Enhancing Diagnostic Precision in Multiple Myeloma: Efficient CD138+ Cell Isolation Using MARS BAR Flex

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ABSTRACT

Innovative solutions that improve diagnostic precision in multiple myeloma patients have potential to improve patient care and research outcomes. In this study we introduced a streamlined method for isolating CD138+ cells for multiple myeloma (MM) diagnosis and cytogenetic analysis. Using CD138 microbeads and the MARS BAR Flex platform, we achieved high recovery rates, purity, and increased detection of abnormal signals compared to conventional methods.

INTRODUCTION

Multiple myeloma (MM) is the second most common blood cancer in the world and forms in a subset of white blood cells known as plasma cells (PC). There is no cure for myeloma and patient median survival rate is 5 years.[1] When healthy, plasma cells reside in the bone marrow and are a crucial part of the immune system by secreting antibodies to help the body fight infections. Cancerous plasma cells create abnormal proteins known as monoclonal proteins, or M proteins, instead of normal antibodies thereby reducing immune responses.[2] MM can be diagnosed through chromosomal abnormalities using fluorescence in situ hybridization (FISH), but other methods are being developed including SNP-array and sequencing.[3] Each method requires isolating PC from bone marrow and achieving sufficient purity to ensure assay reliability.

CD138 (Syndecan-1) is a surface antigen that is highly expressed on differentiated and malignant plasma cells and is a key target for isolation of myeloma cells for downstream cytogenetic analysis.[1] Given its use as a diagnostic tool, there is a need for a consistent CD138+ cell isolation workflow with minimal hands-on time, that results in high purity samples. Using directly conjugated CD138 microbeads along with the MARS BAR Flex platform, purified CD138+ cells can be isolated in a quick and robust process with minimal handling.

RESULTS

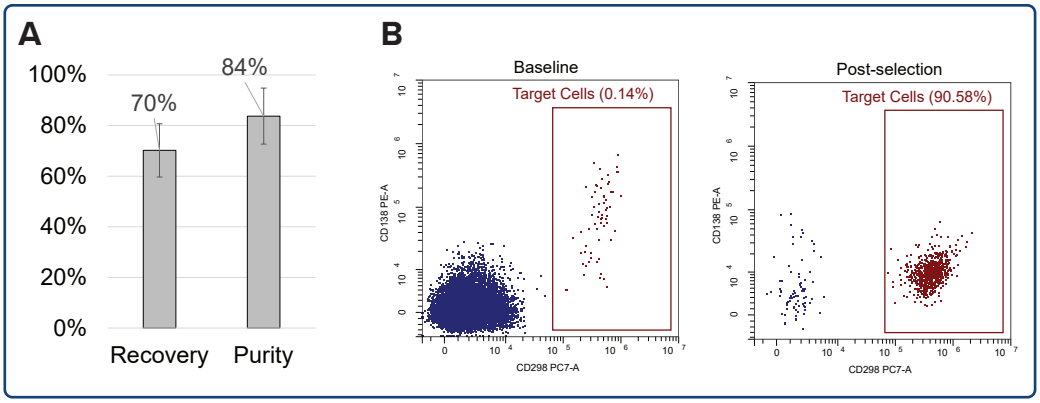

Fig. 1 Selection of rare CD138+ Cells from Whole Blood. MM1.S cell line cells were selected from Ficoll-treated whole blood samples using MARS Magnetic Selection serial program. Across 10 samples from 6 donors (A), recovery averaged 70% with 11% standard deviation, separation averaged 83% with 9% standard deviation, and target cells increased in purity to 84% of WBC with 11% standard deviation from between 0.1-0.5% of WBC in input samples (B).

