Limitations of VS38c labeling in the detection of plasma cell myeloma by flow cytometry

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Abstract
Plasma cell myeloma (multiple myeloma [MM]) is a malignant neoplasm originating from the plasma cells. Besides other methods, flow cytometric analysis of the patient’s bone marrow aspirate has an important role in the diagnosis and also in the response assessment. Since the cell surface markers, used for identifying abnormal plasma cells, are expressed diversely and the treatment can also alter the phenotype of the plasma cells, there is an increasing demand for new plasma cell markers. VS38c is a monoclonal antibody that recognizes the CLIMP-63 protein in the membrane of the endoplasmic reticulum. CLIMP-63 is known to be expressed at high levels in normal and pathologic plasma cells in the bone marrow, thus VS38c antibody can be used to identify them. Although VS38c staining of plasma cells is reported to be constant and strong even in myeloma, we were wondering whether sample preparation can affect the staining. We have investigated the effect of different permeabilization agents and washing of the cells on the quality of the VS38c staining and found that in many cases the staining is inadequate to identify the plasma cells. We measured the VS38c staining of the bone marrow aspirates of 196 MM patients and observed that almost all cases showed bright staining with VS38c. However, permeabilization with mild detergent resulted in the appearance of a significant VS38cdim subpopulation, which showed increased sensitivity to mechanical stress (centrifugation). Our results indicate that VS38cdim MM cells can appear due to the improper permeabilization of the endoplasmic reticulum and this finding raises the possibility of the existence of a plasma cell subpopulation with different membrane properties. The significance of this population is unclear yet, but these cells can be easily missed with VS38c staining and can be lost due to centrifugation-induced lysis during sample preparation.

KEYWORDS
CLIMP-63, flow cytometry, immunophenotyping, intracellular labeling, plasma cell myeloma, VS38c

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1 | INTRODUCTION

Plasma cell myeloma (multiple myeloma [MM]) is a malignant B cell neoplasm characterized by clonal proliferation of plasma cells in bone marrow (BM), typically presented with increased monoclonal immunoglobulin (Ig) and calcium levels in the serum, bone lesions, and renal failure. MM is a relapsing–remitting type of disease, and despite several new treatments (e.g., proteasome inhibitors, immunomodulatory drugs, monoclonal antibodies, and cellular therapies) which can provide longer remission and better handling of relapses, MM remains mostly incurable [1, 2].

For proper response assessment, the detection of residual cancer cells in the BM (measurable residual disease [MRD]) is crucial [3, 4]. Upon diagnosis, myeloma cells can be identified by flow cytometry according to their high CD138 and CD38 expression, often increased CD56, CD20, or CD117 level compared to the normal plasma cells, and loss of CD45 or CD81 in most cases [5]. In contrast, in MRD measurements, plasma cells are usually more difficult to trace due to their reduced number. Moreover, beside the heterogeneous marker expression of MM cells, these marker levels can also be altered during the treatment, or even become hardly detectable (e.g., daratumumab can cause loss of CD38) [6–9]. This problem has driven researchers to seek new markers of normal and pathological plasma cells. VS38c is a monoclonal antibody clone (mAb) that recognizes a luminal epitope of the endoplasmic reticulum (ER) resident cytoskeleton-linking membrane protein 63 (CLIMP-63, also known as cytoskeleton-associated protein 4, CKAP4, or p63) [10]. CLIMP-63 is known to act as a spacer that maintains the width of ER sheets. Due to its function, the expression of CLIMP-63 is strongly correlated with the spread of the ER and also with the protein production of the cells [10–12]. Moreover, the increased expression of CLIMP-63 protein has been connected to the progression of several different cancer types [13–15]. CLIMP-63 expression can be detected very well by using VS38c antibody in normal and neoplastic plasma cells by immunohistochemistry. Since plasma cells show uniquely strong staining with VS38c among hematopoietic cells, it is proposed by Mizuta et al. as an intracellular (ic) marker to support the identification of normal and pathologic plasma cells by flow cytometry even for MRD detection [16].

In this study, we were curious whether sample preparation can affect the quality of VS38c staining. Here we present numerous cases in which a VS38c<sup>dim</sup> plasma cell subpopulation appears in the sample due to methodical reasons, making it possible to erroneously underestimate the percentage of the plasma cells based on the VS38c staining. Furthermore, we would also like to draw attention to some experimental conditions that can reduce the number of the detected plasma cells.

