNS, skin, …

Primary (at diagnosis)
or secondary (at disease progression) EMD

•primary: approximately 1-5% of patients (i.e. soft tissue plasmacytoma)

•secondary: approximately 3-20% of patients (i.e. malignant pleural effusion)

Varettoni M et al. Annals of oncology 2010
Rasche Leo et al. Annals of hematology 2012
Mangiacavalli Silvia et al. Annals of hematology 2017
Rasche, L., et al. Nature communications 2017
Da Vià MC, et al. Oncologist. 2020
Montefusco Vittorio et al. Haematologica 2020
Bansal R, et al. Blood Cancer J. 2021
Jiménez-Segura R, et al. Blood Cancer J. 2022
Bladé J, et al. Blood cancer journal 2022
Zanwar S, et al. Blood advances 2025
Ho M, et al. Current oncology 2025
Tao Y, et al. BMC medicine 2025

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Bansal R, et al. Blood Cancer J. 2021

•Bone marrow **cytogenetics** and protein / gene expression

•**Proliferative** capacities
•EMD: **multiple genes** and signaling **pathways**

Primary EMD
t(4;14)
t(14;16)
del(17p)
gain(1q)

Secondary EMD
del(17p)
del(13q)
Loss of surface CD56
MAFB overexpression
MYC overexpression

Jiménez-Segura R, et al. Blood Cancer J. 2022
Bladé J, et al. Blood cancer journal 2022
Zanwar S, et al. Blood advances 2025
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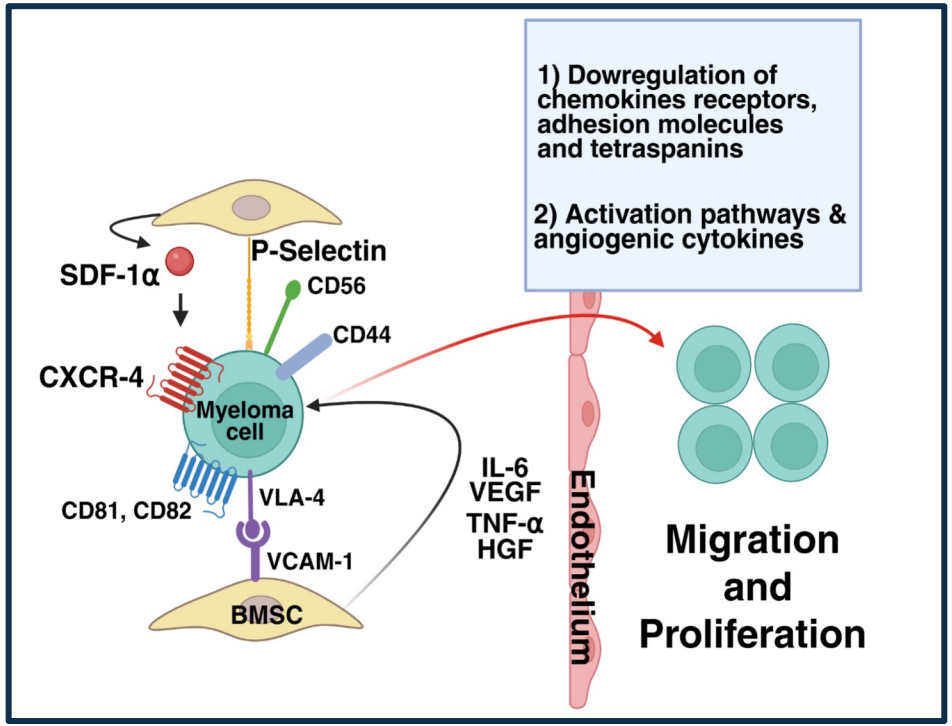
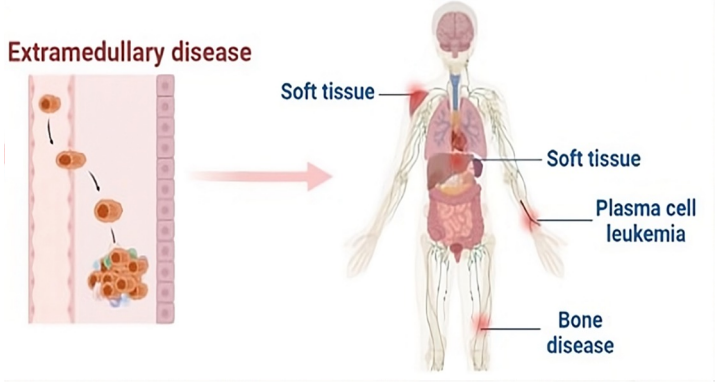
EMD biology and Tumor Microenvironment - TME



- The tumor microenvironment constitutes a **homeostatic niche** that supports PCs proliferation, influences therapeutic response and contributes to disease relapse.
- Disease progression is characterized by the aberrant activation of **intracellular signaling pathways** and the secretion of **pro-angiogenic cytokines**.

Roccaro A M, et al. Cell reports 2015
Bhutani M, et al. Leukemia 2020
Yao, et al. Clin. Epigenetics 2018
Bansal R, et al. Blood Cancer J. 2021
Bianchi G, et al. Blood cancer discovery 2021
Merz M, et al. Blood advances 2023
Notarfranchi L, et al. Haematologica. 2024
Martello M ,et al. Blood cancer journal 2024
Lutz R, et al. Science immunology 2025
Anilkumar S A, et al. Blood cancer discovery 2026

Failure of Modern Therapies in Extramedullary Disease



- Extramedullary disease is characterized by the colonization of **distal anatomical site**, including soft tissues, lymph nodes, the central nervous system, and the dermo-epidermal layer, by malignant plasma cells.

This manifestation represents a clinically **aggressive phenotype**, frequently associated with **high-risk** cytogenetic aberrations, **reduced treatment susceptibility** and a significantly **poorer prognosis**.

Failure of Modern Therapies in EMD (anti-CD38 mAb, BiTEs and CAR-T)

Mailankody S, et al. ASCO 2024
Zanwar, et al. "Journal of hematology & oncology 2024
Kumar S, et al.American journal of hematology 2026
Ho M, et al. Current oncology (Toronto, Ont.) 2025
Kumar S, et al. "The New England journal of medicine 2026

Modern Therapies in Extramedullary Disease

Therapy Class	Representative Studies	Median Prior Lines	ORR in EMM	PFS in EMM	Key Message
BCMA CAR-T (Ide-cel / Cilta-cel)	KarMMa, CARTITUDE, multiple real-world cohorts	~6	**52–60%**	~5 months	Markedly inferior vs non-EMM
Investigational BCMA CAR-T	Early phase trials (China / US)	~3–4	**80–92%**	~5–8 months	Responses but limited durability
BCMA Bispecific (Teclistamab)	MajesTEC-1 + real-world studies	~5–6	**35–47%**	~2 months	Substantially reduced efficacy
Bispecific (mixed analyses)	Meta-analysis of BiTE trials	NA	**~48%**	NA	Lower than CAR-T
Dual targeting (BCMA + GPRC5D)	**RedirecTT-1**	~5	**~79%**	12-mo PFS ~61%	Most promising results

Mailankody S, et al. ASCO 2024
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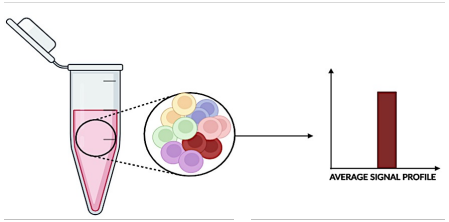
Investigational Problems in Characterizing the TME

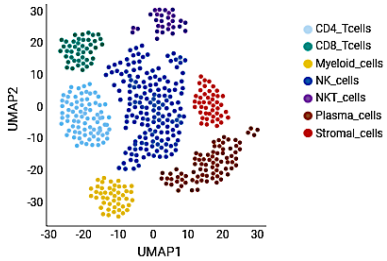
Bulk analysis



Loss of cellular heterogeneity information.