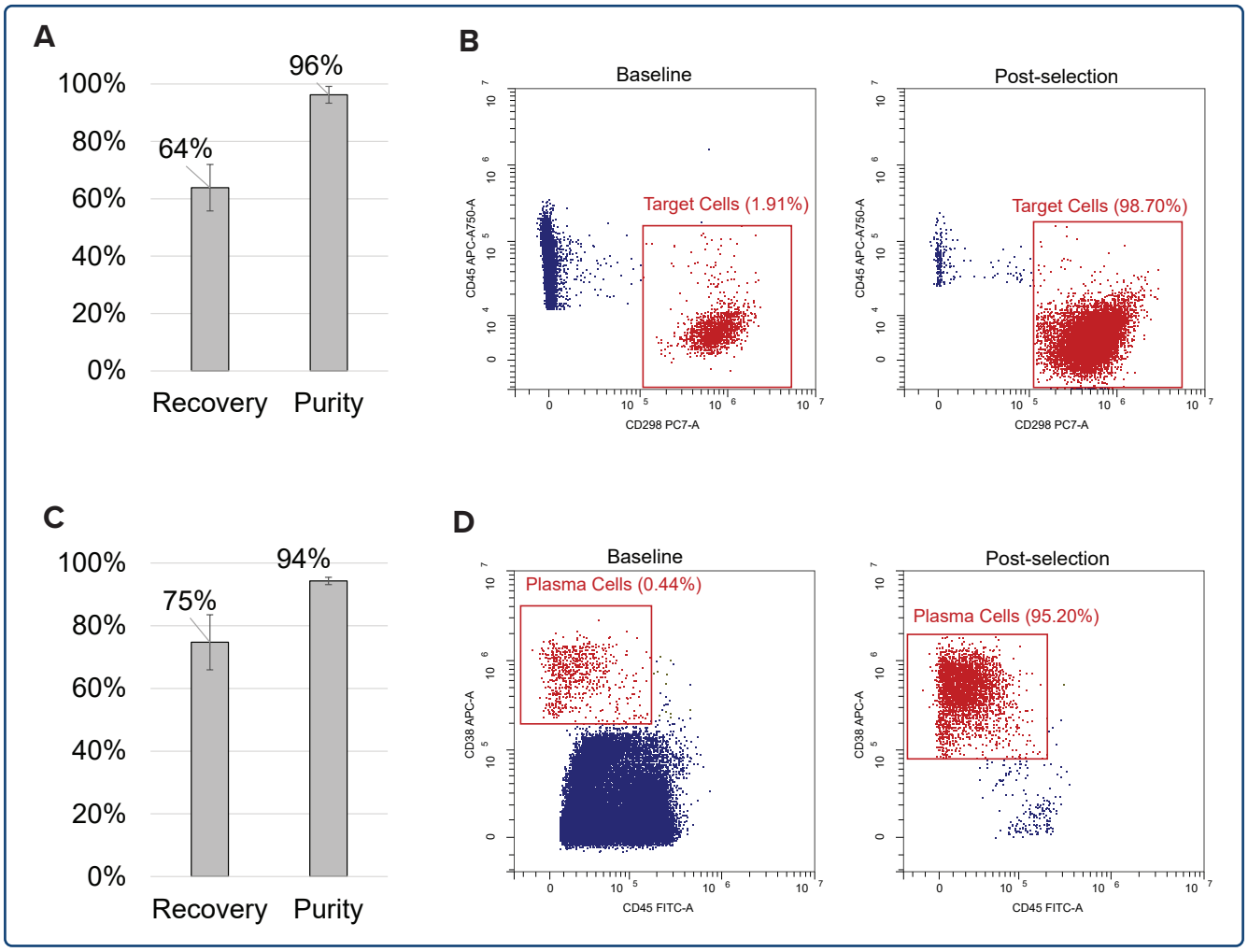

Fig. 2 Selection of CD138+ Cells from Bone Marrow. PBMC samples with MM1.S cell line cells were prepared to mimic Multiple Myeloma patient Bone Marrow and processed using MARS Magnetic Selection bone marrow program to select CD138+ cells (A, B). Across 6 samples from 2 donors (A), recovery averaged 64% with 8% standard deviation, separation averaged 83% with 9% standard deviation, and target cells increased in purity to 96% of WBC with 3% standard deviation from 1% of WBC in input samples (B). CD138+ plasma cells were also selected from healthy whole bone marrow using this protocol (C, D). Across 4 samples from a single healthy donor (A), recovery averaged 75% with 9% standard deviation, separation averaged 77% with 3% standard deviation, and target cells increased in purity to 94% of WBC with 1% standard deviation from 0.44% of WBC in input samples (B).

Table 1. Selection of CD138+ cells from MM Patient Bone Marrow.

Baseline Plasma Cell %	After MARS Plasma Cell %	After Current Column-Based Method Plasma Cell %
0.28%	21.86%	0.62%
0.38%	39.86%	9.52%
0.64%	45.49%	7.18%
1.52%	81.40%	6.33%
2.32%	74.70%	34.88%
3.33%	79.86%	13.64%
7.49%	96.94%	18.39%
12.36%	92.09%	51.86%
18.50%	94.40%	55.75%

Nine multiple myeloma patient bone marrow samples (Table 1) with different plasma cell frequency were magnetically enriched using MARS and the current column base method side by side. After MARS enrichment, plasma cell percentage was higher than current column base enrichment.

