Mate pair sequencing outperforms fluorescence in situ hybridization in the genomic characterization of multiple myeloma

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Abstract

Fluorescence in situ hybridization (FISH) is currently the gold-standard assay to detect recurrent genomic abnormalities of prognostic significance in multiple myeloma (MM). Since most translocations in MM involve a position effect with heterogeneous breakpoints, we hypothesize that FISH has the potential to miss translocations involving these regions. We evaluated 70 bone marrow samples from patients with plasma cell dyscrasia by FISH and whole-genome mate-pair sequencing (MPseq). Thirty cases (42.9%) displayed at least one instance of discordance between FISH and MPseq for each primary and secondary abnormality evaluated. Nine cases had abnormalities detected by FISH that went undetected by MPseq including 6 tetraploid clones and three cases with missed copy number abnormalities. In contrast, 19 cases had abnormalities detected by MPseq that went undetected by FISH. Seventeen were MYC rearrangements and two were 17p deletions. MPseq identified 36 MYC abnormalities and 17 (50.0% of MYC abnormal group with FISH results) displayed a false negative FISH result. MPseq identified 10 cases (14.3%) with IgL rearrangements, a recent marker of poor outcome, and 10% with abnormalities in genes associated with lenalidomide response or resistance. In summary, MPseq was superior in the characterization of rearrangement complexity and identification of secondary abnormalities demonstrating increased clinical value compared to FISH.

Introduction

Multiple myeloma (MM) is a plasma cell neoplasm (PCN) representing the second most common hematopoietic malignancy and accounts for ~20% of all hematologic cancer related deaths in the United States1. During the last decade there have been remarkable improvements in the treatment of patients with MM that have resulted in increased survival, including immunomodulatory compounds, proteasome inhibitors, and immunotherapeutic approaches such as monoclonal antibodies2. Paralleling the advances in novel therapeutic strategies, characterization of the genomic complexities of MM have significantly improved with the implementation of next-generation sequencing (NGS), thus enabling the identification of novel single nucleotide variants (SNV), structural rearrangements and copy number abnormalities (CNA)3–11. Comprehensive genomic characterization studies such as the Multiple Myeloma Research Foundation (MMRF) CoMMpass Trial and other research studies are necessary for the discovery of novel variants of clinical significance that may lead to improved treatment approaches and prognostication strategies12,13.

In contrast to the use of genome-wide NGS strategies employed in the research/investigational trial setting,
most clinical genomics laboratories rely upon traditional cytogenetic methodologies such as conventional chromosome studies and fluorescence in situ hybridization (FISH) to characterize recurrent cytogenetic abnormalities of prognostic significance. High-risk cytogenetic abnormalities as defined by the Mayo Clinic mSMART 3.0 algorithm\(^\text{14}\) include t(4;14), t(14;16), t(14;20) translocations, 17p deletions and 1q gains, while standard-risk cytogenetic abnormalities include hyperdiploidy (gains of odd-numbered chromosomes), t(11;14) and t(6;14) translocations\(^\text{15,16}\). A limited number of laboratories evaluate for MYC and t(6;14) rearrangements, and detection of IGK and IgL rearrangements is not routinely performed in the clinical setting\(^\text{15}\). Although FISH assays have high sensitivity, are relatively inexpensive compared to NGS techniques and provide input for risk stratification\(^\text{17}\), several limitations exist. They allow for the interrogation of only the regions for which FISH probes are available and multiple FISH probes are needed in order to be comprehensive, with each probe requiring a resource-consuming validation. More importantly, FISH has the potential to miss cryptic abnormalities, including rearrangements that result in a position effect due to juxtaposition of enhancers near oncogenes\(^\text{18–22}\). Since many translocations identified in MM involve a position effect (i.e., IGH and MYC) with heterogeneous breakpoints\(^\text{10,23,24}\) and some CNAs may be cryptic, we hypothesize that some clinical FISH probes used in the characterization of PCNs have a high rate of false negative FISH results.

