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Supplementary Data

Isolation of Circulating Plasma Cells from Peripheral Blood of Patients Diagnosed with Clonal Plasma Cell Disorders using Cell Selection Microfluidics

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Experimental Details

Materials and reagents. Pt wires (75 µm diameter) were purchased from Sigma-Aldrich (St. Louis, MO). Cyclic Olefin copolymer, COC (Topas 6013S-04) plates (1/8" thick) and films (250 µm thick) were acquired from Topas Advanced Polymers (Florence, KY). PEEK tubing and connectors were purchased from IDEX Health & Science (Oak Harbor, WA). Chemicals used for COC surface cleaning and modification included reagent grade isopropyl alcohol, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), fetal bovine serum, and 2-(4-morpholino)-ethane sulfonic acid (MES) all of which were acquired from Sigma-Aldrich. Monoclonal anti-CD138 (Clone #359103, 0.5 mg/mL) antibody was obtained from R&D Systems (Minneapolis, MN). Tris-glycine buffer was obtained from Bio-Rad Laboratories (Hercules, CA). PBS buffer and trypsin from porcine were purchased from Sigma-Aldrich. The nuclear stain, DAPI, was obtained from Thermo Pierce Technologies (Rockford, IL). For immunofluorescence analysis, antibodies such as anti-CD138 pacific blue (MI15) (Biolegend, CA, 5.0 µg/mL) , anti-CD45-FITC (HI30 clone, 10 µg/mL), anti-CD56-PE (MEM-188 clone, 20 µg/mL), anti-CD38-APC (HIT2 clone, 2.5 µg/mL), anti-Ig kappa light chain-PE (TB28-2 clone, 5.0 µg/mL), and anti-Ig lambda light chain-APC (1-155-2 clone, 2.5 µg/mL) were purchased from eBiosciences (San Diego, CA) were prepared in 100 µL of PBS. Propidium Iodide for viability analysis was obtained from Thermo Pierce Technologies (Rockford, IL). Bovine serum albumin (BSA) in PBS buffer (pH 7.4) was secured from Sigma-Aldrich. MEM-non essential amino acids were obtained from GIBCO (Grand Island, NY GEAA). QIAamp DNA Mini Kit (Valencia, CA) was used for genomic DNA isolation and purification. Custom made oligonucleotide probes and primers for both PCRs and LDRs were obtained from Integrated DNA Technologies (IDT, Coralville, IA). Taq 2X master mix and *Taq* DNA ligase were purchased from New England Biolabs (NEB; Ipswich, MA). Live/Dead™ cell viability assay (calcein AM and ethidium homodimer-1), was secured from Life Technologies (Carlsbad,CA). Sodium carbonate and bicarbonate were secured from Fisher Scientific (Houston, TX) and dithiothreitol, sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (sulfo-SMCC, No-Weigh Format), Zeba spin desalting columns (7K MWCO) were obtained from Thermo Scientific, (Rockford,IL), Specific Excision Reagent USER ™ enzyme system was obtained from NEB (Ipswich, Massachusetts).

The ssDNA linker used to attach the selection antibody to the activated COC surface contained the sequence 5'NH_2-C_{12}-T_8-CCCTT CCTCC TCACT TCCCT TT-**U**-T_9-C_3-S-S-CH_2-OH-3' with an internal uracil residue and was obtained from Integrated DNA Technologies (Coralville, IA). All solutions were prepared in nuclease-free water purchased from Invitrogen (Carlsbad, CA).

Nuclease-free microfuge tubes were purchased from Ambion (Foster City, CA) and were used for preparation and storage of all samples and reagents

Fabrication of the Circulating Plasma Cell (CPC) selection device. Hot embossing was used for the fabrication of the CPC selection device as described previously (1). Mold masters used for hot embossing were prepared in brass using high precision micromilling (KERN 44, KERN Micro- und Feinwerktechnik GmbH & Co.KG; Murnau, Germany) and standard carbide bits (Performance Micro Tool, Janesville, WI) (1). Hot embossing of polymer-based devices was performed using a HEX03 embossing machine (Jenoptik Optical Systems GmbH, Jena, Germany). The embossing conditions utilized a temperature of 155°C with 30 kN force for 120 s for the cyclic olefin copolymer, COC, substrates.