Dunphy K, et al. Cancers 2023
Zanwar S, et al. Blood advances 2025

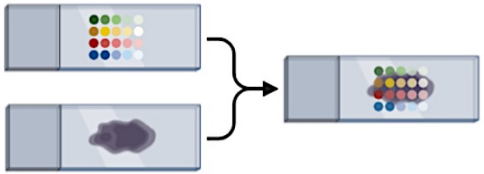
Single-cell analysis



The spatial architecture of the lesion cannot be investigated with this approach

Ryu D, et al. Clinical cancer research 2020
Jelinek T, et al. Leukemia 2024

Spatial transcriptomics



- **Background biological signals**
- **Lack of correlation between mRNA and protein levels**
- **Limited applicability to FFPE samples**
- **Resolution not always high or single-cell**

John M, et al. Blood 2024
Anilkumar S A, et al. Blood cancer discovery 2026

Which Is The Optimal Choice?

Editorial

https://doi.org/10.1038/s41592-024-02565-3

Method of the Year 2024: spatial proteomics

Check for updates

Approaches for profiling the spatial proteome in tissues are the basis of atlas-scale projects that are delivering on their promise for understanding biological complexity in health and disease.

Humans by nature are inquisitive. We love to explore, and as we do, we make maps. Perhaps it is then not surprising that we find ourselves in an age of atlas-building as we explore the incredible complexity of biological systems with cutting-edge methods to better understand how cells, tissues, organs and organisms connect structure and function.

In the spirit of exploring and mapping biological complexity, we have chosen spatial proteomics as our Method of the Year for its critical role in revealing the organization of complex tissues. Spatial proteomics is an umbrella term that covers a broad swath of immunohistochemistry-based methods including, but not limited to, cyclic immunofluorescence (cycIF), co-detection by indexing (CODEX), iterative bleaching extends multiplexity (IBEX), multiplexed ion beam imaging (MIBI) and imaging mass cytometry (IMC). These approaches can be used to generate highly multiplexed images of specimens such as tissue and organ slices to understand their protein composition and spatial organization and are the basis of many global atlas projects. We are also excited about a newer technique known as deep visual proteomics (DVP), in which complex samples are laser dissected and individual dissociated cells are analyzed by mass spectrometry in such a way that their spatial context information is retained to create spatial protein maps. A major benefit of this technique is that it is not limited by the number of available antibodies and thus achieves substantially greater proteome coverage.

Spatial proteomics technologies such as immunofluorescence have been around longer than other spatial biology methods, such as spatially resolved transcriptomics, which was our Method of the Year in 2021, so why are we choosing it as Method of the Year now? For one, we were excited by the recent

development of DVP and other methods seeking new ways to explore the spatial proteome in greater depth and breadth. In addition, we were inspired by the current efforts of large consortia such as the Human BioMolecular Atlas Program (HuBMAP) and the Human Tumor Atlas Network (HTAN) not only to create large atlases of data for the scientific and medical communities, but also to develop tools to process, analyze, visualize and mine the data to go beyond the pretty pictures and deeper into biological discovery. This month's issue features two papers from the HTAN consortium: an Article from Peter Sorger and colleagues[1] describing CyLinter, an improved tool for quality control of highly multiplexed images, and an Article from Benjamin Raphael and colleagues[2] presenting CalicoST, an algorithm to simultaneously infer allele-specific copy number aberrations and reconstruct spatial tumor evolution from spatially resolved transcriptomics data. For this month's News Feature, journalist Vivien Marx asked some researchers about atlas-building and where they see things going next[3].

Our special issue features a series of Comments on the past, present and future of spatial proteomics. The first piece[4], from Bernd Bodenmiller, introduces why proteins are such interesting targets for biological investigations and offers a brief look back at how immunofluorescence has grown into the field of spatial proteomics. The piece further describes how generating large atlases will help reveal the intricacies of complex tissues and pave the way for precision medicine. It ends with a discussion of technological developments that will help move the field forward, briefly commenting on the role artificial intelligence may play in the future.

Computational tools for spatial proteomics are the focus of the second Comment, from Yuval Bussi and Leeat Keren[5]. These authors note that current image processing and analysis workflow are well defined but fragmented, with various steps happening back to back rather than in an integrated fashion. They envision a future for the field where image processing and analysis steps work in concert for improved biological discovery.

The third Comment[6], from Daniela Quail and Logan Walsh, discusses how spatial proteomics has revolutionized cancer research, from our understanding of tissue organization and cell–cell interactions to how it has shaped our thinking on how the immune system interacts with tumors. The piece also covers the potential role of spatial proteomics in combination with artificial intelligence in generating hypotheses for basic research, improving personalized medicine, and guiding future therapeutic strategies to fight cancer.

The fourth Comment, from Thierry Nordmann, Andreas Mund and Matthias Mann[7], introduces deep visual proteomics and the benefits of using mass spectrometry to probe the complexity of the proteome during processes such as development or in disease. They note that future improvements to sensitivity will allow mass spectrometry access to the entire proteome, including post-translational modifications, with single-cell resolution. They also discuss the benefits of combining DVP with other 'omics methods, future efforts to democratize the technology, and moving the technology into the clinic.

Is spatial proteomics enough? The final Comment, from Rong Fan[8], discusses spatial proteomics in the context of other 'omics technologies and the importance of integrating complementary technologies such as spatial transcriptomics and spatial epigenetic profiling to gain a more holistic understanding of biological complexity. This piece

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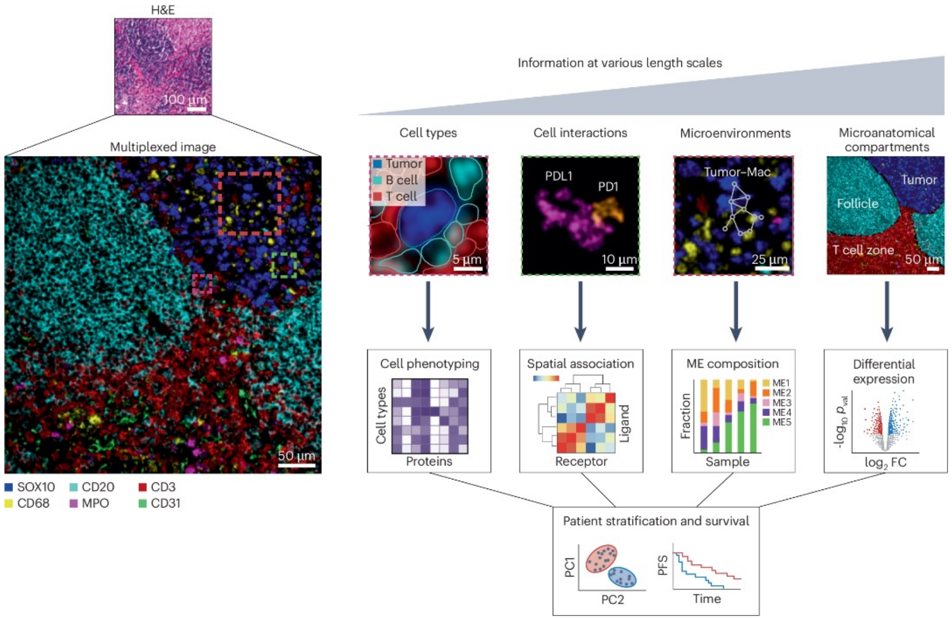
nature methods