2 | MATERIALS AND METHODS

2.1 | Description of the experimental groups

BM aspirates of 196 individuals diagnosed with MM (121 patients with a new diagnosis and 75 patients with remission control or relapse) were analyzed with flow cytometry, while 11 healthy participants with no apparent sign of hematologic malignancy in their BM formed the control group. Demographic data of the investigated group were detailed in Table S1. The diagnosis of MM was established from the clinical data and the corresponding BM biopsies according to the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues [17]. The study was conducted following the Declaration of Helsinki and has been approved by the local ethics committee (number of ethical permission: TUKEB 7/2006).

2.2 | Staining protocol

Preparation and ic staining of BM samples were carried out as follows: 50 μl of samples were incubated with the antibodies against surface epitopes. We used Panel 1 or 2 based on the availability of the mAbs and Panel 3 for ic Igκ and Igλ labeling (for panel information please see Table S2) at 4°C for 13 min followed by fixation (IntraStain reagent A, Dako-Agilent, CA) for 15 min at room temperature (RT). After washing (5 min, ×400 g, RT) with phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH = 7.4), the cells were permeabilized by IntraStain reagent B as a mild permeabilization agent. For six samples, we also applied 0.25% Triton X-100 (TX-100) as a strong permeabilization agent. During permeabilization, the cells were stained with the antibody against ic epitope: anti-CLIMP-63 FITC (VS38c, Dako) alone or mixed with anti-icIgκ-PE, and anti-icIgλ-APC (Triple-Color Reagent, Dako) for 15 min at RT in the dark. Afterward, the samples were washed once more before analysis.

In seven cases, soluble components of the samples were removed by washing (50 μl of BM aspirates were washed two times in PBS, 5 min, ×400 g, RT) before staining. These samples are called prewashed samples and were stained either with Panel 1 (six samples) or Panel 3 (five samples). Differently stained samples from the same patient were considered technical replicates.

Five samples were treated with seven different dilutions of TX-100, to titrate their effective concentration (see the protocol in the Data S1).

Three samples, aside from staining with VS38c, we also stained the actin as a positive control (see the protocol in the Data S1).

All used antibodies were pre-titrated. All fluorescent stainings were carried out in the dark and stained samples were handled light protected. Stained cells were measured with an eight-color Beckman Coulter Navios benchtop flow cytometer. The instrument settings were regularly controlled by Flow-Check Pro QC beads (Beckman Coulter). To obtain a satisfactory number of plasma or myeloma cells, we acquired 100,000–500,000 total events from the samples.

2.3 | Data analysis and statistics

Flow data were analyzed using Kaluza 2.1.1 software (Beckman Coulter). Gating strategies were applied according to Figures S1–S3. For
graphing and statistical analysis SigmaPlot 12.5 (Systat Software Inc., San Jose, CA) was used. We used related sample t-tests to compare the difference in the percentage of MM cells in the IntraStain kit versus TX-100 permeabilized and prewashed versus not-washed sample pairs. All other samples were compared with the Kruskal-Wallis test followed by the Dunn post hoc test. Differences were considered significant at \( p < .05 \).

3 | RESULTS AND DISCUSSION

3.1 | Mild permeabilization can lead to the appearance of a false VS38c\textsuperscript{dim} population

Introducing new markers to flow cytometric (FCM) panels may improve the sensitivity and specificity, but these new markers can also be a source of missed events due to improper experimental setup. In this article, we tested VS38c, a mAb clone against the ER-resident CLIMP-63 protein to detect normal and abnormal plasma cells in BM aspirates with flow cytometry. We examined 196 samples from MM patients using a commercially available fixation and permeabilization kit (IntraStain kit), to see how plasma and myeloma cells can be stained with VS38c mAb. Compared to isotype and fluorescence minus one (FMO) controls, all plasma cells (both normal and pathological) showed positive staining with VS38c mAb (Figure 1B). However, in almost all samples the staining was not homogenous: usually, a VS38c\textsuperscript{dim} population with various sizes also appeared among the normal plasma and MM cells, besides the VS38c\textsuperscript{br} cells (Figure 1). These VS38c\textsuperscript{dim} MM cells were stained with similar intensity to the granulocytes (Figure 1A). Thus, we used granulocytes as internal control to separate VS38c\textsuperscript{dim} and VS38c\textsuperscript{br} plasma cells in case they did not form a distinguishable subpopulation. The fraction of VS38c\textsuperscript{dim} plasma cells compared to all plasma cells showed broad distribution, and there was no significant difference between the normal and MM cases (Figure 1C). These results were in contrast with other papers reporting that plasma or MM cells were labeled by VS38c with high intensity either with immunohistochemistry or with FCM in BM samples \[10, 12\]. To resolve this contradiction, we first checked whether the permeabilization of the cells was successful. For ic stainings to fix and permeabilize cells we used the IntraStain kit, which provides a two-step fixation and permeabilization procedure that allows immunological detection of ic antigens while the cellular structure, morphologic light scatter and cell surface immunoreactivity remain mostly intact \[18\]. Since the IntraStain kit provides gentle permeabilization, we chose actin as an ic target to test the satisfactory permeabilization of the cell membrane. In the case of all tested MM samples, anti-actin antibody showed homogenous and strong staining in all MM cells when the IntraStain kit was used (Figure 2). This proves that the