After hot embossing, the CPC selection device and cover plate, both of which were made from COC, were flood exposed to broadband UV/O_3 light at ~16 mW/cm^2 (254 nm) for 15 min using a home-built light box employing a low pressure Hg lamp (GLF-42, Jelight Company Inc., Irvine, CA). UV/O_3 exposure produced carboxylic acid surface functional groups that were used for the covalent attachment of monoclonal antibodies (anti-CD138 monoclonal antibodies) for CPC selection (2,3). After UV/O_3 exposure, the substrate was enclosed with the UV/O_3 light activated cover plate by thermal bonding at a temperature of 132°C and a pressure of ~1 N/cm^2. Bonding conditions were selected to achieve high bond strength to accommodate the high pressures generated by pumping whole blood at relatively high volume flow rates (~25 µL/min) through the device, and preserve device structural integrity.

Cell culture. The RPMI-8226 (multiple myeloma) cancer cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured according to recommended conditions. Briefly, the cell line was incubated at 37˚C under a 5% CO_2 atmosphere. RPMI 1640 with 2.5 mM L-glutamine supplemented with 10% FBS (GIBCO, Grand Island, NY) was used. For sub-culturing, RPMI-8226 cells were grown as non-adherent cell suspensions in T25 culture flasks (Corning) by maintaining a cell density between 5 x 10^5 and 2 x 10^6 viable cells/ml with fresh media changes every 2-3 d either via dilution or replacement of new medium.

Flow cytometry and RPMI-8226 cell line surface antigen characterization. All flow studies were performed at the UNC Flow Cytometry Core. A Beckman Coulter (Dako) Cyan ADP instrument equipped with 11 parameter analysis capability – forward and side scatter and 9 colors of fluorescence using 405 nm, 488 nm and 635 nm excitation – was used. The software, which provided instrument control and data acquisition for the Cyan, was Summit. For sample prep,

approximately 10^6 RPMI-8226 cells obtained from culture media were centrifuged and resuspended in 1 ml cold 0.5% BSA/PBS buffer. Prior to staining, the cells' surfaces were blocked with human IgG and incubated at 4ºC. Ten-µl of 0.1 mg/ml fluorescently-labeled antibody or isotype control was added to the cell suspension and left to incubate for 45 min at 4˚C under dark conditions. Upon completion of immobilization of the surface antibody, cells were washed 3 times by centrifuging and replacing the buffer with cold 0.5% BSA/PBS.

Release of selected cells for downstream processing. After capture of CPCs using the CPC selection device, they were released from the capture surface containing monoclonal antibodies by cleavage of a single-stranded oligonucleotide bifunctional linker containing a uracil residue using the USER™ enzyme system. The CPC selection device was infused with USER™ enzyme (2U/10 µl PBS; pH = 7.4) and incubated on a thermal heater for 35 min at 37ºC. Immediately after incubation, cells were washed from the selection device and collected into flat bottom wells.

FISH. For control samples, RPMI-8226 cells in media were spun down and the supernatant was replaced with KCl hypotonic solution (0.056 M). After 5 min, an equal volume of fixative (3:1 methanol:acetic acid) and cell suspension was mixed and the cells were spun down again. The aforementioned procedure was repeated 3 times. The cells were stored in methanol:acetic acid until required for FISH analysis.