To test this hypothesis, we evaluated the performance of a genome wide mate-pair sequencing (MPseq) assay in comparison to FISH panel testing for MM. Since MPseq utilizes long input DNA (2–5 Kb) followed by circularization and fragmentation to the size of paired-end fragments (200–500 bp) that are sequenced at reduced depth, this assay is designed to detect structural rearrangements and CNAs throughout the genome resulting in a cost-effective strategy amenable to a clinical genomics laboratory. Furthermore, as MPseq has higher resolution than FISH and is not limited to specific genomic footprints for interrogation, this assay could provide an alternative technique to comprehensively detect structural rearrangements and CNAs in a single assay. Herein we describe the performance, along with the added clinical utility, of MPseq in 70 samples previously characterized by FISH to detect chromosome rearrangements and CNAs in patients with a PCN.

**Methods**

**Patient samples**

All samples were referred to the Mayo Clinic Genomics Laboratory as part of routine clinical testing and further evaluated by MPseq as part of a Mayo Clinic Institutional Review Board approved study. There were multiple sources of samples obtained either from fresh or frozen whole bone marrow (BM), or from fixed cell pellets (FCP) from an abnormal BM chromosome study. Some specimens had undergone plasma cell enrichment from fresh whole BM that was either flow sorted or subjected to CD138+ magnetic-enrichment from patients that had an abnormal plasma cell FISH result. Additional methodology, including conventional chromosome analysis and flow cytometry are included in supplemental data.

**Fluorescence in situ hybridization**

Plasma cell proliferative disorder FISH (PCPDF) of immunoglobulin (cIg)-stained positive PCs studies were performed as previously described\(^\text{23}\) using the following probes to detect primary and secondary MM abnormalities: monosomy 13 or 13q deletion (Abbott Molecular, Abbott Park, IL), monosomy 17 or TP53 deletion (Abbott Molecular), trisomy 3, 7, 9 or 15 (Abbott Molecular), 1q gain (in house, custom developed), MYC rearrangement (Abbott Molecular), IGH rearrangement (in house, custom developed), t(11;14) CCND1/IGH (Abbott Molecular), t(4;14)(p16.3;q32) FGFR3/IGH (Abbott Molecular), t(6;14)(p21;q32) CCND3/IGH (Abbott Molecular), t(14;16)(q32;q23) IGHI/MAF (Abbott Molecular), and t(14;20)(q32;q12) IGHI/MAFB (Abbott Molecular). The PCN FISH panel is indicated in supplemental Table 1 with footprints and probe source shown in supplemental Table 2.

**Plasma cell enrichment**

BM cells (20 × 10^6) were lysed in ACK lysis buffer for 5 min. This was followed by 2 wash steps in PBS (lyse-wash procedure) and the cell pellet was re-suspended in 3% BSA/PBS. 10 × 10^6 cells were then incubated for 15 min with the following antibodies: CD19-PerCP 5.5 (clone SJ25C1, BD Biosciences), CD38-APC (clone REA671, Miltenyi Biotec), CD45-BB515 (clone HI30, BD Biosciences), CD56-PE-Cy7 (clone NCAM16.2, BD Biosciences), CD138-BV421 (clone MI15, BD Biosciences), CD45-BB515 (clone HI30, BD Biosciences), CD38-APC (clone REA671, Miltenyi Biotec), and CD319-PE (clone REA150, Miltenyi Biotec). The specimen was centrifuged and re-suspended in 1.5 mL of PBS. Sorting was performed on BD FACSMelody cell sorter (BD Biosciences, San Jose, CA). Sorting streams were defined for each case separately, using gates to include CD138-positive, CD319-positive, CD38-bright, CD56-positive and/or CD45-negative plasma cells, and separate them from normal plasma cells. A minimum of 2 × 10^5 cells were collected, with the purity of at least 95%, verified by Kaluza software (Beckman Coulter Life Sciences, Indianapolis, IN). In some cases, plasma cells were separated by positive selection using CD138-coated magnetic beads (MACS; Miltenyi Biotec, CA) in a RoboSep system (STEMCELL Technology, Canada) as described in Jang et al.\(^\text{26}\).
DNA extraction and library preparation

DNA extraction and mate pair library preparation methods have been previously described\textsuperscript{18,27,28}. Briefly, DNA was isolated using either the Qiagen Puregene extraction kit (for samples < 2 mL), Autopure LS Automated high quality DNA extraction (for samples > 2 mL) or the QIAmp Tissue kit for fixed cell pellet samples. DNA was processed using the Illumina Nextera Mate Pair library preparation kit and sequenced on the Illumina HiSeq 2500 in rapid run mode as described in Aypar, library preparation kit and sequenced on the Illumina DNA was processed using the Illumina Nextera Mate Pair or the QIAmp Tissue kit for mated high quality DNA extraction (for samples > 2 mL) extraction kit (for samples < 2 mL), Autopure LS Auto reads and paired end sequencing.

