CORRESPONDENCE OPEN

Check for updates

Deep immune cell profiling in blood and bone marrow of early stage monoclonal gammopathy: an iStopMM and ECRIN-M3 collaborative study

© The Author(s) 2025

Blood Cancer Journal (2025)15:46 ; https://doi.org/10.1038/s41408-025-01255-3

Dear Editor,

Increasing evidences support a key role of the immune system in the development and progression of monoclonal gammopathies (MG), i.e., from premalignant stages—e.g., MG of undetermined significance (MGUS), smoldering multiple myeloma (SMM) and smoldering Waldenström's macroglobulinemia (SWM)—to overt malignant diseases—e.g., multiple myeloma (MM) and Waldenström's macroglobulinemia (WM)–[1–3]. In this regard, an altered crosstalk in the bone marrow (BM) between neoplastic cells—clonal plasma cells (cPC) and clonal B-cells (cB-cells)—and the surrounding immune cell populations may lead to an impaired response that favors immunoescape, clonal growth and disease progression [1–3]. However, the specific mechanisms involved in loss of immunosurveillance, together with those immune alterations that emerge at the earliest stages of MGUS, remain largely unknown. Here, we used next-generation flow cytometry (Table S1) to we investigate the distribution of a broad variety of immune cell populations ($n = 360$) in paired blood and BM samples of 75 (otherwise) healthy donors (HD) from the general population who screened positive for MGUS—$n = 55$ (18 IgM-MGUS; 37 non-IgM-MGUS)–, SMM ($n = 12$) and SWM ($n = 8$), within the iStopMM program [4] (Data S1).

Except for increased eosinophil ($p = 0.01$) and decreased dendritic cells (DC) numbers ($p = 0.02$), MGUS cases showed normal blood counts of all other major myeloid cell populations. In parallel, a decreased neutrophil production was found in BM of MGUS ($p < 0.001$), SMM ($p < 0.001$) and SWM ($p = 0.009$), which only translated into abnormally low blood counts at the more advanced disease stages—i.e., SMM ($p = 0.003$)–, in line with previous observations in MM [5]. Unlike neutrophils, an increased percentage of total BM monocytes was observed in MGUS ($p = 0.02$) and SMM ($p = 0.006$) [6], associated with normal total monocyte counts in blood, but decreased levels of non-classical—i.e., $CD36^-$ and $Slan^+$ in MGUS ($p \le 0.003$); $CD36^+$ and $Slan^-$ in MGUS ($p \le 0.004$) and SMM ($p \le 0.008$)—monocytes ($p < 0.001$) in MGUS, which suggests a preserved monocyte production in BM associated with a potentially increased migration of blood monocytes to tissues, preferentially affecting the more mature (i.e., non-classical) monocyte compartment [6]. Conversely, SWM patients displayed significantly increased monocyte counts in blood at the expense of the major subsets of classical—$CD62L^-$, $CD62L^+$ and $FcεRI^-$ ($p \le 0.005$)—monocytes ($p \le 0.001$). In parallel, decreased numbers of blood circulating Axl^+ DC, associated with lower blood counts of both $CD1c^+$ myeloid (m)DC and $CD141^+$ mDC, only $CD141^+$ mDC, or just plasmacytoid (p)DC, were found in MGUS ($p \le 0.03$), SMM ($p \le 0.04$) and SWM ($p \le 0.03$) subjects, respectively, supporting an increased migration to the tumor sites of Axl^+ DC [2, 3, 7], together with different subsets of mDC and pDC in MGUS, SMM and SWM, respectively. This might (locally) promote different immune cell microenvironments [2, 8] and (potentially also) unique downstream T-cell and adaptive immune response profiles in MGUS, SMM and SWM, modulated in SWM by locally increased monocytic-myeloid-derived suppressor cells (M-MDSC) ($p = 0.02$) (Fig. 1 and S1; Tables S2-5).