Volume 21 | December 2024 | 2195–2196 | 2195



Method of the Year 2024: spatial proteomics.Nature methods 2024

Bussi Y, et al. Nat Methods. 2024

EMD Unmasked: A Clinical Pilot Study

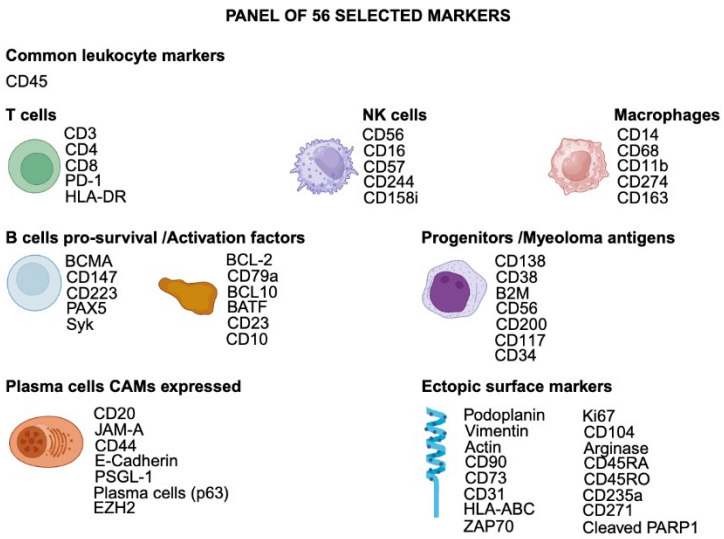
Five patients with primary EMD (paired BM and EMD)

Strengths of Spatial Proteomics

- Direct measurement of proteins
- Better correlation with cellular function
- High spatial resolution
- Multiplexing capability
- Preservation of tissue morphology
- Applicability to FFPE samples

ID	Sex	Age	Isotype	Restriction	Site	ISS	R-ISS	CRAB-SLiM	Cytogenetics
MM#1	M	50	IgD	Lambda	Right lung and soft tissue	2	2	AB-LiM	del(17p13)
MM#2	M	60	LC	Lambda	Skin	3	2	CRAB - S	t(11;14)
MM#3	F	66	IgA	Lambda	Kidney	2	3*	A - Li	del(17p13) and t(4;14)
MM#4	F	66	IgA	Lambda	Back muscle	2	2	CA -	no alterations
MM#5	M	72	LC	Lambda	Deltoid muscle	3	2	R - SLi	no alterations

M: male; F: female; Ig: immunoglobulin; LC: light chain; ISS: International Staging System; R-ISS: Revised International Staging System. C: hypercalcemia; R: renal disease; A: Anemia; B: osteolytic lesions at CT-scan, S: >60% on bone marrow biopsy, Li: light chain ratio > 100, M: at least 1 lesion >5mm on magnetic resonance

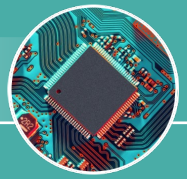


Desantis V, et al. J Hematol Oncol 2025

The MACSima™ Spatial Biology Platform

Miltenyi Biotec

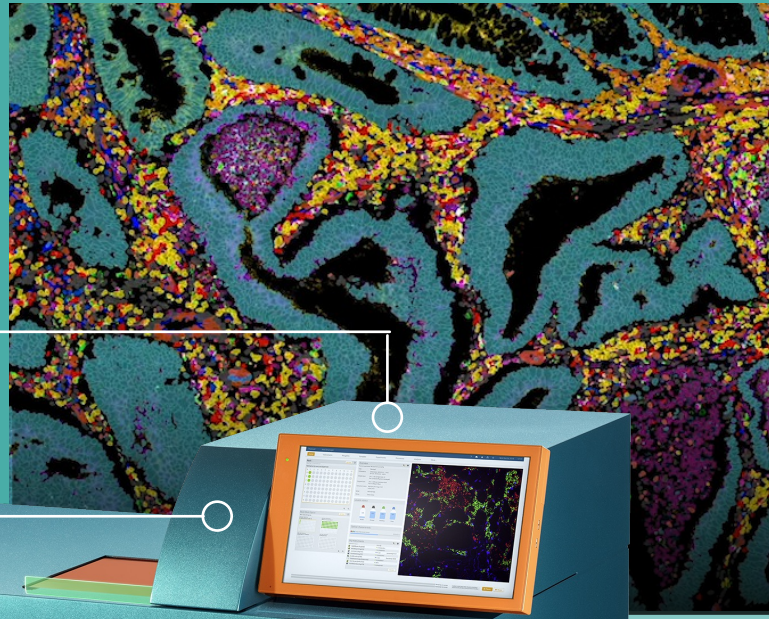
Computers



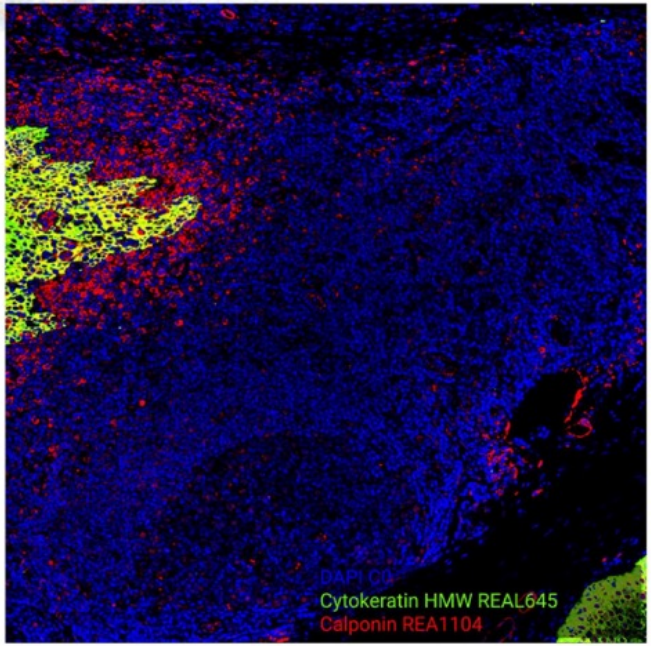
Liquid handling system

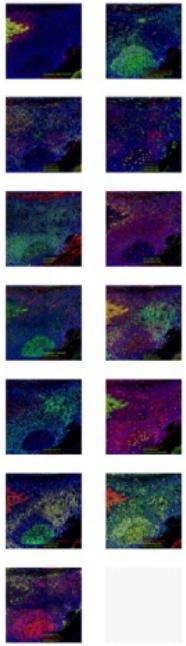
Ultraprecise stage

State-of-the-art microscope and camera



MACSima™ Platform
MICS technology (MACSima Imaging Cyclic Staining)