Structural variant bioinformatics pipeline and visualization

The sequencing data was analyzed for the detection of structural variants (SVs), which are large genomic changes (>30Kb) that involve breakpoint junctions and/or CNAs. The sequencing data was mapped to the reference genome (GRCh38) using BIMA\textsuperscript{29} and the output was analyzed using SVAtools. This set of algorithms can detect and report the breakpoint locations of both junctions and CNAs at high resolution and accuracy (Schematic in supplemental Fig. 1)\textsuperscript{18,27,28}. Junctions and CNVs were graphically illustrated using genome, junction and region plots as previously described\textsuperscript{18,27,30}.

Results

Patient characteristics

A total of 70 cases referred to the Mayo Clinic Genomics Laboratory for routine clinical PCN FISH testing were selected for further evaluation by MPseq (Tables 1, 2 and supplemental Table 3). Criteria for inclusion included the type of primary cytogenetic abnormality to ensure representation of each recurring rearrangement and sample source to evaluate various methods of sample attainment, including PC enrichment (Tables 1, 2). The median age was 66 years (range 42–88) demonstrating male predominance with a 1.3:1 (M:F) ratio. Fifty-seven cases (81.4%) had either a diagnosis of MM (N = 35) or a reason for referral (RFR) of MM or PCN indicated at the time of clinical testing (N = 22) (Tables 1, 2). Of thirty-five cases with complete clinical data, 13 (37.1%) were newly diagnosed (ND) and 22 (62.9%) had relapsed and/or refractory disease (RR).

MM abnormalities identified by FISH

Recurrent primary MM cytogenetic abnormalities identified by FISH in samples 1–65 were t(11;14) (21.4%), t(4;14) (11.4%), t(14;16) (5.7%), t(14;20) (5.7%), t(6;14) (2.9%), and hyperdiploidy (45.7%) either without an IGH rearrangement (32.9%) or with an IGH rearrangement that did not involve CCND1, FGFR3, MAF, MAFB or CCND3 (12.9%) (Tables 1, 2). Five samples (cases 66–70) had undefined primary abnormalities including one case of tetraploidy with a relative 1q gain, one case with monosomies 13 and 14, two cases with monosomy 15 by FISH and a single case with normal FISH results in a patient with a diagnosis of amyloidosis (Tables 1, 2). Conventional chromosome studies were performed on 42 (60.0%) cases and an abnormal PC clone was identified in 33.3% of the cases with chromosome studies performed (Supplemental Table 3).

We have previously determined tumor content requirements for MPseq requiring 10% tumor for the detection of structural rearrangements and 25% tumor for the detection of CNAs\textsuperscript{18}. Since variable and sometimes low clonal PC percentages can be identified in the BM aspirates of patients with NDMM\textsuperscript{31}, we performed two enrichment strategies for samples with low PCs including magnetic enrichment or flow sorting. For some samples, no enrichment was performed. Thirty-nine (55.7%) samples with a median 23.0% PCs were subjected to either flow sorting (N = 24) or CD138 + magnetic bead for PC enrichment (N = 15). For the remaining 31 samples (44.3%) with a median 58% PC, no PC enrichment was performed.

Identification of recurrent, primary cytogenetic abnormalities using MPseq

To determine the accuracy of MPseq in comparison to our PCN FISH panel (Supplemental Table 1) in the detection of recurrent, primary MM abnormalities (IGH rearrangement and/or hyperdiploidy), we analyzed DNA extracted from either a fixed cell pellet (FCP) from a chromosome study (n = 8), from fresh (n = 18) or frozen (n = 5) BM aspirates or from fresh BM specimens that had been flow sorted (n = 24) or subjected to CD138 + magnetic enrichment (n = 15) (Supplemental Fig. 1, Tables 1, 2). For samples 1–65, MPseq confirmed the primary abnormality identified by FISH in each case demonstrating 100% concordance between both assays for the classification of recurrent, primary cytogenetic abnormalities (Fig. 1). For those cases without evidence of a recurrent, primary abnormality (samples 66–69), MPseq did not identify tetraploidy in case 66 and monosomy 15 in case 68–69, but identified monosomies 13 and 14 in case 67 and confirmed no recurrent abnormality in case 70 with normal FISH results. As a negative control, no recurrent primary MM abnormalities (MM specific IGH rearrangements and/or hyperdiploidy with gains of odd numbered chromosomes) were identified by MPseq in a previously described cohort of 88 patients with a reason for referral of acute myeloid leukemia (data not shown)\textsuperscript{18}.