Selected CPCs from patient blood samples were, after release, spun down at 300g for 7 min and the supernatant was removed. A 3:1 (v/v) methanol:acetic acid solution was added and this process was repeated twice. Cells in methanol:acetic acid were transferred onto a glass microscope slide. Slides were immediately placed on a hot plate at 42ºC and left to dry for ~15 min. The slides were treated with 0.05% NP-40 in 2X SSC (pH 7.3) at 37ºC for 30 min and then dehydrated successively in 70%, 85% and 100% ethanol at room temperature for 2 min each and dried completely. 7.5-µl of probe (DLEU 13q14 Kreatech probe) was applied to each slide and the probe was covered with a cover slip and sealed with rubber cement. Cells with probes were denatured at 75ºC for 7 min and hybridized at 37ºC overnight in a HYBrite oven. After removal of the cover slip, the slides were washed in 0.4X SSC/0.3% NP-40 at 73ºC for 2 min and then in 2X SCC/0.1% NP-40 at room temperature for 1 min. The slides were air-dried and 10 µl (0.1 ng/ml) of DAPI II counterstain (Vysis) was applied to each slide. The cells were analyzed using a Zeiss Axioplan 2 Microscope with a 63X or 100X Zeiss oil immersion objective.

Polymerase Chain Reaction and Ligase Detection Reaction (PCR/LDR). LDR relies on the high fidelity of DNA ligase to discriminate single base mismatches (4). There are 2 primers used in the assay: One is designed to be complementary to the polymorphism (*i.e.*, discriminating primer), and the other to a region directly adjacent to the polymorphism (*i.e.*, common primer; see Table S1 for sequences). If there is a perfect match between the nucleotide at the 3' end of the discriminating primer and the polymorphic site being queried, the primers will be ligated by a ligase enzyme. A single base mismatch at the junction inhibits ligation and thus, single-base mutations can be distinguished (4,5). Discriminating and common primers were designed to produce ligation products with different sizes; they could be separated using capillary gel electrophoresis and the mutation identified based on the size of the product detected.

Table S1. PCR and LDR primer sequences for identification of *KRAS* mutations.

***KRAS* PCR or LDR Primer**	**5'-sequence-3'**	**nt**	**T_m(°C)**	**LDR product size (nt)**
Forward PCR	TTAAAAGGTACTGGTGGAGTATTTGATA	28	54.0	-
Reverse PCR	AAAATGGTCAGAGAAACCTTTATCTGT	27	54.8	-
Common_12.1	pGTGGCGTAGGCAAGAGTGCCAA-**Cy5**	22	62.2	-
Common_12.2	pTGGCGTAGGCAAGAGTGCCT-**Cy5**	20	61.5	-
Common_13.3	pGCGTAGGCAAGAGTGCCTTGA-**Cy5**	21	59.9	-
12.1wt	TTTTTTTTTTTTTTTTTATATAAACTTGTGGTAGTTGGAGCTG	43	58.4	65
12.1A (G34A)	TTTTATATAAACTTGTGGTAGTTGGAGCTA	30	54.5	52
12.1C (G34C)	TTTTTTATATAAACTTGTGGTAGTTGGAGCTC	32	55.8	54
12.1T (G34T)	TTTTTTTTATATAAACTTGTGGTAGTTGGAGCTT	34	56.1	56
12.2wt	TTTTTTTTTTTTTTTTTTAAACTTGTGGTAGTTGGAGCTGG	41	60.0	61
12.2A (G35A)	TTAAACTTGTGGTAGTTGGAGCTGA	25	56.3	45
12.2C (G35C)	TTTTAAACTTGTGGTAGTTGGAGCTGC	27	57.8	47
12.2T (G35T)	TTTTTTAAACTTGTGGTAGTTGGAGCTGT	29	57.3	49

13.3wt	TTTTTTTTTTTTTTTTTTTTGTGGTAGTTGGAGCTGGTG	39	59.9	60
13.3A (G37A)	CTTGTGGTAGTTGGAGCTGGTA	22	56.4	43

p-phosphorylated

Genomic DNA (gDNA) was extracted and purified from HT-29, RPMI-8226 cells and CPCs using the QIAamp DNA Mini Kit. PCR amplifications were carried out to generate 290 bp amplicons of gDNA from HT-29, RPMI-8226 and CPCs from clinical samples using gene-specific primers (Table S1). The PCR cocktail consisted of a 50 µl reaction suspended in 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 5% Glycerol, 0.08% IGEPAL® CA-630, 0.05% Tween® 20, 25 units/ml *Taq* DNA Polymerase, (pH 8.6) and cellular gDNA. Amplification was carried out in an Eppendorf Thermal Cycler (Brinkmann Instrument Inc., Westbury, NY); 35 cycles at 94˚C for 30 s, 59˚C for 2 min and a final extension at 72˚C for 3 min.