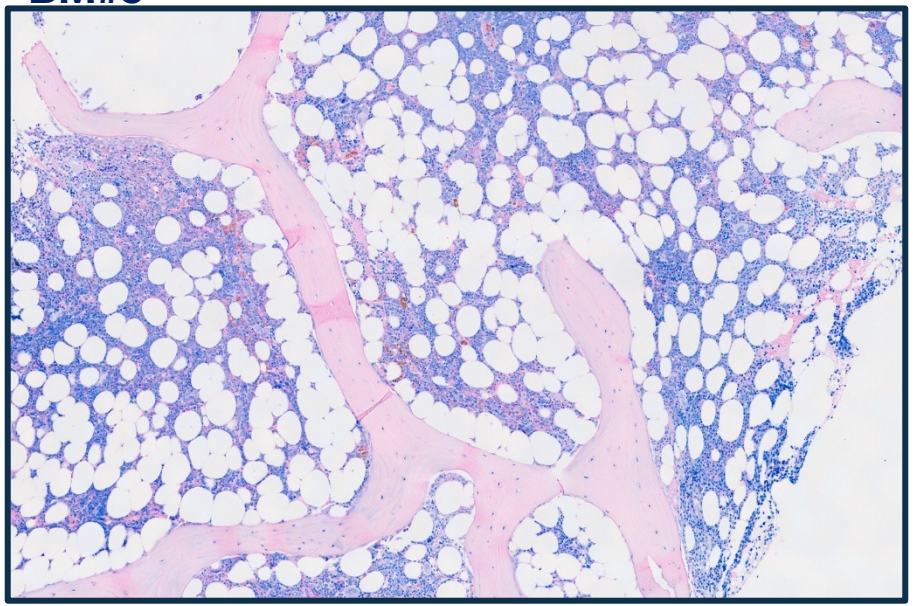




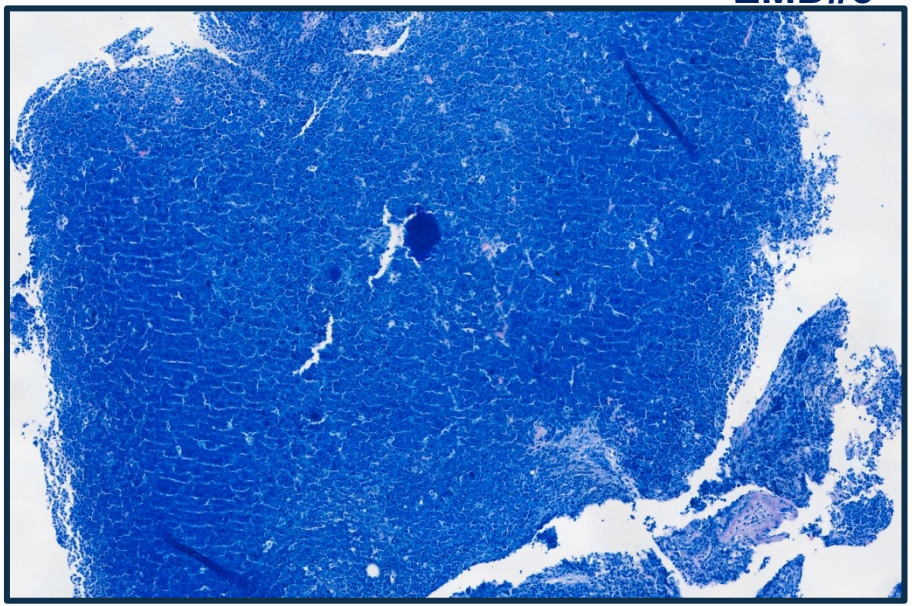
200+ markers!

Spatial Analysis - H&E Stained Sections

BM#5



EMD#5



Desantis V, et al. J Hematol Oncol 2025

Spatial Analysis - H&E Stained Sections

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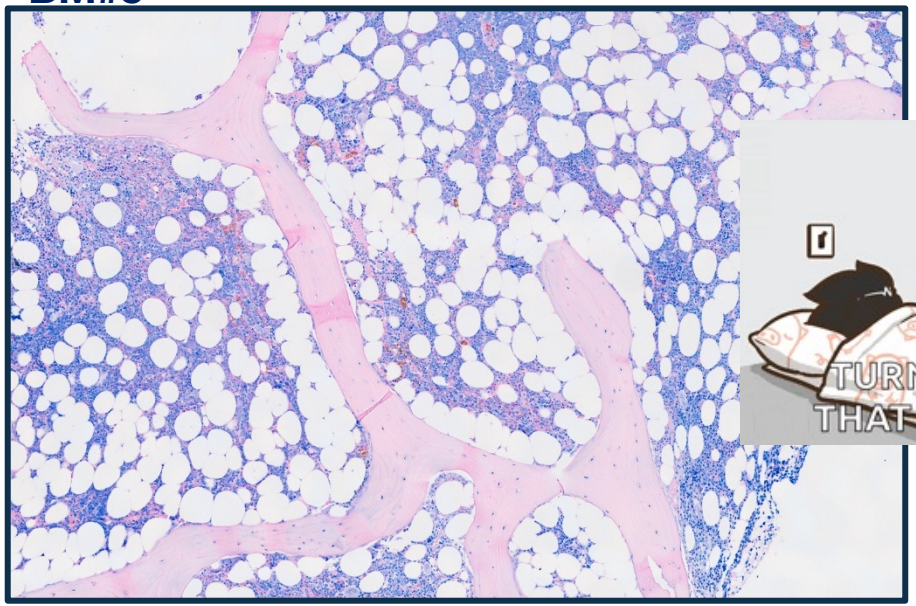

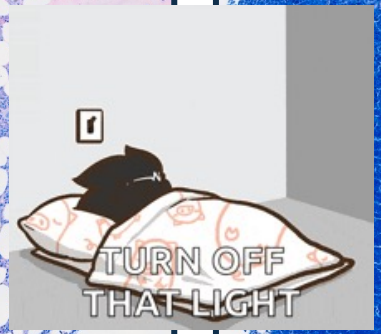

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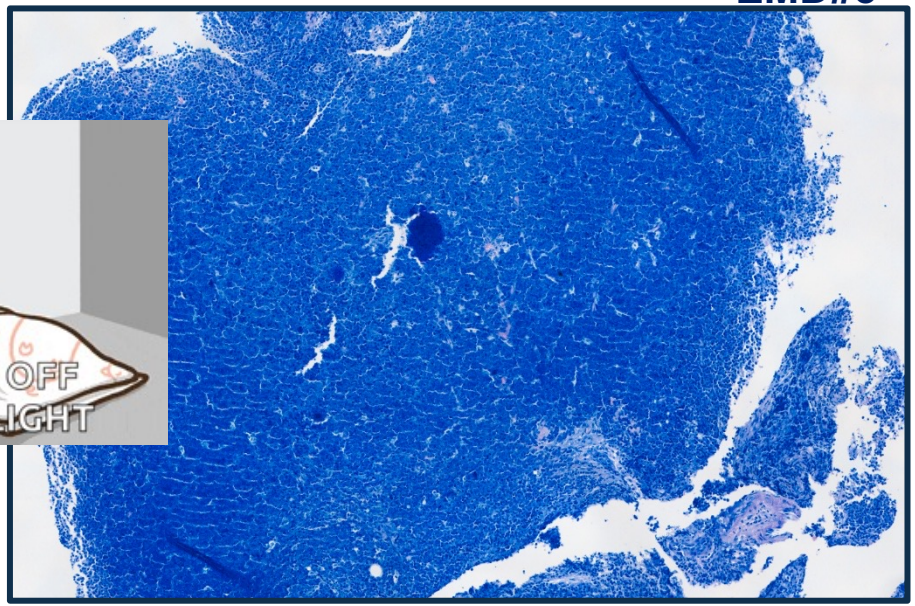