Table 2. Abnormal signals detected by FISH in multiple myeloma patients after CD138 selection

BSL Plasma Cell %	After MARS Probe 1p21/1p32 abnormal rate	After Column Probe 1p21/1p32 abnormal rate	After MARS Probe IGH abnormal rate	After Column Probe IGH abnormal rate	After MARS Probe D13S319 abnormal rate	After Column Probe D13S319 abnormal rate	After MARS Probe p53 abnormal rate	After Column Probe p53 abnormal rate	After MARS Probe IGH/FGFR3 abnormal rate	After Column Probe IGH/FGFR3 abnormal rate	After MARS Probe IGH/CCND1 abnormal rate	After Column Probe IGH/CCND1 abnormal rate	After MARS Probe IGH/MAF abnormal rate	After Column Probe IGH/MAF abnormal rate	Number of Positive Probes After MARS	Number of Positive Probes After Column
0.12%	0%	0%	6%	1%	1%	3%	2%	3%	2%	2%	2%	2%	2%	1%	1	0
0.15%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0	0
0.20%	0%	0%	57%	0%	3%	3%	4%	4%	34%	3%	49%	2%	52%	2%	4	1
0.20%	20%	0%	50%	5%	33%	4%	3%	3%	27%	3%	22%	3%	23%	2%	6	3
0.26%	10%	6%	0%	0%	3%	0%	0%	2%	2%	0%	2%	2%	0%	0%	1	1
0.28%	20%	0%	50%	6%	33%	3%	3%	3%	27%	3%	22%	2%	23%	2%	6	2
0.28%	28%	7%	15%	0%	36%	5%	3%	2%	13%	3%	9%	3%	9%	2%	6	4
0.41%	42%	7%	2%	0%	2%	0%	37%	0%	0%	0%	40%	0%	2%	0%	4	1
0.42%	21%	1%	15%	1%	14%	1%	3%	3%	17%	0%	9%	0%	11%	0%	6	0
0.51%	81%	22%	81%	34%	12%	11%	61%	20%	55%	40%	69%	42%	56%	41%	7	7
0.53%	7%	1%	9%	3%	9%	5%	3%	5%	11%	0%	11%	2%	12%	1%	6	0
0.64%	70%	60%	65%	30%	67%	42%	1%	3%	64%	27%	61%	28%	56%	26%	6	6
1.05%	1%	2%	3%	0%	37%	1%	3%	2%	2%	5%	2%	0%	0%	2%	1	0
1.33%	61%	25%	0%	1%	63%	38%	3%	2%	0%	1%	2%	2%	2%	2%	2	2
2.17%	93%	38%	96%	30%	97%	51%	6%	1%	95%	37%	91%	43%	92%	92%	6	6
2.23%	0%	0%	62%	32%	3%	4%	1%	0%	56%	33%	67%	34%	59%	34%	4	4
3.36%	0%	0%	87%	50%	3%	2%	4%	3%	36%	21%	90%	48%	39%	21%	5	5
3.84%	89%	30%	19%	8%	79%	29%	4%	0%	14%	4%	15%	6%	18%	6%	6	6
4.00%	0%	0%	84%	22%	83%	21%	87%	31%	0%	0%	90%	25%	18%	11%	5	5
7.49%	88%	23%	92%	23%	54%	15%	4%	2%	93%	13%	87%	17%	91%	21%	6	6

Twenty multiple myeloma patient bone marrow with different plasma cell frequency were magnetically enriched using MARS and the current column base method side by side. After that FISH was used to detect the abnormal signals of seven different probes, 1p21/1p32 (positive threshold 5%), IGH (positive threshold 5%), D13S319 (positive threshold 8%), p53 (positive threshold 8%),IGH/FGFR3 (positive threshold 3%), IGH/CCND1(positive threshold 3%), IGH/MAF (positive threshold 3%). Abnormal cell percentages of each probe detected by MARS and current column based magnetic enrichment methods were listed in table 2A. Abnormal cell percentage equal or higher than corresponding threshold was highlighted in blue and marked as positive. The percentage of cytogentically abnormal cells by FISH after MARS enrichment was higher than FISH after the current column based enrichment. The counts of positive probe of both methods were listed in the last two columns (blue and green color). For patients with higher than 1.1% plasma cells in the baseline, the positive probe counts were the same for MARS and the current column based method. For patients with lower than 1.1% plasma cells in the baseline, four patients had the same positive probe counts from both results. MARS detected more positive probes than the current method in nine patients. Furthermore, out of these nine patients, the current method detected no positive probe in three patients. But MARS detected six positive probes in two patients and one positive probe in one patient.