Slab gel electrophoresis was performed on an aliquot of PCR products using a 3% agarose gel (Bio-Rad Laboratories, Hercules, CA) pre-stained with ethidium bromide. Amplicons were indexed against a DNA sizing ladder (50 – 1,000 bp, Molecular Probes, Eugene, OR). Separation was performed at 4.8 V/cm in 164 1X TBE (Tris/Boric Acid/EDTA, Bio-Rad Laboratories). After separation, the gels were imaged using a Logic Gel imaging system (Eastman Kodak). For *KRAS* mutational analysis, the PCR amplicons were either submitted for Sanger sequencing by Genewiz Technologies or used for LDR analysis.

LDRs were performed in a total volume of 20 µl in 0.2 ml polypropylene microtubes using a commercial thermal cycling machine (Eppendorf Thermal Cycler; Brinkmann Instruments, Westbury, NY). The reaction cocktail consisted of 10 mM TRIS–HCl (pH ~8.3), 25 mM KCl, 10 mM $MgCl_2$, 0.5 mM NAD+ (nicotinic adenine dinucleotide, a cofactor for ligase enzyme) and 0.01% Triton X-100, 2 µL of 50 nM of the discriminating primers, fluorescently labeled phosphorylated common primer, and 2 µl of the PCR product as template. Primer sequences used for both discriminating and common primers for each possible mutation were reported by Khanna *et al.* and are listed in Table S1 (6).

Forty units of *Taq* DNA Ligase (New England Biolabs) was added to the cocktail under hot-start conditions and the reactions were thermally cycled 20 times for 30 s at 94˚C and 2 min at 60˚C. The LDR products were separated using a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). Data acquisition was performed using the Beckman P/ACE software.

RESULTS

Flow cytometry characterization of RPMI-8226 as a model cell line for CPCs. Primary myeloma cells strongly express CD38, CD138 and in some cases, CD56. In addition, weak to no

expression of CD45 is observed in most myeloma cells (7). A number of studies have utilized cell lines as models for primary myeloma cells as they share some essential features, such as Ig gene re-arrangements, cytoplasmic Ig expression and common surface antigens (7). Fig. S1 shows the results of a flow cytometry study of the RPMI-8226 cell line used as a model in these studies.

We performed phenotypic characterization of these cells using flow cytometry in order to evaluate the fraction of RPMI-8226 cells expressing key myeloma surface antigens. Results indicated that out of a defined population of approximately 60,000 RPMI-8226 cells, 98% expressed CD38, 90% expressed CD138, 74% expressed CD56 while <0.01% showed low expression of CD45. This agrees with previous studies on this cell line (7).

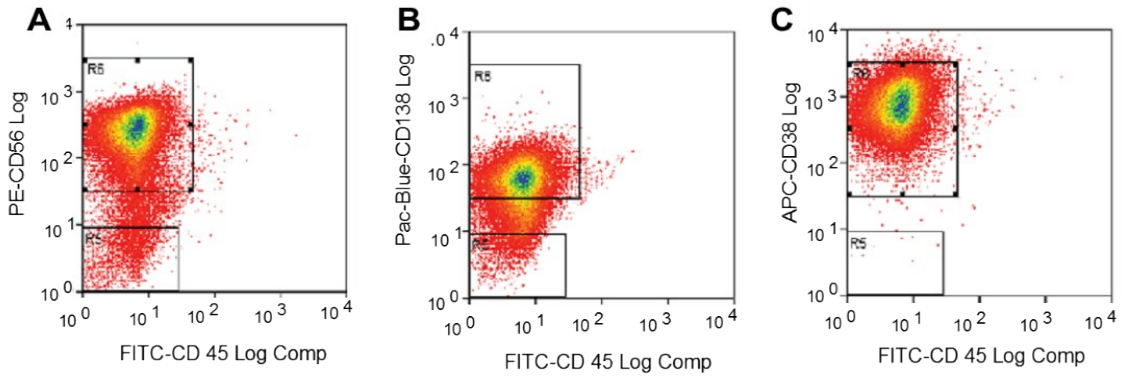

Figure S1. Fluorescence scatter plots for surface antigen expression of the CPC model cell line, RPMI-8226. All scatter plots were gated on CD45. Quadrant R6 of each plot indicated the distribution of cells expressing: (A) CD56; (B) CD138; and (C) CD38 antigens.