In fact, MGUS cases showed decreased T-cell counts in blood ($p = 0.03$), particularly at the expense of $TCD4^+$ naive cells ($p = 0.02$), Treg-like follicular helper (TFH) cells ($p = 0.006$), Tregs ($p = 0.002$)—and their naive, Th1-, Th2- and Th17-like subsets ($p = \le 0.007$)–, Th2 ($p = 0.008$)—and Th2-effector memory cells ($p < 0.001$)—and Th1-17 cells ($p = 0.001$)—Th1-Th17-central, transitional and effector memory cells ($p \le 0.003$)—together with increased numbers of blood-circulating TFH-naive cells ($p < 0.001$). In turn, decreased $TCD4^+$ naive cells ($p = 0.04$) and Treg-naive, Th1-like, Th2-like and Th17-like Tregs ($p \le 0.02$), together with increased TFH-naive ($p = 0.006$) and Th22 cells ($p = 0.004$)—Th22-central and effector memory cells ($p \le 0.04$)—were found in SMM, whereas Treg-like ($p = 0.05$) and Th1-17-like ($p = 0.05$) TFH-cells, together with Tregs ($p = 0.03$)—and their Th1-, Th17- and Th22-like subsets ($p \le 0.05$)– were those $TCD4^+$ subsets altered in SWM. Overall, these findings suggest a reduced $TCD4^+$ cell and Treg production, with an increased recruitment of specific functional compartments of these latter cells to the tumor tissue (e.g., BM) already at the earliest stages of MG, in line with previous observations in overt disease [3, 9, 10]. Despite this, only slightly (non-significantly) elevated percentages of $TCD4^+$ cells were found in BM of MGUS and SMM. However, more detailed analysis of the distribution of the functional subsets of $TCD4^+$ cells suggests a pro-inflammatory response, with increased memory and/or effector Th2 and Th1-17 cells in MGUS, together with decreased numbers of innate lymphoid cells (ILC) ($p < 0.001$) in blood, at the expense of their Th2- and Th17-inducing ILC2 ($p = 0.01$) and ILC3 ($p < 0.001$) subsets, respectively (Figs. 1–2 and S1; Tables S2–3) [11]. Interestingly, BM infiltration by immunosuppressive Tregs reported in SMM and SWM [2] appears to occur already in MGUS cases, which may trigger local immunosuppression already at an early stage, prior to disease progression.

In parallel, $TCD8^+$ naive cells, total NK-cells and their major $CD56^{lo}$ cytotoxic NK-cell compartment [12] were all decreased in blood of MGUS ($p \le 0.007$) and SMM ($p \le 0.01$), while in WM, only the NK-cell populations were altered ($p = 0.03$). Likewise, the cytokine-secreting immunomodulatory [12] $CD56^{hi}$ (cyGranzyme-

Received: 2 January 2025 Revised: 19 February 2025 Accepted: 11 March 2025
Published online: 27 March 2025