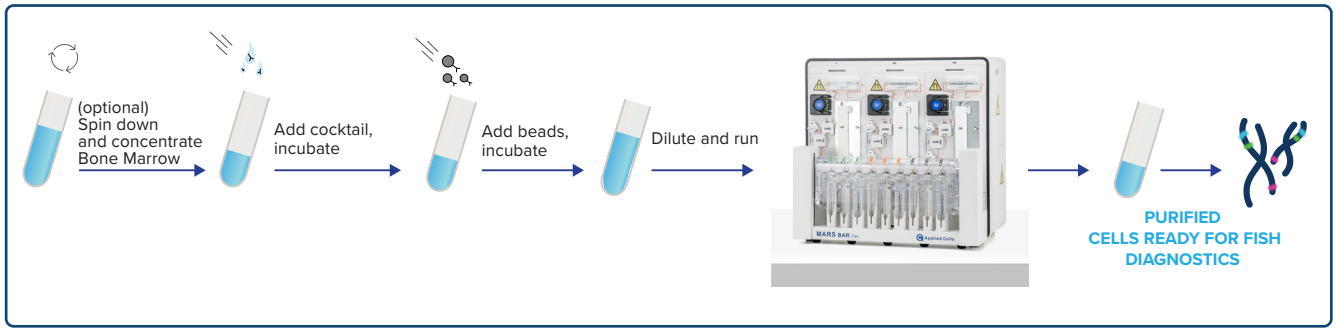

Fig. 3 Selection of CD138+ Cells from Bone Marrow with MARS Bar Flex.

MATERIALS AND METHODS

Cell Line Culture

MM.1S cells (ATCC CRL-2974) were cultured in complete RPMI_1640 growth medium, in an atmosphere of 5% CO2 at 37C. Complete RPMI_1640 consisted of RPMI_1640 (Gibco #11875-093) plus 10% v/v Fetal Bovine Serum (Gibco #26140-079) and 1% v/v 100x Penicillin-Streptomycin mixture (Gibco #15140-122). Media was refreshed every 2-3 days. Cells were harvested from collected media by centrifugation at 500g for 10 minutes and resuspended in MARS Magnetic Buffer. These cells were pre-stained with PE/Cy7 anti-human CD298 (Biolegend #341708), excess antibody removed by centrifugation at 500g for 10 minutes, and finally cells were resuspended to appropriate concentration before addition sample preparations.

Cell Sample Preparation

PBMC were prepared by density gradient centrifugation with Ficoll-Paque (Cytiva #17144003). Pooled blood was diluted 1:1 with 1xPBS, then layered over Ficoll-Paque. Layered mixture was centrifuged at 400g for 30 minutes with brake off. PBMC layer was transferred and washed with 1xPBS then centrifuged again at 500g for 15 minutes with brake on. Supernatant was removed and cells were resuspended and stored in RPMI freezing media (complete RPMI with 10% DMSO). Cells were frozen at -80C until use. PBMC for use in selection were thawed in 37C water bath, diluted in MARS Magnetic Buffer and centrifuged to remove freezing medium, then resuspended at 100 million cells/mL. After resuspension, MM.1S cells were added at desired spike-in percentage of total WBC.

Magnetic Cell Selection – Peripheral Blood Protocol

Samples prepared by Ficoll procedure (above) were first incubated with MARS Ingenuity Human CD138 Positive Selection Kit (ACI,R-MP005) , 100uL cocktail per 1mL of sample volume for 8 minutes while rocking on a nutating mixer in the dark at room temperature. Following cocktail incubation, magnetic beads were added to the mixture at 50uL per 1mL of sample volume. Incubation was repeated for 8 minutes rocking in the dark at room temperature. After incubation, each sample was mixed with an equal volume of MARS Magnetic Buffer (ACI, #MWB01). Samples were processed on the MARS Bar, Tube-In Tube-Out configuration, using the MARS CD138 program. Following selection, both positive and negative fractions were collected for analysis.

Magnetic Cell Selection – Bone Marrow Protocol

Fresh samples were diluted with 10x volume of MARS Magnetic Buffer, then centrifuged at 300g for 10 minutes. White blood cells were resuspended at 20 million cells/mL, then were incubated with MARS Ingenuity Human CD138 Positive Selection Kit (ACI,R-MP005), 50uL cocktail per 1mL of sample volume for 8 minutes while rocking on a nutating mixer in the dark at room temperature. Following cocktail incubation, magnetic beads were added to the mixture at 50uL per 1mL of sample volume. Incubation was repeated for 8 minutes rocking in the dark at room temperature. After incubation, each sample was mixed with 5x volume of MARS Magnetic Buffer (ACI, #MWB01). Samples were processed on the MARS Bar, Tube-In Tube-Out configuration, using the MARS CD138 program. Following selection, both positive and negative fractions were collected for analysis.

REFERENCES

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