Comparison of MPseq to FISH for detection of recurrent, secondary abnormalities

For each primary and secondary abnormality that was identified by either MPseq or FISH, 40 cases (57.1%)
| Site | Sex | Age (years) | Dx or RFR | ND RR | % PC | Light chain | Sample type | Primary abnormality (FISH) |
|------|-----|-------------|-----------|-------|------|-------------|-------------|--------------------------|
| MCL  | M   | 77          | MM\*      | U     | 83   | Kappa       | FCP         | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 65          | MM        | ND    | 23   | Lambda      | Sort        | nuclTP73x1,1q23x1(MYCl2,MYC)x1,MYC/3MYClx1,CCND1-XT(x1),IGH/XT(x1) |
| F    | 70  | POV\*       | U         | 19    |      | Kappa       | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 71          | MM        | ND    | 37   | Lambda      | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MCL  | M   | 69          | MM\*      | U     | 50   | Lambda      | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 68          | MM        | RR    |      | Lambda      | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 83          | MM        | ND    | 37   | Kappa       | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MCL  | M   | 61          | MM\*      | U     | 73   | Kappa       | Fresh       | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 69          | MM        | ND    | 65   | Fresh       | Fresh       | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 77          | MM\*      | U     | 68   | Kappa       | Fresh       | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MCL  | M   | 77          | MM        | ND    | 50   | Lambda      | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 89          | AL        | ND    | 5    | Lambda      | CD138+      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 63          | POCPD     | U     | 5    | Lambda      | CD138+      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 54          | MM        | ND    | 47   | CD138+      | CD138+      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 67          | IgA gammapathy\* | U | 40 | Lambda | CD138+ | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 75          | MM        | RR    | 13   | Kappa       | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 73          | MM\*      | U     | 34   | Lambda      | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MCL  | M   | 68          | Monoclonal gammapathy\* | U | 20 | Lambda | Sort        | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 72          | PLC        | U     | 70   | Kappa       | Fresh       | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 42          | MM        | RR    | 11   | Kappa       | Fresh       | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 57          | MM        | RR    | 41   | Kappa       | Fresh       | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MCL  | M   | 78          | MM        | RR    | 77   | Lambda      | Frozen      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 68          | MM        | ND    | 22   | Lambda      | CD138+      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 40          | MM        | ND    | 50   | Lambda      | CD138+      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 67          | MM        | ND    | 60   | CD138+      | CD138+      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | M   | 75          | MM        | U     | 27   | Kappa       | FCP         | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MCL  | M   | 88          | MM\*      | U     | 56   | Lambda      | CD138+      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 65          | R/O MM\*  | U     | 63   | Lambda      | CD138+      | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| MAYO | F   | 74          | MM        | ND    | 5    | Kappa       | Fresh       | nuclcMYC/BLAP1,TP53x1,CCND1-XT(x1),IGH/XT(x1) |
| Site | Sex | Age (years) | Dx or RR* | ND RR | % PC | Light chain Sample type | Primary abnormality (FISH) | FISH performed on |
|------|-----|-------------|----------|-------|------|-----------------------|---------------------------|---------------------|
| 32   | M   | 74          | MM*      | U     | N/A  | N/A                   | FCP                       | 6/14                |
| 33   | M   | 51          | R/O, MA, hypercalcemia, renal failure | U | N/A  | N/A                   | FCP                       | 6/14                |
| 34   | F   | 58          | MM*      | 33    | MCL | M 51 R/O MM, hypercalcemia, renal failure | FCP                       | Hyper + IGH sep   |
| 35   | F   | 34          | MM*      | 34    | MCL | F 58 MM a U 21 Kappa FCP Hyper IGH sep | FCP                       | Hyper + IGH sep   |
| 36   | MAYO | 82         | MM       | R     | 7     | Lambda                | Sort                       | Hyper + IGH sep   |
| 37   | MAYO | 61         | MM       | R     | 27    | Kappa                 | Sort                       | Hyper + IGH sep   |