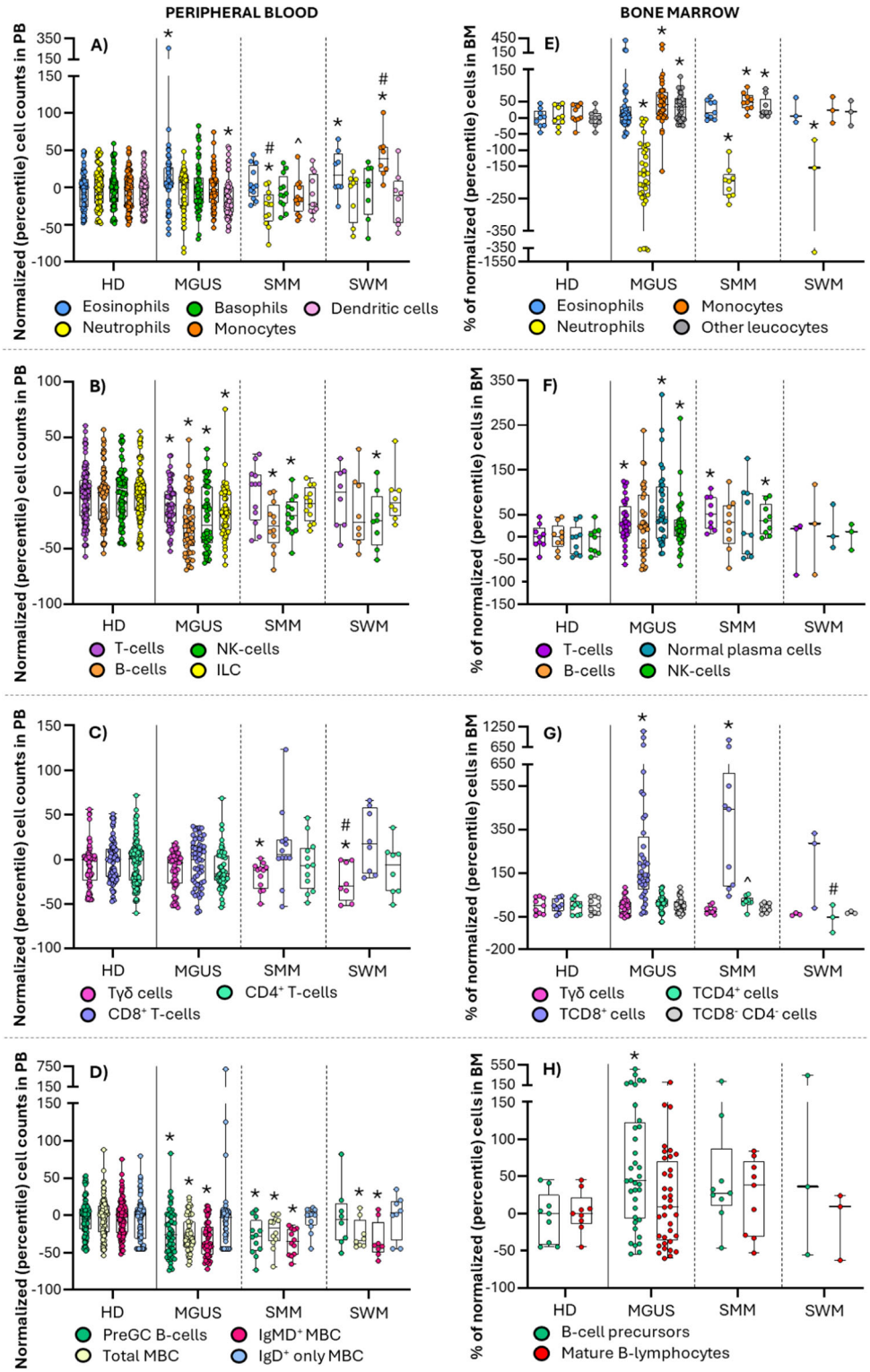
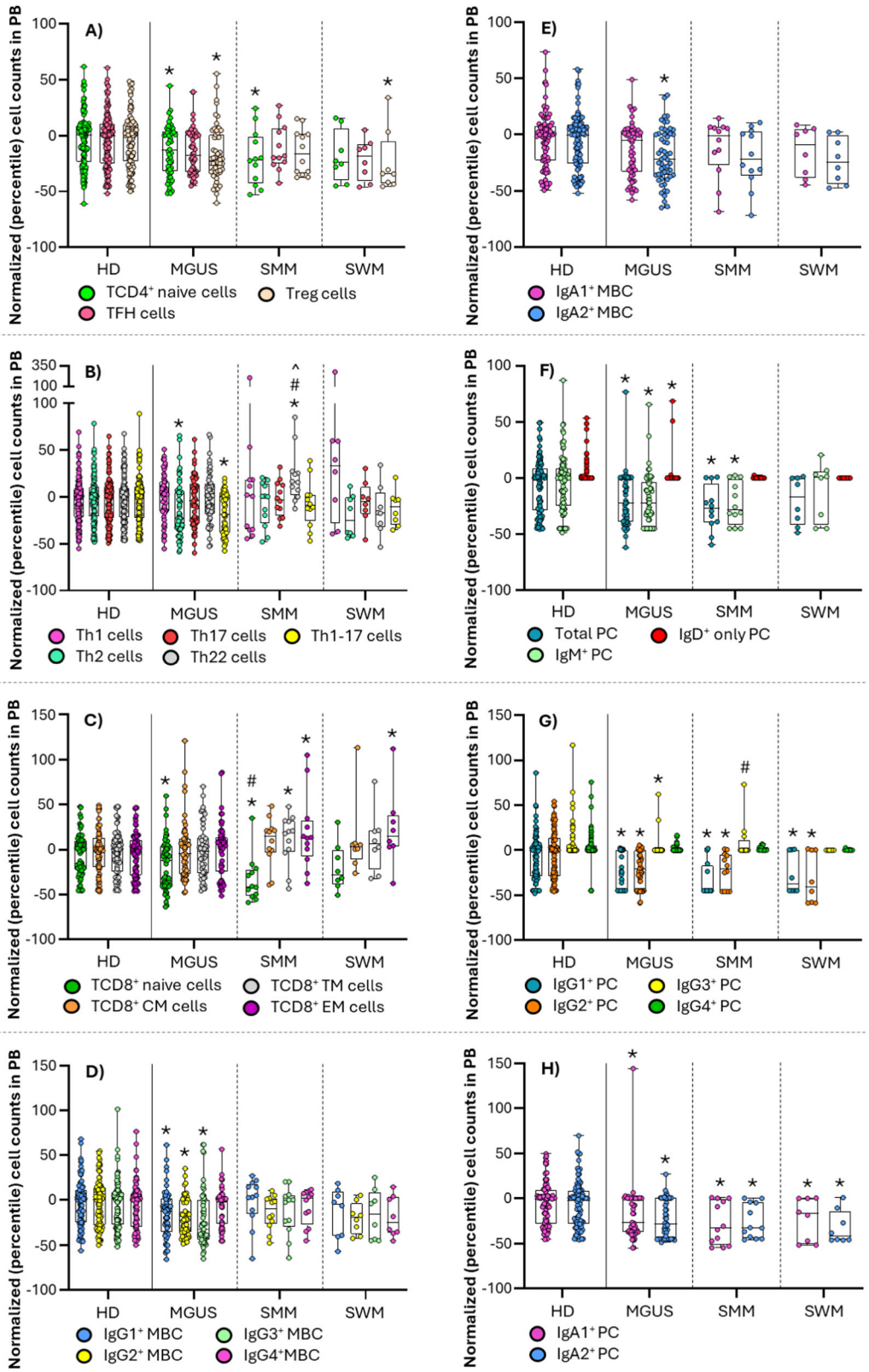