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{VS38c staining of MM cells permeabilized by IntraStain kit. A representative dot plot shows that MM cells (A, black) can be split into VS38c\textsuperscript{br} (55%) and VS38c\textsuperscript{dim} (45%) subpopulations. The intensity of the dim subpopulation is similar to the granulocytes (A, gray). The fluorescence of both subpopulations is higher than the isotype and FMO controls (B, histogram heights are normalized to peak values). The box plot (C) shows the distribution of the VS38c\textsuperscript{dim} population among the MM cells in the investigated patients.}
\end{figure}
IntraStain kit permeabilizes the cell membrane sufficiently and works well with an intracytoplasmic target.

Since CLIMP-63 is an ER-resident protein, we were curious about whether the improper permeabilization of the ER membrane can be responsible for the heterogeneous staining with the VS38c mAb. When we used 0.25% TX-100 as a strong permeabilization agent, all normal plasma and MM cells became VS38cbr, forming a single population (Figure 3A,B). This suggests that some of the normal plasma and MM cells have more resistant ER, and the permeabilization agent of the IntraStain kit failed to permeabilize the ER of these cells. Note that by comparing the IntraStain and TX-100 treated samples, we found that among the MM cells the VS38cdim fraction decreased as much as the bright fraction increased. In the TX-100 treated samples the fraction of the VS38cbr MM cells increased by 3.3 ± 2.6 percentage points \((p \leq 0.027)\), while the VS38cdim MM cells decreased by 2.2 ± 2.0 p.p. \((p = 0.048)\) compared to the IntraStain treated samples (Figure 3C, values are means ± SD in all further mentioned data). Besides, the ratio of all MM cells among the BM cells did not change significantly (elevated only by 1.1 ± 2.4 p.p. \((p = 0.326)\), Figure 3C, insert). Thus, the different results were not caused by losing the dim population during the procedure. These results support our hypothesis that some of the plasma cells had ER that was more resistant to mild permeabilization, and the permeabilizing agent in the IntraStain kit was not potent enough. Although the manufacturer did not share the composition of the permeabilizing agent of the IntraStain kit, we suspect that it is titrated to be effective to permeabilize the cell membrane, but also to be as gentle as possible to maintain the light scattering parameters of the cells [19, 20]. Besides this, it is known that some permeabilizing agents, like the glycoside saponin [21] or digitonin [22] prefer cholesterol-rich membranes, such as the plasma membrane, meanwhile other non-ionic detergents, for example, the TX-100, rather acts outside of the cholesterol-rich regions of the membranes [23–25]. We hypothesize that the IntraStain kit
contains similar molecules to saponin and digitonin, which would explain why it was less effective in the case of the cholesterol-poor ER, but worked well in the case of the cytoplasm (compare anti-actin and VS38c mAbs in Figure 2) [21, 23, 24]. Another cause could be that BM aspirates of plasma cell myeloma patients are special samples since they can contain an extremely high number of cells and also increased amounts of proteins (mostly Igs). These factors can also interfere with the proper fixation and permeabilization. Interestingly, the VS38c<sup>dim</sup> population also appeared, when we used TX-100, just at a smaller concentration compared to that we used for strong permeabilization. At 0.06% a portion of the MM cells remained VS38c<sup>dim</sup>, while the others become bright forming an elongated population in the CD38-VS38c dot-plot. The coefficient of variation of this population was about five times higher than at other concentrations, also indicating the cells were distributed among dim and bright ones (Figure 3D). It proves that VS38c<sup>dim</sup> plasma cells can appear with other permeabilization agents too, and their presence strongly depends on the concentration of the agent.