| 38   | MAYO | 58         | MM       | R     | 11    | Lambda                | Sort                       | Hyper + IGH sep   |
| 39   | MAYO | 64         | MM       | U     | 20    | Lambda                | Sort                       | Hyper + IGH sep   |
| 40   | MAYO | 60         | MM       | U     | 81    | Lambda                | Sort                       | Hyper + IGH sep   |
| 41   | MAYO | 60         | MM       | U     | 98    | Lambda                | Sort                       | Hyper + IGH sep   |
| 42   | MAYO | 70         | MM       | R     | 19    | Lambda                | Sort                       | Hyper + IGH sep   |
| 43   | MAYO | 56         | PCPO     | U     | 4     | Indeterminate         | Sort                       | Hyper + IGH sep   |
| 44   | MAYO | 57         | MM       | RR    | 12    | Kappa                 | Sort                       | Hyper + IGH sep   |
| 45   | MAYO | 75         | M       | U     | 14    | Lambda                | Sort                       | Hyper + IGH sep   |
| 46   | MAYO | 66         | M       | ND    | 19    | Lambda                | Sort                       | Hyper + IGH sep   |
| 47   | MAYO | 72         | M       | ND    | 10    | Lambda                | Sort                       | Hyper + IGH sep   |
| 48   | MAYO | 67         | Lambda   | U     | 10    | Lambda                | Sort                       | Hyper + IGH sep   |
| 49   | MAYO | 71         | Lambda   | U     | 33    | Lambda                | Sort                       | Hyper + IGH sep   |
| 50   | MAYO | 64         | Lambda   | U     | 2     | Lambda                | FCP                       | Hyper + IGH sep   |
| 51   | MAYO | 49         | MM       | RR    | 84    | Lambda                | FCP                       | Hyper + IGH sep   |
| 52   | MAYO | 75         | PCPO     | U     | 73    | Lambda                | FCP                       | Hyper + IGH sep   |
| 53   | MAYO | 66         | MM       | U     | 52    | Lambda                | FCP                       | Hyper + IGH sep   |
| 54   | MAYO | 61         | MM       | U     | 52    | Lambda                | FCP                       | Hyper + IGH sep   |
| 55   | MAYO | 57         | Solitary plasmacytoma | U | 99    | Lambda                | FCP                       | Hyper + IGH sep   |
| 56   | MAYO | 56         | MM       | U     | 34    | Lambda                | FCP                       | Hyper + IGH sep   |
| 57   | MAYO | 61         | Lambda   | U     | 55    | Lambda                | FCP                       | Hyper + IGH sep   |
| 58   | MAYO | 59         | MM       | RR    | 79    | Indeterminate         | FCP                       | Hyper + IGH sep   |
| 59   | MAYO | 74         | MM       | RR    | 60    | Lambda                | FCP                       | Hyper + IGH sep   |
| 60   | MAYO | 53         | MM       | ND    | 90    | Lambda                | FCP                       | Hyper + IGH sep   |
| 61   | MAYO | 61         | MM       | RR    | 25    | Lambda                | FCP                       | Hyper + IGH sep   |
| 62   | MAYO | 48         | MM       | RR    | 75    | Lambda                | FCP                       | Hyper + IGH sep   |
| 63   | MAYO | 49         | MM       | RR    | 5     | Lambda                | FCP                       | Hyper + IGH sep   |
| 64   | MAYO | 48         | MM       | RR    | 70    | Lambda                | FCP                       | Hyper + IGH sep   |
| 65   | MAYO | 66         | MM       | RR    | 25    | Lambda                | FCP                       | Hyper + IGH sep   |
| 66   | MAYO | 70         | Lambda   | U     | 39    | Lambda                | FCP                       | Hyper + IGH sep   |
| 67   | MAYO | 74         | Gammopathy | U | 36    | Lambda                | Sort                       | Monosomy 13/14 |
displayed concordance between FISH and MPseq. Thirty cases (42.9%) displayed at least one instance of discordance between FISH and MPseq (Figs. 1, 2a). Nine of these 30 discordant cases had abnormalities detected by FISH that went undetected by MPseq (Figs. 1, 2a, “FISH advantage”). Of these nine cases, six had a tetraploid clone that was not detectable by MPseq and in three cases MPseq failed to detect CNAs that were identified by FISH (trisomy 3, trisomy 9 and 1q gain). In six cases, FISH identified a CNA involving chromosome 15 that was not confirmed by MPseq. These abnormalities included monosomy 15 identified by FISH without evidence of monosomy 15 by MPseq (cases 26, 47, 68, 69), or one (case 34) or two (case 52) copies of chromosomes 15 identified by FISH in cases with trisomy 15 identified by MPseq (Figs. 1, 2a). In contrast, 19 of the 30 discordant cases had abnormalities detected by MPseq that went undetected by FISH (Figs. 1, 2a, “MPseq advantage”). Of these 19 cases, 17 were MYC rearrangements and two were 17p deletions (cases 4 and 21), including a 17p translocation involving the TPS3 gene in one case (Figs. 1, 2a).