Fig. 1 Distribution of the main immune cell populations in blood and BM of monoclonal gammopathy of undetermined significance (MGUS) ($n = 55$), smoldering multiple myeloma (SMM) ($n = 12$) and smoldering Waldenström's macroglobulinemia (SWM) ($n = 8$) patients compared to healthy donors (HD) ($n = 118$) and normal BM ($n = 57$). In (**A**–**D**), the distribution in peripheral blood of total eosinophils, neutrophils, basophils, monocytes and dendritic cells (**A**), total T-cells, B-cells, NK-cells and ILC (**B**), Tγδ$^+$, TCD8$^+$ and TCD4$^+$ cells (**C**), and pre-germinal center B-cells and IgMD$^+$ and IgD$^+$ memory B-cells (MBC) (**D**) is shown, while in panels E-H the percentage of bone marrow total eosinophils, neutrophils, monocytes and nucleated red cells (**E**), total T-cells, B-cells, normal plasma cells and NK-cells (F), Tγδ$^+$, TCD8$^+$, TCD4$^+$ and TCD8$^-$ CD4$^-$ cells (**G**), and pre-germinal center B-cells and mature B-lymphocytes (**H**) is displayed. Box plots extend from the 25th to the 75th percentile values, while the horizontal lines indicate median values and both the minimum and maximum values (whiskers) of cell counts in blood and percentage numbers in bone marrow normalized for each individual case (inner circles) by the median percentile values of aged-matched HD. HD, healthy donor; ILC, innate lymphoid cells; MBC, memory B-cells; MGUS, monoclonal gammopathy of undetermined significance; preGC, pre-germinal center; SMM, smoldering multiple myeloma; SWM, smoldering Waldenström's macroglobulinemia. *$p < 0.05$ vs HD; #$p < 0.05$ vs MGUS; ^$p < 0.05$ vs SWM.