**FIGURE 4** Washing, which was necessary for Ig light chain staining, removed VS38c<sup>dim</sup> MM cells. VS38c and intracellular λ light chain staining of the MM (black) cells in a representative sample without (not-washed sample) and with (prewashed sample) washing before the staining procedure. In the not-washed sample, 54% of the MM cells were VS38c<sup>dim</sup> (A), but no icIg<sub>λ</sub> staining could have been detected (C). In contrast with this, in the prewashed sample only 11% of the MM cells were VS38c<sup>dim</sup> (B), but the icIg<sub>λ</sub> staining was successful (D). The box plot (E) shows the average differences of the VS38c<sup>dim</sup>, VS38c<sup>br</sup> MM cell populations and all MM cells (insert) between prewashed and not-washed samples (n = 7). *p < .05
3.2 | Special features of the VS38c$^{\text{dim}}$ MM cells

The appearance of the VS38c$^{\text{dim}}$ population was an unexpected result of our experiments and to the best of our knowledge, this is the first report of such a population [16, 26, 27]. In some samples, the VS38c$^{\text{dim}}$ MM cells were completely separated from the bright ones (see Figure 3A), which raised the question of whether these dim cells belong to a distinct subpopulation. Although the presence of VS38c$^{\text{dim}}$ MM cells seems to be a completely artificial phenomenon, we found a few features that make these cells differ from the VS38c$^{\text{br}}$ ones. When the samples were washed before staining to remove the soluble Igs, we noticed that the VS38c$^{\text{dim}}$ fraction significantly decreased in size or disappeared completely (Figure 4A,B). At the same time, washing was necessary for the successful staining of ic Ig light chains (Figure 4C,D). Interestingly, in contrast to the permeabilization issue discussed above, in this case, the VS38c$^{\text{dim}}$ MM cells were actually lost. In the prewashed samples the fraction of the VS38c$^{\text{br}}$ MM cells among all BM cells increased only by 0.72 ± 1.91 p.p. ($p = .357$), while the fraction of the VS38c$^{\text{dim}}$ MM cells decreased by 1.66 ± 1.59 p.p. ($p = .032$, compared to the not-washed samples, values are means ± SD, Figure 4E). This cell loss can also be seen in the fall of the total MM cell ratio as the fraction of the MM cells decreased by 1.04 ± 0.87 p.p. ($p = .020$), (Figure 4E, insert). Compared to the not-washed samples in the prewashed samples more than 40% of the VS38c$^{\text{dim}}$ cells were lost, which represented almost 20% of the MM cells (Figure S4).

A possible explanation for this phenomenon could be that those plasma cells, which have more detergent-resistant ER (i.e., the VS38c$^{\text{dim}}$ population) differ from the others and tolerate less mechanical stress caused by washing and centrifugation. Besides, this cell loss also raises an important concern because VS38c has an ic target [10], and as such, it is advised to use together with other mAbs against ic targets, for example, anti-ic-lgκ or anti-ic-lgλ mAbs (as it is available in mixture [see Section 2]). However, detection of Igκ or Igλ light chains on the cell surface and also in the cytoplasm requires the removal of soluble Igs by washing the cells before the staining procedure (Figure 4C,D) [28, 29]. We demonstrated that washing unfortunately eliminates a significant portion of VS38c$^{\text{dim}}$ MM cells (Figure 4 and Figure S4). Therefore, in the case of washing, we must expect reduced sensitivity. This makes the VS38c and anti-ic-lgκ or anti-ic-lgλ combination kits less suitable for detecting small amounts of residual plasma cells.

We have demonstrated that plasma and MM cells permeabilized with IntraStain kit and stained with VS38c mAb showed a heterogeneous staining pattern, and among these, the ratio of the dim and bright cells showed broad distribution. Furthermore, this pattern was not constant over time. We compared the VS38c staining of 0- (measured on the sampling day), 1- and 2-days old samples (the groups consist of 29, 79, and 14 samples respectively, 122 in total). Two days old samples showed a significantly smaller average VS38c$^{\text{dim}}$ percentage than fresh ones (Figure 5A). Since these samples were independent, we were curious about how the fraction of the VS38c$^{\text{dim}}$ cells changes in the same sample over time. Hence, in a subsequent experiment we measured five samples three times: on the day when it had been taken (day 0), then 1 day, and 2 days later (samples were stored on RT, protected from light). We then compared the fraction of the VS38c$^{\text{dim}}$ MM cells (among the MM cells) to the day 0 sample. On day 2, it decreased on average below 40% of the original amount (Figure 5B). It can also be seen that the ratio of all MM cells did not change significantly (Figure S5), therefore the VS38c$^{\text{dim}}$ MM cells did not get lost, just became bright. Both experiments suggest that the ratio of the VS38c$^{\text{dim}}$ MM cells among all MM cells is decreasing over time. These results also support our suspicion that the resistance to permeabilization of the ER is maintained by a biological process, which may begin to decline 2 days after the sample was taken.