Shown in Fig. S2 are CPCs isolated using the CPC selection device from a symptomatic MM patient. The CPCs were subjected to brightfield microscopy and/or immunophenotyping. As can be seen from the micrographs, these cells were classified as CPCs because of their lack of CD45 expression, but expressing CD38 (the CPCs were selected using anti-CD138 monoclonal antibodies and thus, express this antigen).

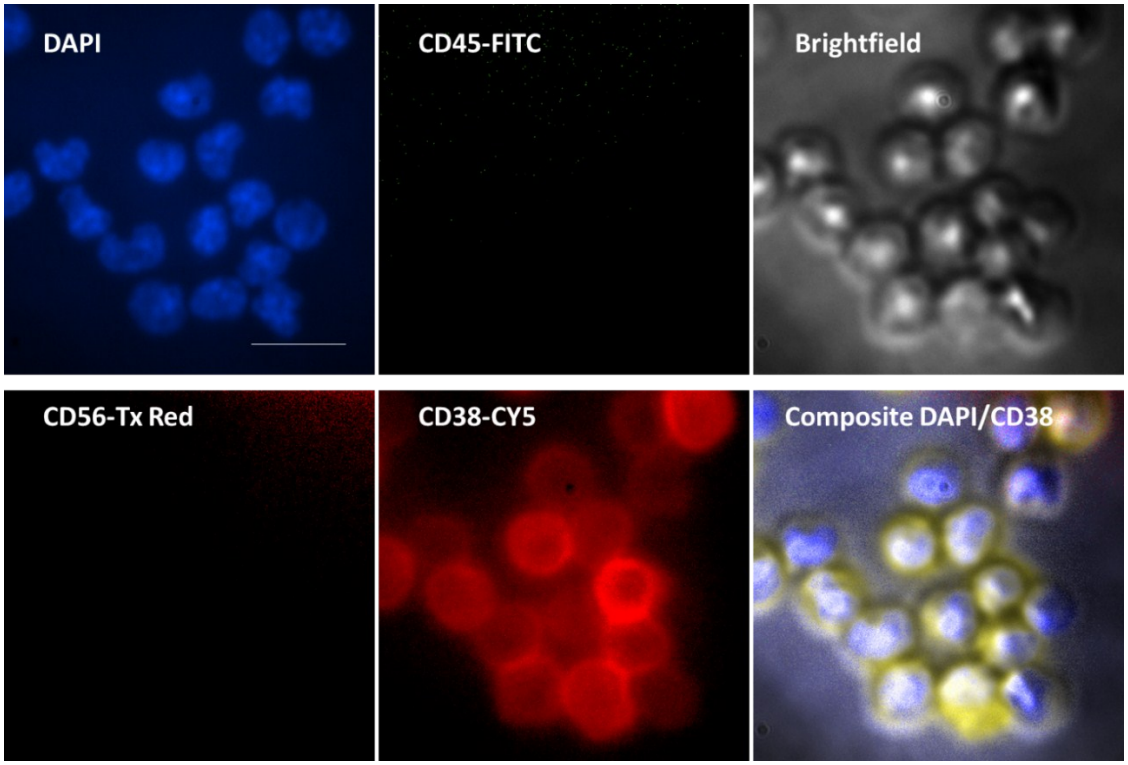

Figure S2. Immunophenotyping results from staining of CPCs isolated from a symptomatic MM patient showing a CD38+/CD56-/CD45- phenotype. Scale bar is 15 µm.

Clinical data summary

Table S2. Classification of controls, MGUS and smoldering MM patients and raw CPCs enumeration data.