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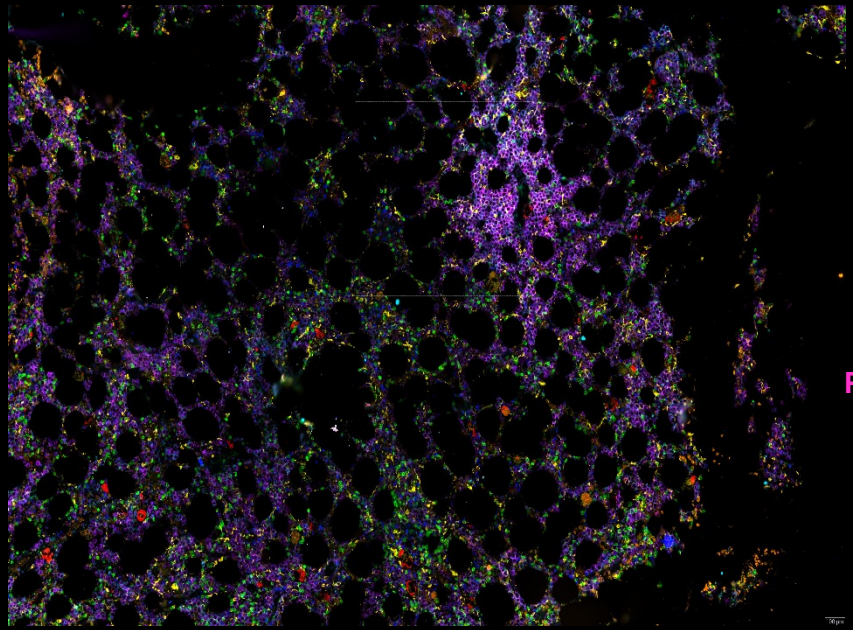
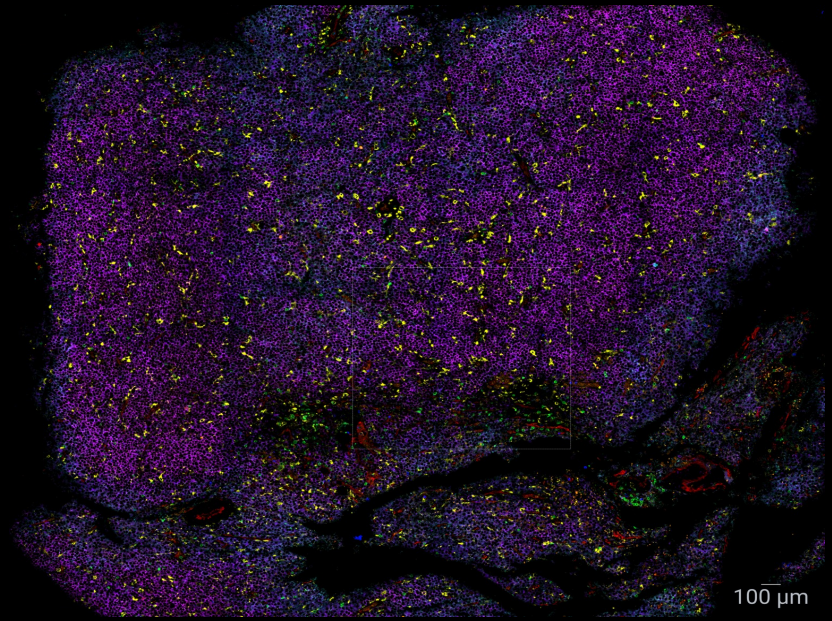
Spatial Profiling of BM niche and EMD in MM

BM#5



CD3
CD38
CD31
CD138
CD235a
CD68
CD11b
Plasma Cells

EMD#5



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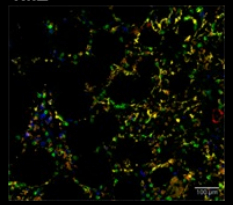
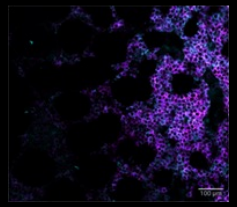
Pilot study on five extramedullary disease patients

Spatial analysis – Selected ROI on samples

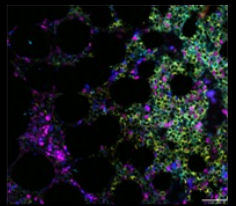
TME



PCs

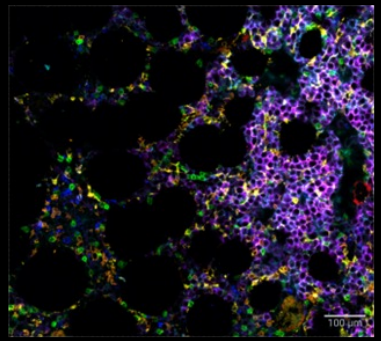


PCs biomarker expression

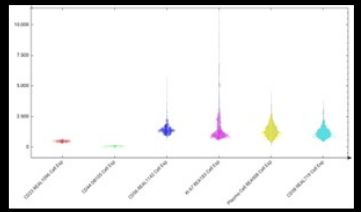


Desantis *et al. J Hematol Oncol* 2025

BM#5



Strip + Violin Plot of PCs



CD3
CD38
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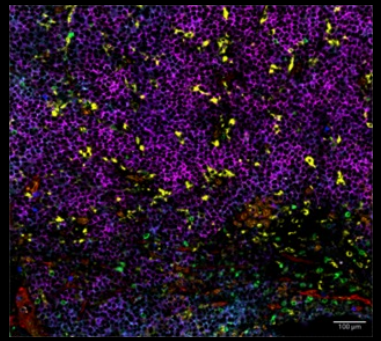
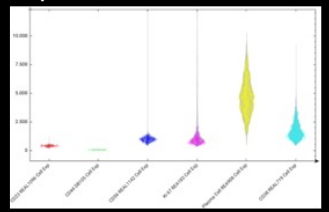
Plasma Cells