4 | SUMMARY

In this article, we showed that VS38c mAb is useful to detect normal and pathologic plasma cells by flow cytometry. However, due to its intra-ER target, too gentle permeabilization can cause the appearance of a false VS38c$^{\text{dim}}$ subpopulation, which can cause erroneous estimation of the percentage of the plasma cells. We also showed that
despite the presence of the dim subpopulation among plasma cells seems an artificial phenomenon, these cells have some special characteristics (decreased mechanical resistance and time-dependent decrease of VS38c\textsuperscript{dim} fraction). It raises the possibility that their ER is more resistant to gentle permeabilization, which may have some biological or pathological significance. This should be investigated in future work.

Our findings prove that the careful setup of a staining method is crucial, especially in the case of ic staining. This makes the VS38c mAb, similarly to other antibodies with ic epitopes, less suitable for detecting small amounts of residual pathologic cells. Still, VS38c has its rightful place in ic panels, as together with conventional backbone markers, like CD38, CD138, and CD45, it can be useful to identify plasma cells. However, it should be noted that ic staining must be used beside at least one panel which contains mAbs against cell surface targets that provide better sensitivity to detect small populations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR’S CONTRIBUTION

Ágnes Czeti: Data curation; formal analysis; investigation; validation; writing-original draft; writing-review & editing; Gábor Szaloki: Conceptualization; data curation; formal analysis; investigation; methodology; software; supervision; validation; writing-original draft; writing-review & editing; Gergely Varga: investigation; validation; writing-review & editing; Virág Réka Szita: investigation; validation; writing-review & editing; Zsolt István Komlósi: investigation; validation; writing-review & editing; Ferenc Takács: supervision; validation; writing-review & editing; Ágnes Márk: supervision; validation; writing-review & editing; Botond Timár: investigation; validation; writing-review & editing; András Matolcsy: funding acquisition; resources; writing-review & editing; András Pálffy: Conceptualization; data curation; funding acquisition; methodology; project administration; resources; supervision; validation; writing-review & editing.