Classification/Disease	Sample ID	CPC/ml*	Other Cells/ml **	% Purity
Healthy Control	0	1.0	5.0	-
Non-cancer	0	0.5	10.5	-
Healthy Control	0	0.0	13.5	-
Non-cancer	51	0.0	200.0	-
Non-cancer	58	0.0	230.0	-
MGUS	14	228.0	45.0	83.5
MGUS	46	16.0	134.0	10.7
MGUS	48	43.0	1075.0	3.8
MGUS	49	105.1	548.9	16.1
MGUS	53	107.0	1873.0	5.4
MGUS	55	0.0	312.0	-
MGUS	59	44.6	349.4	11.3
MGUS	61	0.0	686.0	-
MGUS	67	34.0	397.0	7.9

Smoldering MM	12	5.0	77.0	6.1
Smoldering MM	21	14.0	122.0	10.3
Smoldering MM	24	22.0	70.0	23.9
Smoldering MM	25	16.0	43.0	27.1
Smoldering MM	36	22.0	142.0	13.4
Smoldering MM	41	169.2	676.8	20.0
Smoldering MM	43	148.1	1851.9	7.4
Smoldering MM	45	99.8	324.2	23.5
Smoldering MM	52	5.8	168.2	3.3
Smoldering MM	56	62.5	797.4	7.3
Smoldering MM	63	192.3	1249.7	13.3

*The following criteria was used to identify CPCs (Circulating Plasma Cells): CD138+/CD38+/CD45-/CD56(+/-)/DAPI+. **Other Cells: cells were not classified as CPCs if showed CD38 negative staining and/or were CD45+. The following phenotypes were included: CD38+/CD45+/DAPI, CD56+/DAPI+/CD38-/CD45-, CD45+/DAPI, CD56+/CD45+/DAPI, CD56+/CD38+/CD45+/DAPI. MGUS - monoclonal gammopathy of undetermined significance, MM- multiple myeloma.

Table S3. Classification of symptomatic MM patients and raw CPCs enumeration data.

	Classification/Disease	Sample ID	CPC/ml*	Other Cells/ml**	% Purity
Symptomatic Multiple Myeloma	NDMM	1	102.0	25.5	80.0
	RRMM	2	325.5	38.5	89.4
	MM in sCR	4	5.0	9.0	35.7
	RMM	9	12.5	285.5	4.2
	NDMM	10	170.0	64.0	72.6
	RMM	11	257.0	115.0	69.1
	RRMM	15	398.0	426.0	48.3
	RRMM	18	108.0	80.0	57.4
	RRMM	20	742.0	82.0	90.0
	RMM	32	330.0	204.0	61.8
	NDMM	33	908.0	162.0	84.9
	RMM	35	554.0	274.0	66.9
	NDMM	38	530.0	82.0	86.6
	RRMM	42	5820.0	180.0	97.0
	NDMM	44	100.7	987.3	9.3
	RRMM	50	42.4	237.6	15.2

	NDMM	54	305.3	112.6	73.1
	RMM	64	14.4	187.6	7.1
	NDMM	65	4825.0	2270.9	68.0
	RRMM (Treatment)	3	100.0	30.0	76.9
	RRMM (Treatment)	13	715.0	333.0	68.2
	RRMM (Treatment)	16	36.0	37.0	49.3
	RRMM (Treatment)	17	398.0	104.0	79.3
	RRMM (Treatment)	19	38.0	196.0	16.2
	RRMM (Treatment)	26	52.0	64.0	44.8
	RRMM (Treatment)	34	4.0	74.0	5.1
	RRMM (Treatment)	47	10.6	583.4	1.8

* The following criteria was used to identify CPCs (Circulating Plasma Cells): CD138+/CD38+/CD45-/DAPI+. **Other Cells: cells were not classified as CPCs if showed: CD38 negative staining and/or were CD45+. The following phenotypes were included: CD38+/CD45+/DAPI, CD56+/DAPI+/CD38-/CD45-, CD45+/DAPI, CD56+/CD45+/DAPI, CD56+/CD38+/CD45+/DAPI. NDMM-newly diagnosed multiple myeloma, RRMM-relapsed/refractory multiple myeloma, RMM-relapsed multiple myeloma, sCR-critical remission.