B^+ and $CD57^-$) NK-cell population was also decreased in blood of MGUS ($p = 0.03$), while increased in BM of MGUS ($p = 0.006$) and SMM ($p = 0.001$) (but not SWM), suggesting an enhanced recruitment of progressively higher numbers of effector cytotoxic (e.g., $TCD8^+$ and NK) cells to the tumor niche [1, 2, 9, 13]. Altogether, these findings point to an enhanced cytokine-secreting and immune-regulatory vs cytotoxic response at the earliest (MGUS) vs more advanced (e.g., SMM) disease stages, that might prevent excessive T-cell responses to proinflammatory stimuli already in MGUS [12, 14]. Of note, $TCD8^+$ effector memory

Fig. 2 Distribution of the major T-cell, B-cell and plasma cell populations in blood of monoclonal gammopathy of undetermined significance (MGUS) ($n = 55$), smoldering multiple myeloma (SMM) ($n = 12$) and smoldering Waldenström's macroglobulinemia (SWM) ($n = 8$) patients compared to healthy donors (HD) ($n = 118$). **A** $TCD4^+$ naive, TFH and T-regulatory (Treg) cells; **B** Th1, Th2, Th17, Th22 and Th1-17 $TCD4^+$ cells; **C** $TCD8^+$ naive, central memory, transitional memory and effector memory cells; **D** $IgG1^+$, $IgG2^+$, $IgG3^+$ and $IgG4^+$ MBC; **E** $IgA1^+$ and $IgA2^+$ MBC; **F** total plasma cells (PC), IgM^+ and IgD^+ PC; **G** $IgG1^+$, $IgG2^+$, $IgG3^+$ and $IgG4^+$ PC; and **H** $IgA1^+$ and $IgA2^+$ PC. Box plots extend from the 25th to the 75th percentile values, while the horizontal lines indicate median values and both the minimum and maximum values (whiskers) of cell counts in blood normalized for each individual case (inner circles) by the median percentile values of aged-matched HD. HD, healthy donor; MBC, memory B-cells; MGUS, monoclonal gammopathy of undetermined significance; PC, plasma cells; preGC, pre-germinal center; SMM, smoldering multiple myeloma; SWM, smoldering Waldenström's macroglobulinemia; TFH, follicular helper T-cell; Th, helper T-cell; Treg, regulatory T-cell. *$p < 0.05$ vs HD; #$p < 0.05$ vs MGUS; ^$p < 0.05$ vs SWM.

cells increased in SMM ($p = 0.04$) and SWM ($p = 0.03$), mostly showed a cyGranzyme-B^- $CD57^{-/+}$ exhausted/immunosenescent phenotype, which might result from chronic exposure to target antigens in the cPC niche [9, 13]. Likewise, abnormally high $TCD8^+$ cell numbers were found in BM of MGUS ($p < 0.001$) and SMM ($p < 0.001$), but not SWM. Globally, these data confirm previous observations pointing to the association between the emergence of oligoclonal expansions of cytotoxic T-cells in blood and in the tumor BM environment (as an attempt to control the outgrowth of cPC) in patients with MG [9, 13], and a better prognosis [9, 13]. In contrast, decreased $T\gamma\delta^+$ cytotoxic cell counts were identified in blood of SMM ($p = 0.05$) and in both blood ($p = 0.02$) and BM ($p = 0.04$ for $CD3^{hi}$ $T\gamma\delta^+$ cells) of SWM, pointing towards a limited role of $T\gamma\delta^+$ cells in BM tumor surveillance at the earliest MG stages (Fig. 2 and S1; Tables S2-5).