**Increased detection rate of MYC rearrangements by MPseq**

From 70 total cases, we identified 36 cases (51.4%) that displayed a MYC rearrangement by MPseq (Fig. 1). Of these 36 cases, 34 had FISH data evaluating the MYC locus. Seventeen cases (50.0% of MYC abnormal group with FISH results) displayed a false negative MYC FISH result where a MYC rearrangement was identified by MPseq, but was negative by FISH (Fig. 1). The most common partner gene/enhancer segment identified were IGH (n = 7), FAM46C (n = 5), IGK (n = 4), NSMCE2 (n = 4), TXNDC5 (n = 4) and IGL (n = 4) (Table 3). Of the 36 MYC rearrangements, multiple mechanisms resulting in positioning of MYC near enhancer sequences including small insertions, inversions, simple, balanced or complex translocations were identified (Table 3). The most common method of rearrangement identified in 15 cases included a small insertion of enhancer sequences near the MYC gene or, alternately, the insertion of MYC near enhancer sequences. These insertions typically involve the duplication of genetic material of similar size at both the source location and the insertion location, whereby the source DNA is inserted between flanking duplications at the insertion location32 (Fig. 2b). Thirteen of these 15 insertion cases co-occurred with hyperdiploidy (hyperdiploidy only or hyperdiploidy with IGH separation) and two of these cases were identified by FISH studies. Of the 17 MYC cases that were missed by FISH, 11 represented these small insertions (Table 3).

**Detection of additional genomic alterations by MPseq that are not evaluated by FISH**

We next evaluated for the presence of rearrangements involving non-recurrent IGH MM partners (excluding
CCND1, FGFR3, MAF, MAFB or CCND3) and the IGK and IGL loci by MPSeq. There were 19 additional IGH rearrangements identified in 18 cases (25.7% of cohort) with partner chromosomes at 8q24.21 (MYC) (n = 7) as the only recurrent rearrangement (Table 4, Fig. 3). Of the nine cases classified as “hyperdiploidy with IGH separation”, an IGH partner was identified in six cases, while the other three cases had a loss within the IGH locus. Two cases (cases 50 and 54) classified as hyperdiploidy without an IGH rearrangement to one of the common partner chromosomes had the “small insertion” type of MYC/IGH rearrangement (Tables 3, 4, Fig. 3). There were three cases with a CCND1 rearrangement to a locus other than IGH (IGK/CCND1 in case 43, IGL/CCND1 in case 4 and BRINP3/CCND1 in case 57) that had additional copies of CCND1 observed by FISH in case 4 and 43. FISH for CCND1 was not performed in case 57 and in case 4, the signal pattern for CCND1 was scored as amplification (Table 4, Figs. 1, 3).

There were five cases with IGK rearrangements (7.1% of cohort) mainly with partner chromosome 8q24.21 (MYC) (n = 4) and a single case with partner chromosome at 11q13.3 (CCND1) (Table 4, Fig. 3). In addition, 10 cases (14.3% of cohort) had IGL rearrangements with partner chromosomes at 8q24.21 (MYC) (n = 4), 11q13.3 (CCND1) (n = 1), 8q24.22 (ST3GAL1/NDRG1), 3q26.2 (MECOM) (n = 1) and 17q25.1 (GRB2) (n = 1) (Table 4, Fig. 3). Of these 15 cases with either an IGK or IGL rearrangement, 12 (80.0%) co-occurred with hyperdiploidy (hyperdiploidy only or hyperdiploidy with IGH separation).