Table S4. Summary of clinical results for CD138+/CD38+/CD45- CPCs.

	Health Controls (n = 5)	MGUS (n = 9)	Smoldering MM (n = 11)	Symptomatic MM (n = 19)	Treated Symptomatic MM (n = 8)
Average (CPCs/ml)	0.3	64.2	68.8	818.4	169.3
Median (CPCs/ml)	0.0	43.0	22.0	305.3	45.0
Range (CPCs/ml)	0.0 – 1.0	0.0 – 228.0	5.0 – 192.3	5.0 – 5820.0	4.0 – 715.0
% of $CD56^+$ among CD138+/CD38+ population	0.0 ±0.0 %	33.5 ±10.1 %	19.7 ±7.7 %	35.7 ±9.4 %	34.8 ±13.25 %

Table S5. *KRAS* mutation analysis in clinical samples. Listed are the number of mutations found in CPC isolated from controls and patients #35 (symptomatic MM), #36 (smoldering MM) and #38 (symptomatic MM) blood.

Sample or Patient #	Number of *KRAS* Mutations identified via PCR/LDR/CGE assay	*KRAS* Mutation Type identified	Mutation identified via Sanger sequencing
HT-29	0	Wild type	none
RPMI-8226	1	G35C	G35C
35	5	G34A, G34C, G34T, G35A, G35T	none
36	4	G34A, G35A, G35T, G37A	G37A
38	4	G34C, G34T, G35A, G37A	none

Table S6. Summary of isotypic restriction results.

PCD Type /Pt ID	κ^+ / λ^-CD45$^-$ expressing CPC (%)	κ^- / λ^+ CD45$^-$ expressing CPC (%)	κ^+ and λ^+ CD45$^-$ expressing CPC (%)
AMM/ 20	33	17	50
AMM/ 26	13	0	87
AMM/ 19	No clonal cell populations seen		100
AMM/ 31	No clonal cell populations seen		100
AMM/ 63	50	0	50
AMM/ 64	0	50	50

References

1. Hupert, M., Guy, W.J., Llopis, S., Shadpour, H., Rani, S., Nikitopoulos, D. and Soper, S. (2007) Evaluation of micromilled metal mold masters for the replication of microchip electrophoresis devices. *Microfluid Nanofluid*, **3**, 1-11.
2. Wei, S.Y., Vaidya, B., Patel, A.B., Soper, S.A. and McCarley, R.L. (2005) Photochemically patterned poly(methyl methacrylate) surfaces used in the fabrication of microanalytical devices. *Journal Of Physical Chemistry B*, **109**, 16988-16996.
3. McCarley, R.L., Vaidya, B., Wei, S., Smith, A.F., Patel, A.B., Feng, J., Murphy, M.C. and Soper, S.A. (2004) Resist-Free Patterning of Surface Architectures in Polymer-Based Microanalytical Devices. *Journal of the American Chemical Society*, **127**, 842-843.
4. Barany, F. (1991) Genetic disease detection and DNA amplification using cloned thermostable ligase. *Proceedings of the National Academy of Sciences*, **88**, 189-193.
5. Khanna, M., Park, P., Zirvi, M., Cao, W.G., Picon, A., Day, J., Paty, P. and Barany, F. (1999) Multiplex PCR/LDR for detection of K-ras mutations in primary colon tumors. *Oncogene*, **18**, 27-38.
6. Khanna, M., Cao, W., Zirvi, M., Paty, P. and Barany, F. (1999) Ligase detection reaction for identification of low abundance mutations. *Clinical Biochemistry*, **32**, 287-290.
7. Gooding, R.P., Bybee, A., Cooke, F., Little, A., Marsh, S.G.E., Coelho, E., Gupta, D., Samson, D. and Apperley, J.F. (1999) Phenotypic and molecular analysis of six human cell lines derived from patients with plasma cell dyscrasia. *British Journal of Haematology*, **106**, 669-681.