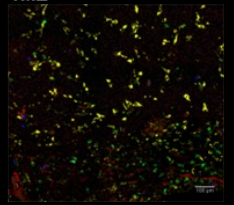
EMD#5



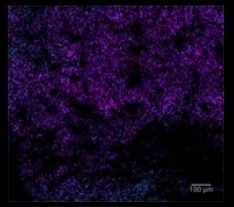
Strip + Violin Plot of PCs



TME

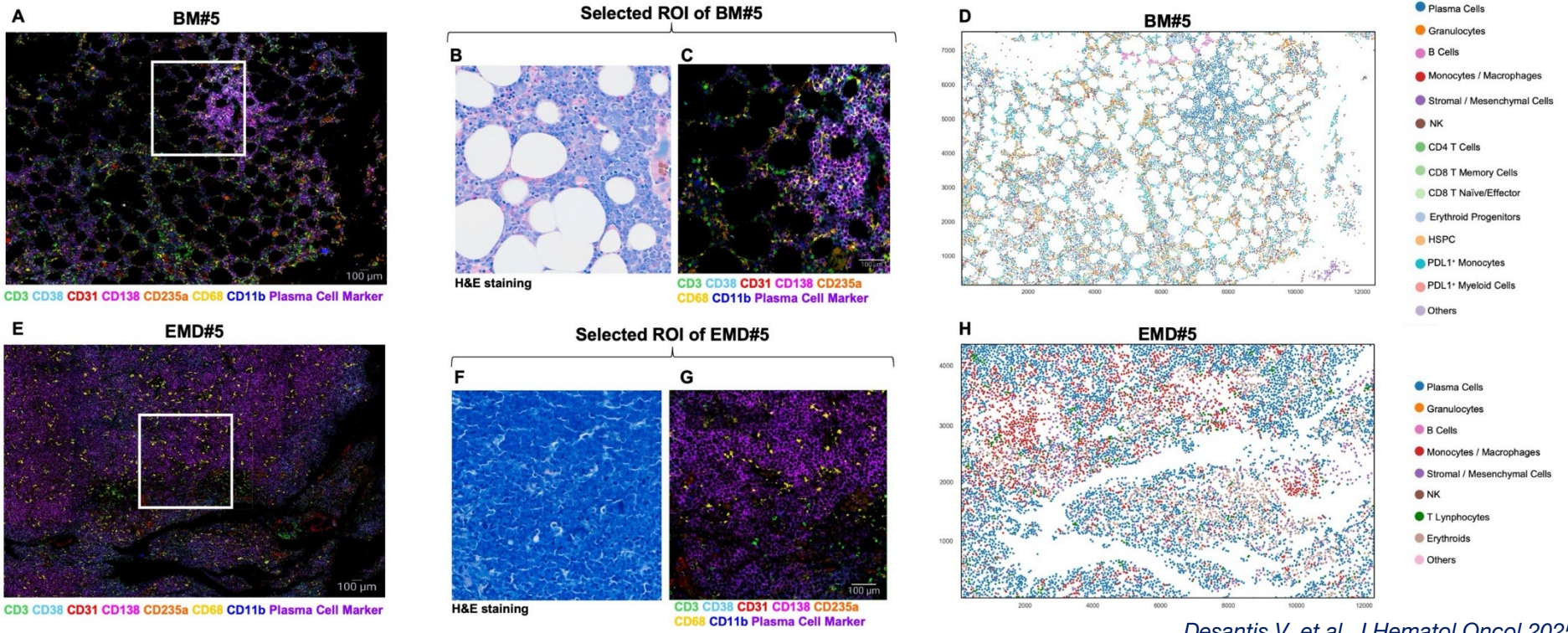


PCs



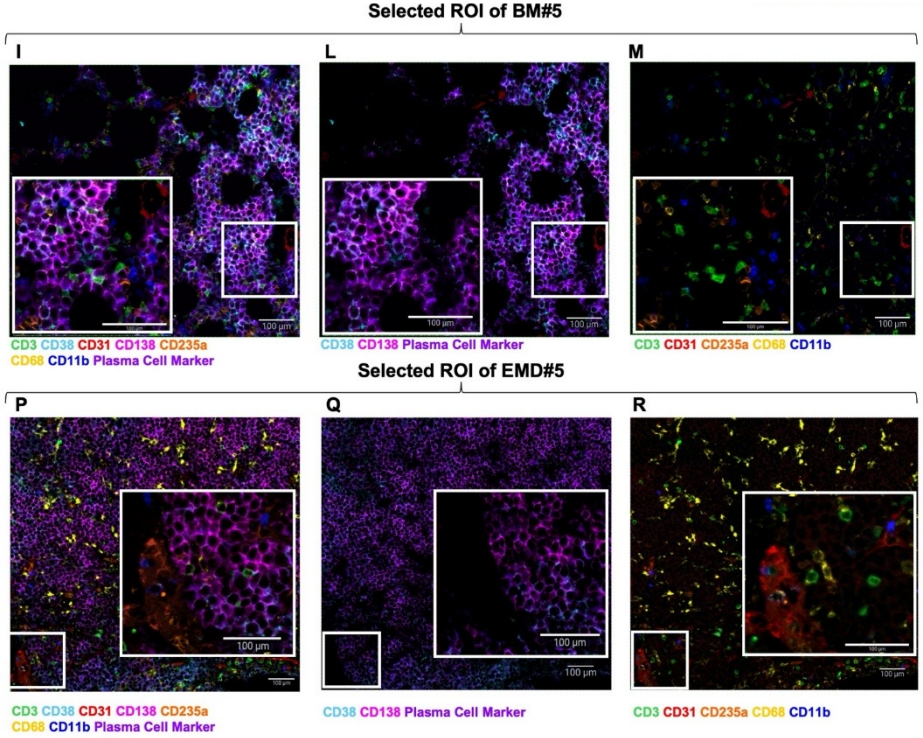
PCs biomarker expression

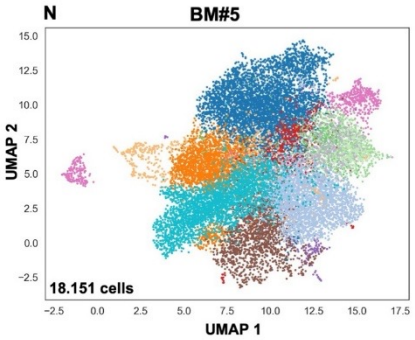
Cell annotation of bone marrow and EMD

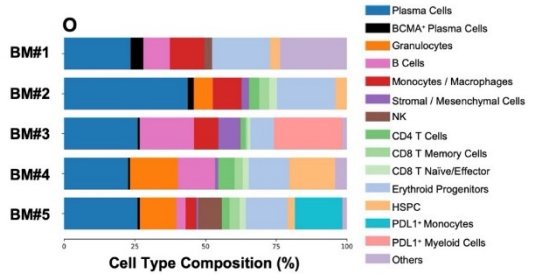


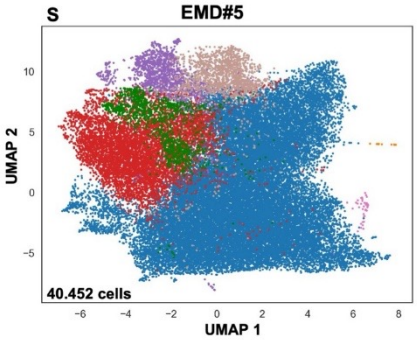
Desantis V, et al. J Hematol Oncol 2025

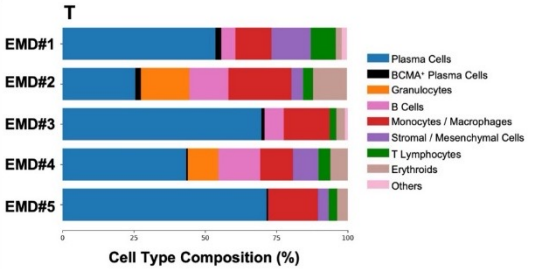
Loss of TME Diversification In EMD Sites











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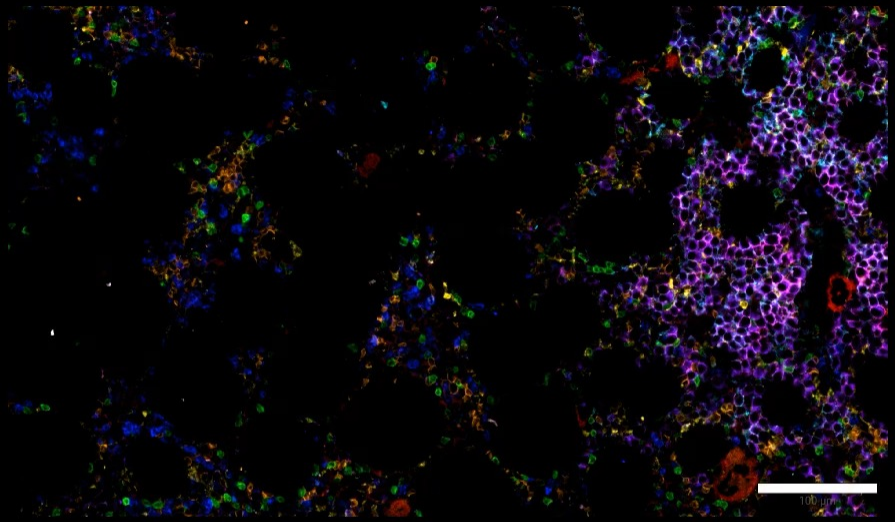