Among all immune cells, B-cells were the most strikingly altered compartment. Thus, decreased counts of both total (normal residual) PC and B-lymphocytes—at the expense of pre-germinal center (GC) B-cells and memory B-cells (MBC)–, were found in blood of MGUS ($p < 0.001$) and SMM ($p \leq 0.01$), while only MBC ($p = 0.01$) were decreased in SWM. Of note, unswitched-$IgMD^+$ MBC ($p \leq 0.004$) were decreased in blood across all patient groups, together with lower numbers of different subsets of switched-MBC in MGUS—$IgG_{1\text{-}3}$ and $IgA2^+$ ($p \leq 0.02$)–, but not SMM and SWM. Interestingly, except for an increased percentage of precursor B-cells ($p = 0.03$) in MGUS, normal B-cell percentages were found in BM of all patient groups, suggesting an impaired generation of both switched-MBC and unswitched-MBC in MGUS, potentially due to a progressively decreased production and/or survival of (the more mature $CD5^-$) naive B-lymphocytes. In SWM, however, the altered B-cell profile points to an intrinsically altered production and/or expansion of unswitched MBC, similar to SMM. Altogether, these results might reflect the generation of increased percentages of MBC is from pre-existing switched-MBC, due to a progressively more limited response to new antigens, and an increased risk of infection in more advanced disease stages [15]. In this regard, decreased numbers of recently-produced normal PC were observed in blood of MGUS ($p < 0.001$) and SMM ($p = 0.01$), which might be responsible in the medium-to-long-term immunoparesis observed in high-risk SMM and MM [15]. Interestingly, decreased IgM^+, $IgG_{1\text{-}2}$ and $IgA_{1\text{-}2}$ PC counts were found in blood of MGUS ($p < 0.001$) and SMM ($p \leq 0.02$), together with decreased serum IgM in non-IgM-MGUS ($p = 0.03$) and SMM ($p < 0.001$), and decreased serum IgG in IgM-MGUS ($p < 0.001$) (Figure S2). Conversely, SWM cases displayed decreased PC numbers –i.e., $IgG_{1\text{-}2}$ ($p \leq 0.03$) and $IgA_{1\text{-}2}$ ($p \leq 0.007$) switched-PC– associated with decreased serum IgG ($p = 0.04$) (but not uninvolved IgM) antibody levels, supporting an Ig class-switch defect in SWM, rather than a decreased ability to respond to new antigens (Figs. 1–2 and S2; Tables S2–3). Globally, these findings suggest a progressively defective production of naive B-lymphocytes associated with a decreased generation of PC and production of (IgM) antibodies against new antigens in (non-IgM) MGUS and SMM, leading to progressively decreased IgM serum levels and a potentially greater susceptibility to infection [15]. In contrast, in SWM, a specific PC Ig-class switch defect would exist, with decreased $IgG_{1\text{-}2}$ and $IgA_{1\text{-}2}$ PC counts, and serum IgG titers (similarly to IgM-MGUS), associated with normal preGC, switched-MBC and total PC numbers. Whether the defective IgM vs IgG immune responses observed here at the very early non-IgM-MGUS (and SMM) vs IgM-MGUS (and SWM) stages are a predisposing condition or a consequence of the clonally expanded tumor B-cell/PCs, remains to be elucidated.

Overall, our results revealed uniquely altered immune profiles already at the earliest MGUS stages, which specifically involved the B-cell and PC compartments, among other adaptative and innate immune cells. Of note, these altered immune profiles partially overlapped, but differed significantly from those observed in SMM and, more prominently even, in SWM (Figs. S3–4).