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REFERENCES

1. Rajkumar SV. Multiple myeloma: 2020 update on diagnosis, risk-stratification and management. Am J Hematol. 2020;95:548–67.
2. Kumar SK, Rajkumar V, Kyle RA, van Duin M, Sonneveld P, Mateos MV, et al. Multiple myeloma. Nat Rev Dis Primers. 2017;3:17046.
3. Carulli G, Tarasco A, Sammuri P, Oattaviano V, Domenichini C, Ciamia EM, et al. Assessment of response to therapy in multiple myeloma by multiparameter flow cytometry. Usefulness of an eight-color single tube with monoclonal antibodies in dried formulation. Clin Ter. 2019;170:e352-e356.
4. Galteva IV, Davydiva YO, Kapranov NM, Julhakyan HL, Mendeleeva LP. Minimal residual disease in multiple myeloma: benefits of flow cytometry. Int J Lab Hematol. 2018;40:12–20.
5. Kumar S, Kimlinger T, Morice W. Immunophenotyping in multiple myeloma and related plasma cell disorders. Best Pract Res Clin Haematol. 2010;23:433–51.
6. Paiva B, Corchete LA, Vindrales MB, Puig N, Maiso P, Rodriguez I, et al. Phenotypic and genomic analysis of multiple myeloma minimal residual disease tumor cells: a new model to understand chemoresistance. Blood. 2016;127:1896–906.
7. Cao W, Goolsby CL, Nelson BP, Singhal S, Mehta J, Peterson LAC. Instability of immunophenotype in plasma cell myeloma. Am J Clin Pathol. 2008;129:926–33.
8. Sullivan HC, Gerner-Smidt C, Nooka AK, Arthur CM, Thompson L, Mener A, et al. Daratumumab (anti-CD38) induces loss of CD38 on red blood cells. Blood. 2017;129:3033–7.
9. Krejci J, Freichs KA, Nijhof IS, van Kessel B, van Velzen JF, Bloem AC, et al. Monocytes and granulocytes reduce CD38 expression levels on myeloma cells in patients treated with Daratumumab. Clin Cancer Res. 2017;23:7498–511.
10. Banham AH, Turley H, Pulford K, Gatter K, Mason DY. The plasma cell associated antigen detectable by antibody VS38 is the p63 rough endoplasmic reticulum protein. J Clin Pathol. 1997;50:485–9.
11. Sandoz PA, van der Goot FG. How many lives does CLIMP-63 have? Biochem Soc Trans. 2015;43:222–8.
12. Turley H, Jones M, Erber W, Mayne K, de Waele M, Gatter K, VS38: a new monoclonal antibody for detecting plasma cell differentiation in routine sections. J Clin Pathol. 1994;47:418–22.
13. Banerjee SS, Shanks JH, Hasleton PS, VS38 immunostaining in neuroendocrine tumours. Histopathology. 1997;30:256–9.
14. Sulzbacher I,uchs M, Chott A, Lang S. Expression of VS38 in osteoblasts and stroma cells of bone tumors. Pathol Res Pract. 1997;193:613–6.
15. Shanks JH, Banerjee SS, VS38 immunostaining in melanocytic lesions. J Clin Pathol. 1996;49:205–7.
16. Mizuta S, Kawata T, Kawabata H, Yamane N, Mononobe S, Komai T, et al. VS38 as a promising CD38 substitute antibody for flow cytometric detection of plasma cells in the daratumumab era. Int J Hematol. 2019;110:322–30.
17. World Health Organization Classification of Tumour. In: SH Swerdlow, E Campo, NL Harris, ES Jaffe, SA Pileri, & H Stein, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer; 2017.
18. Kappelmayer J, Gratawa JW, Karasi E, Menéndez P, Ciudad J, Rivas R, et al. Flow cytometric detection of intracellular myeloperoxidase, CD3 and CD79a. Interaction between monoclonal antibody clones, fluorochromes and sample preparation protocols. J Immunol Methods. 2000;242:33–65.
19. Verdier M, Jayat C, Ratinou MH, Trouaud D. Optimization of cell permeabilization for multiparameter flow cytometric analysis with lectin staining. Cytometry. 2000;41:55–61.
20. Camelejohn RS. The measurement of intracellular antigens and DNA by multiparameter flow cytometry. J Microsc. 1994;176( Pt 1):1–7.
21. Wassler M, Jonasson I, Persson R, Fries E. Differential permeabilization of membranes by saponin treatment of isolated rat hepatocytes. Release of secretory proteins. Biochem J. 1987;247:407–15.

22. Plutner H, Davidson HW, Saraste J, Balch WE. Morphological analysis of protein transport from the ER to Golgi membranes in digitonin-permeabilized cells: role of the PS8 containing compartment. J Cell Biol. 1992;119:1097–116.

23. Schuck S, Honsho M, Ekroos K, Shevchenko A, Simons K. Resistance of cell membranes to different detergents. Proc Natl Acad Sci U S A. 2003;100:5795–800.

24. Gombos I, Bacsó Z, Detre C, Nagy H, Goda K, Andrásfalvy M, et al. Cholesterol sensitivity of detergent resistance: a rapid flow cytometric test for detecting constitutive or induced raft association of membrane proteins. Cytometry A. 2004;61:117–26.

25. Banfalvi G. Methods of permeabilization. Permeability of biological membranes. Cham: Springer International Publishing; 2016. p. 129–200.

26. Medina F, Segundo C, Campos-Caro A, González-García Í, Brieva JA. The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. Blood. 2002;99:2154–61.

27. Sanoja-Flores L, Flores-Montero J, Garcés JJ, Paiva B, Puig N, García-Mateo A, et al. Next generation flow for minimally-invasive blood characterization of MGUS and multiple myeloma at diagnosis based on circulating tumor plasma cells (CTPC). Blood Cancer J. 2018;8:117.

28. O’Donahue M, Johnson L, Hedley B, Vaughan E. Flow Cytometric Testing for Kappa and Lambda light chains. 2018 [cited 2020. 06. 03.]. Available from: https://www.cytometry.org/web/modules/Module%206.pdf

29. Reynolds WM, Williamson AM, Smith GJ, Lane AC. A simple technique for the determination of kappa and lambda immunoglobulin light chain expression by B cells in whole blood. J Immunol Methods. 1992;151:123–9.

SUPPORTING INFORMATION
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