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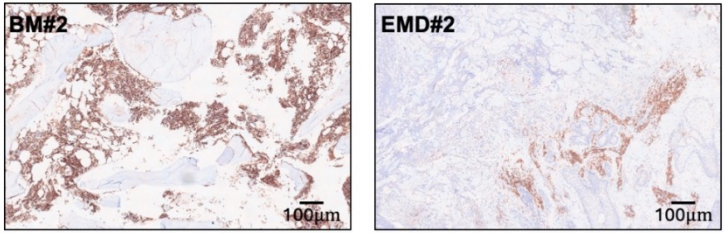
CD38 Expression on PCs in BM and EMD Sites

A direct 56-marker profiling of matched BM and EMD disease explains why EMD patients show worse outcomes in the era of current therapies

	Mean BM CD38 expression	Mean EMD CD38 expression	P value
MM#1	1874	198	< 0.0001
MM#2	824	119	< 0.0001
MM#3	740	246	< 0.0001
MM#4	878	382	< 0.0001
MM#5	665	328	< 0.0001

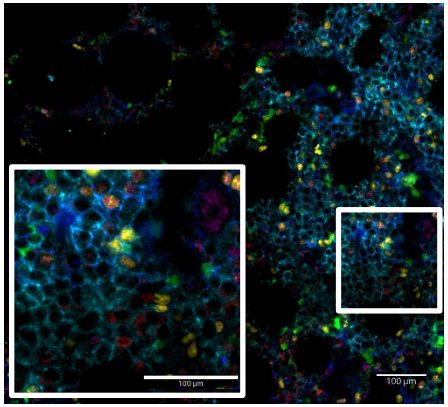


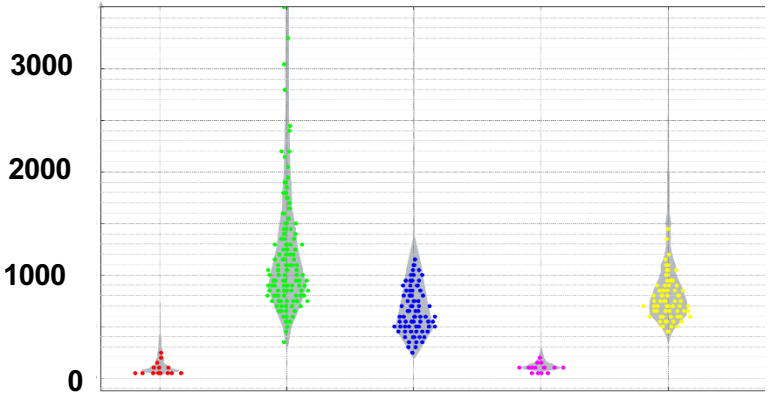
CD38 is a PCs surface marker

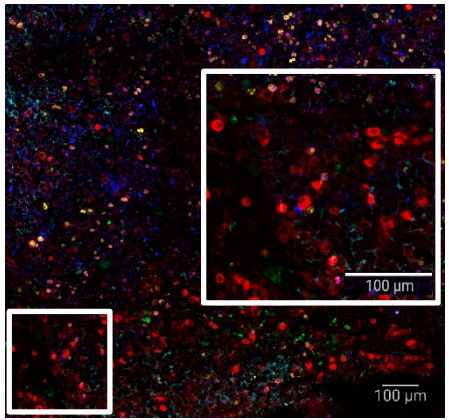
There is a **reduced expression of CD38 in EMD samples**

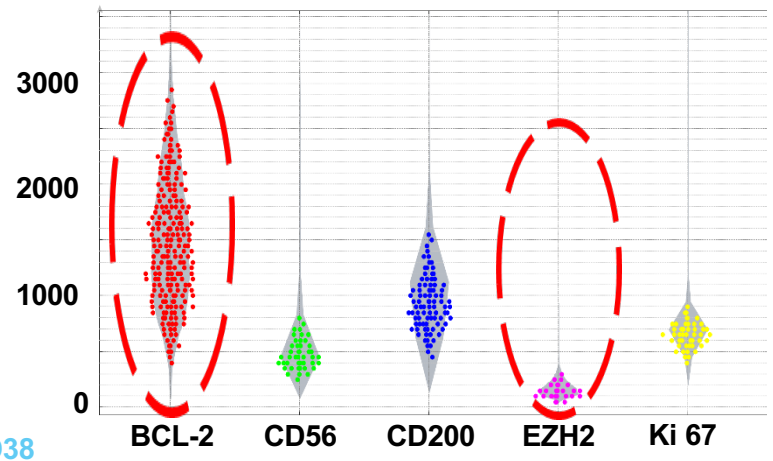
Resistance to anti-CD38 therapies

Saltarella I, et al. Cells 2020
Jelinek T, et al. Leukemia 2024
Notarfranchi L, et al. Haematologica. 2024
Desantis V, et al. J Hematol Oncol 2025





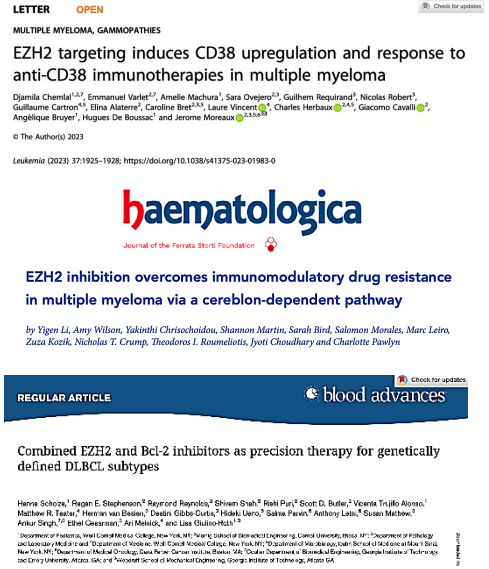




BCL-2 CD56 CD200 EZH2 Ki 67 CD38

EZH2 activation may drive CD38 downregulation and lenalidomide resistance

Concurrent BCL2 upregulation may create a therapeutic vulnerability exploitable with BCL2-targeted strategies



LETTER OPEN

MULTIPLE MYELOMA, GAMMOPATHIES

EZH2 targeting induces CD38 upregulation and response to anti-CD38 immunotherapies in multiple myeloma

Djamila Chemlal[1,2,7], Emmanuel Varlet[1,2,7], Amelie Machura[1], Sara Ovejero[2,3], Guilhem Requirand[1], Nicolas Robert[1], Guillaume Cartron[4,5], Elina Alaterre[6], Caroline Bret[1,2,5], Laure Vincent[4], Charles Herbaux[4,5,6], Giacomo Cavalli[2], Angélique Bruyer[1], Hugues De Boussac[1] and Jerome Moreaux[1,2,3,5,6,8]

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haematologica

Journal of the Ferrata Storti Foundation

EZH2 inhibition overcomes immunomodulatory drug resistance in multiple myeloma via a cereblon-dependent pathway

by Yigen Li, Amy Wilson, Yakinthi Chrisochoidou, Shannon Martin, Sarah Bird, Salomon Morales, Marc Leiro, Zuza Kozik, Nicholas T. Crump, Theodoros I. Roumeliotis, Jyoti Choudhary and Charlotte Pawlyn