Oihane Pérez-Escurza[1,2,3], Juan Flores-Montero[1,2,3,4,5], Jón Þórir Óskarsson[6], Luzalba Sanoja-Flores[4,7], Julio Pozo[1], Quentin Lécrevisse[1,2,3,4], Silvia Martín[1], Elín Ruth Reed[6], Guðlaug Katrín Hákonardóttir[6], Stephen Harding[8], Sigrún Þorsteinsdóttir[6,9], Sæmundur Rögnvaldsson[6,10], Thorvardur Jon Love[6,10], Brian Durie[11], Sigurdur Yngvi Kristinsson[6,12], Alberto Orfao[1,2,3,4]✉ and on behalf of the iStopMM and ECRIN-M3 consortia*

*[1]Translational and Clinical Research Program, Cancer Research Center (IBMCC, CSIC—University of Salamanca); Cytometry Service, NUCLEUS; Department of Medicine, University of Salamanca (Universidad de Salamanca), Salamanca, Spain. [2]Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. [3]Department of Medicine, University of Salamanca (Universidad de Salamanca), Salamanca, Spain. [4]Biomedical Research Networking Centre Consortium of Oncology—CIBERONC (CB16/12/00400)–, Instituto de Salud Carlos III, Madrid, Spain. [5]Department of Hematology, University Hospital of Salamanca, Salamanca, Spain. [6]Faculty of Medicine, University of Iceland, Reykjavík, Iceland. [7]Institute of Biomedicine of Seville, Department of Hematology, University Hospital Virgen del Rocío of the Consejo Superior de Investigaciones Científicas (CSIC), University of Seville, Seville, Spain. [8]The Binding Site Group, Birmingham, UK. [9]Department of Hematology, Rigshospitalet, Copenhagen, Denmark. [10]Department of Science, Landspitali University Hospital, Reykjavík, Iceland. [11]Department of Hematology and Oncology, Cedars-Sinai Medical Center, Los Angeles, CA, USA. [12]Department of Hematology, Landspitali University Hospital, Reykjavík, Iceland. *A list of authors and their affiliations appears at the end of the paper.*

✉*email: orfao@usal.es*

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Dhodapkar MV. The immune system in multiple myeloma and precursor states: lessons and implications for immunotherapy and interception. Am J Hematol. 2023;98:S4–12.

2. McCachren SS, Dhodapkar KM, Dhodapkar MV. Co-evolution of immune response in multiple myeloma: implications for immune prevention. Front Immunol. 2021;12:1–13.
3. García-Ortiz A, Rodríguez-García Y, Encinas J, Maroto-Martín E, Castellano E, Teixidó J, et al. The role of tumor microenvironment in multiple myeloma development and progression. Cancers. 2021;13:1–22.
4. Rögnvaldsson S, Love TJ, Thorsteinsdottir S, Reed ER, Óskarsson JÞ, Pétursdóttir Í, et al. Iceland screens, treats, or prevents multiple myeloma (iStopMM): a population-based screening study for monoclonal gammopathy of undetermined significance and randomized controlled trial of follow-up strategies. Blood Cancer J. 2021;11:94.
5. Schmidt-Hieber M, Pérez-Andrés M, Paiva B, Flores-Montero J, Perez JJ, Gutierrez NC, et al. CD117 expression in gammopathies is associated with an altered maturation of the myeloid and lymphoid hematopoietic cell compartments and favorable disease features. Haematologica. 2011;96:328–32.
6. Damasceno D, Almeida J, Teodosio C, Sanoja-Flores L, Mayado A, Pérez-Pons A, et al. Monocyte subsets and serum inflammatory and bone-associated markers in monoclonal gammopathy of undetermined significance and multiple myeloma. Cancers. 2021;13:1–18.
7. Giannotta C, Autino F, Massaia M. The immune suppressive tumor microenvironment in multiple myeloma: the contribution of myeloid-derived suppressor cells. Front Immunol. 2023;13:1102471.
8. Verheye E, Bravo Melgar J, Deschoemaeker S, Raes G, Maes A, De Bruyne E, et al. Dendritic cell-based immunotherapy in multiple myeloma: challenges, opportunities, and future directions. Int J Mol Sci. 2022;23:904.
9. Pasiarski M, Grywalska E, Kosmaczewska A, Góźdź S, Steckiewicz P, Garus B, et al. Assessment of peripheral blood and bone marrow T, NK, NKT and dendritic cells in patients with multiple myeloma. Postepy Hig Med Dosw Online. 2015;69:1435–42.
10. Bryant C, Suen H, Brown R, Yang S, Favaloro J, Aklilu E, et al. Long-term survival in multiple myeloma is associated with a distinct immunological profile, which includes proliferative cytotoxic T-cell clones and a favourable Treg/Th17 balance. Blood Cancer J. 2013;3:1–7.
11. Eberl G, Colonna M, Di Santo JP, McKenzie ANJ. Innate lymphoid cells: a new paradigm in immunology. Science. 2015;348:aaa6566.
12. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001;22:633–40.
13. Allegra A, Innao V, Allegra AG, Pugliese M, Di Salvo E, Ventura-Spagnolo E, et al. Lymphocyte subsets and inflammatory cytokines of monoclonal gammopathy of undetermined significance and multiple myeloma. Int J Mol Sci. 2019;20:1–25.
14. Laroni A, Uccelli A. CD56bright natural killer cells: a possible biomarker of different treatments in multiple sclerosis. J Clin Med. 2020;9:1450.
15. Blimark C, Holmberg E, Mellqvist UH, Landgren O, Björkholm M, Hultcrantz M, et al. Multiple myeloma and infections: a population-based study on 9253 multiple myeloma patients. Haematologica. 2015;100:107–13.