REGULAR ARTICLE

blood advances

Combined EZH2 and Bcl-2 inhibitors as precision therapy for genetically defined DLBCL subtypes

Hanna Schöne,[1] Regan E. Stephenson,[2] Raymond Reynolds,[2] Shivani Shah,[2] Rishi Puri,[2] Scott D. Butler,[2] Vicente Trujillo Alonso,[1] Matthew R. Teater,[4] Herman van Besien,[2] Destin Gibbs-Cuna,[2] Hideki Uero,[2] Salma Parekh,[4] Anthony Letai,[4] Susan Mathew,[2] Ankur Singh,[1,2] Ethel Cesarman,[2] Ari Melnick,[4] and Lisa Giulino-Roth[1,3]

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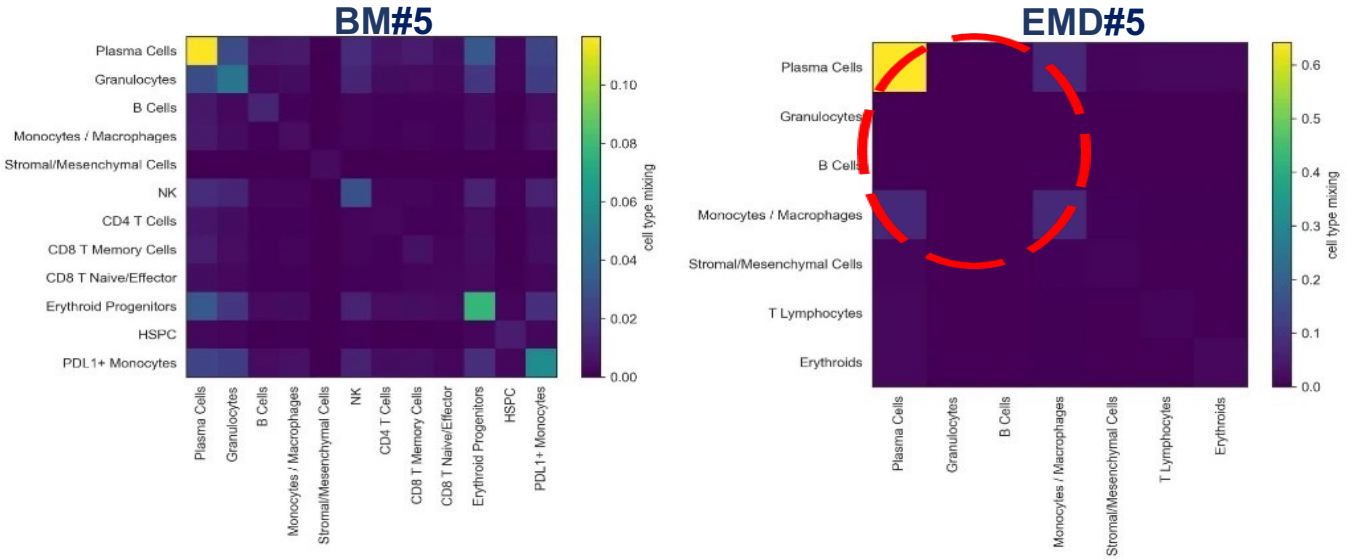
Comparative neighborhood analysis in BM and EMD lesions



Heatmaps illustrate the proportion of links across all potential cell-type pairs to measure cell-type mixing. EMD lesions exhibit a widespread loss of cellular interactions, with the **sole exception** of macrophage-mediated ones.

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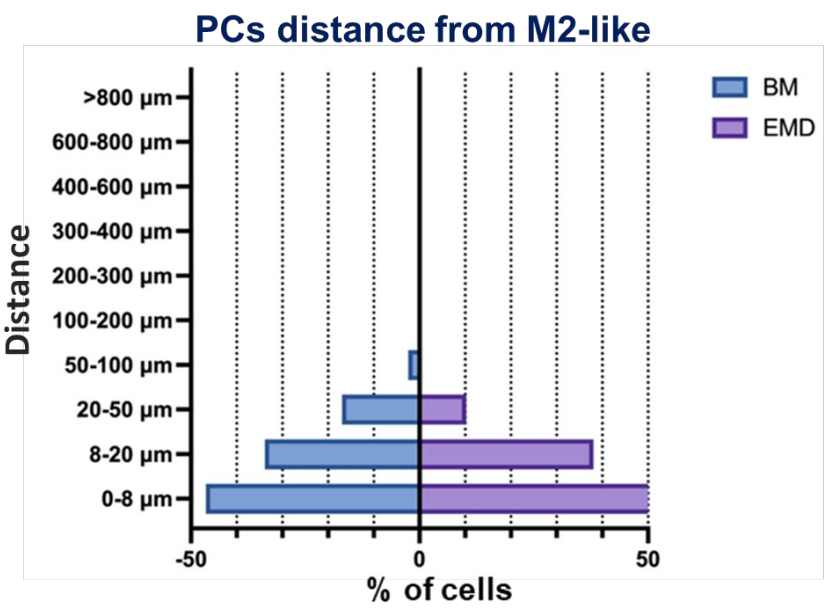
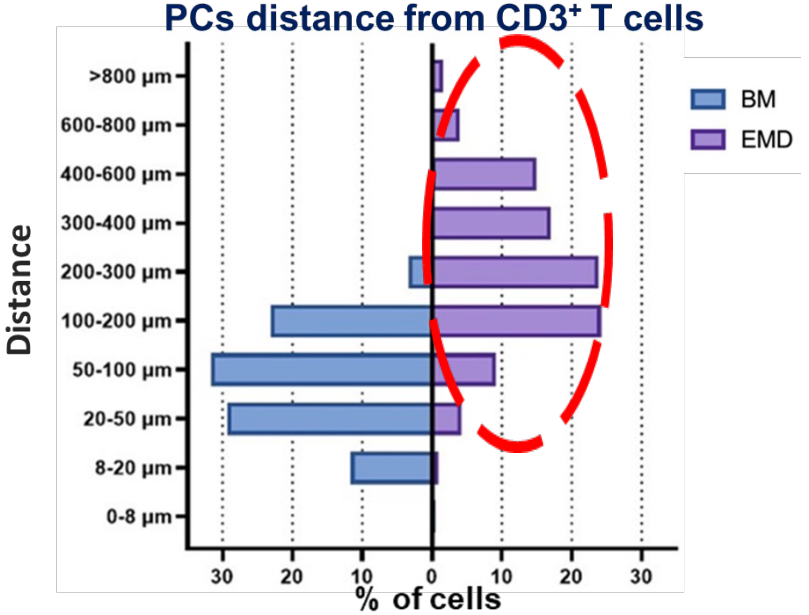
Immune desertification in EMD niche





T-cell exclusion drives immune desertification and limits CAR-T and BiTE therapy efficacy.

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Summary

- **Spatial proteomics** provides deeper insights into the pathogenetic and immune-evasion mechanisms of multiple myeloma, uncovering **spatial relationships** between MM cells and immune subsets within lesions.

- Mapping protein expression and intercellular distances may elucidate the causes of **moAb**, **BiTEs**, **CAR-T** therapy failure in extramedullary disease (EMD); furthermore, identifying **EMD "drivers"** could refine risk stratification and guide the development of targeted treatments.

- Spatial proteomics holds promise as a **Companion Diagnostic** tool, defining patient-specific proteomic profiles to support **personalized** therapeutic strategies.

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