ACKNOWLEDGEMENTS

This work was supported by a grant of the International Myeloma Foundation, the EuroFlow Consortium and The Binding Site Group. It was also partially supported by the "Early Cancer Research Initiative Network on MBL (ECRIN-M3)" ACCELERATOR award -CRUK (UK), Fundación AECC (Spain) and Associazione Italiana per la Ricerca Sul Cancro (Italy)- and the PI22/00674 grant from the Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación, (Madrid, Spain). O. Pérez-Escurza was supported by a grant of the Government of Castilla y León (Orden EDU/601/2020), Spain. We thank the members of the iStopMM program for their support in sample and data collection. The iStopMM study is funded by the Black Swan Research Initiative by the International Myeloma Foundation and the Icelandic Centre for Research (grant agreement No 173857, 217897-051 and 228521-051). We thank our laboratory colleagues for their collaboration in the recruitment of healthy adult samples. Screening tests were performed by The Binding Site Ltd. Birmingham, UK.

AUTHOR CONTRIBUTIONS

OP-E, JF-M, SÞ, SR, TJL, SYK and AO contributed to the design, review of the protocol and supervision of the work. OP-E, JÞO, LS-F, JP, SM, ERR and GKH performed the experiments and data acquisition. OP-E, JF-M, JÞO, LS-F, QL, ERR, GKH, SH, BD, SYK and AO contributed to data analysis, reporting and interpretation of the results. OP-E and AO wrote the paper. All authors critically revised and approved the final version of the manuscript.

COMPETING INTERESTS

SR, honoraria for scientific talks from Siemens Healthineers, Johnson & Johnson. ST, honoraria for scientific talks from Abbvie, Thermo Fisher. SYK, research funding from Amgen and Celgene; Independent Data Monitoring Committee for Jansen.

ETHICAL APPROVAL

Written informed consent was given by each donor according to the Declaration of Helsinki. The study was approved by the National Bioethics Committee (NCT03327597) and the Icelandic Data Protection Agency; the Spanish Ministry of Health granted permission (Registration number: 2020/252) for the transfer of the biological samples.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41408-025-01255-3.

Correspondence and requests for materials should be addressed to Alberto Orfao.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2025

THE ISTOPMM AND ECRIN-M3 CONSORTIA

Guðlaug Katrín Hákonardóttir[6], Sigrún Þorsteinsdóttir[6,9], Sæmundur Rögnvaldsson[6,10], Thorvardur Jon Love[6,10] and Sigurdur Yngvi Kristinsson[6,12]