We explored alterations in additional genes contributing to dysregulation of multiple pathways such as WNT or NF-kB signaling including genes CYLD at 16q12.1, BIRC2 and BIRC3 at 11q22.2, NFKB1 at 4q24, NFKB2 at 10q24.32, TRAF2 at 9q34.3, TRAF3 at 14q32.32 and MAP3K14/NIK at 17q21.31 or other tumor suppressor genes such as CDKN2C (p18) at 1p32.3 or TENT5C/FAM46C at 1p12 (33,34) (Table 5, Fig. 3). Twenty-five cases

| Table 2 Patient characteristics | Total (N = 70) |
|----------------------------------|---------------|
| Characteristic                   | N (%)         |
| Sex                              |               |
| Male                             | 40 (57.1)     |
| Female                           | 30 (42.9)     |
| Age                              |               |
| Median                           | 66 years      |
| Range                            | 42–88 years   |
| 40–49                            | 4 (5.7)       |
| 50–59                            | 14 (20.0)     |
| 60–69                            | 26 (37.1)     |
| 70–79                            | 21 (30.0)     |
| 80–89                            | 5 (7.1)       |
| Diagnosis or RFR                 |               |
| MM, PCN diagnosis or RFR         | 57 (81.4)     |
| Amyloidosis                      | 3 (4.3)       |
| Plasma cell leukemia             | 2 (2.9)       |
| Plasma cell proliferative disorder | 3 (4.3) |
| Other                            | 5 (7.1)       |
| Site                             |               |
| Mayo Clinic-local                | 37 (52.9)     |
| Mayo Clinic Laboratories-outside | 33 (47.1)     |
| PC percentage (N = 68)           |               |
| Median                           | 35.5%         |
| Range                            | 4–99%         |
| 4–19                             | 19 (27.9)     |
| 20–39                            | 19 (27.9)     |
| 40–59                            | 11 (16.2)     |
| 60–79                            | 12 (17.6)     |
| 80–99                            | 7 (10.3)      |
| Sample type                      |               |
| No enrichment                    | 31 (44.3)     |
| Fixed cell pellet (FCP)          | 8 (11.4)      |
| Fresh sample                     | 18 (25.7)     |
| Frozen sample                    | 5 (7.1)       |
| Enrichment                       | 39 (55.7)     |
| Flow sorting                     | 24 (34.3)     |
| CD138+ magnetic                  | 15 (21.4)     |
| Light chain                      |               |
| Kappa                            | 46 (65.7)     |
| Lambda                           | 20 (28.6)     |
| Indeterminate or unknown         | 4 (5.7)       |
| Primary cytogenetic abnormality  |               |
| t(11;14)                         | 15 (21.4)     |
| t(4;14)                          | 8 (11.4)      |
| t(14;16)                         | 4 (5.7)       |
| t(14;20)                         | 4 (5.7)       |
| t(6;14)                          | 2 (2.9)       |
| Hyperdiploid only                | 23 (32.9)     |
| Hyperdiploid with an unknown IGH rearrangement | 9 (12.9) |
| Tetraploid without primary abnormality | 1 (1.4)     |
| Monosomy 13/14 alone             | 1 (1.4)       |
| Monosomy 15 alone                | 2 (2.9)       |
| Normal                           | 1 (1.4)       |

| Table 2 continued |
|--------------------|
| Characteristic      | N (%)         |
| Conventional chromosome study |               |
| Not performed       | 28 (40.0)     |
| Performed           | 42 (60.0)     |
| Normal or loss of Y | 27 (38.6 of total, 64.3 of performed) |
| Abnormal with PC abnormalities | 14 (20.0 of total, 33.3 of performed) |
| Abnormal with non-PC abnormalities | 1 (1.4 of total, 2.4 of performed) |

Patient characteristics of the 70 patients within the cohort evaluated
had an alteration in *TENT5C/FAM46C* with 6 cases with translocations (five of these to *MYC*) and 19 cases had a heterozygous deletion of *TENT5C/FAM46C* ranging in size from 3.4 Mb to 120 Mb. Nineteen cases (27.1%) had alterations ranging in size from 3.4 Mb to 120 Mb. Nineteen cases had a heterozygous deletion of *CDKN2C* and/or *FAF1*. Fourteen were heterozygous deletions involving *CDKN2C* ranging in size from 587 Kb to 120 Mb, four cases had focal biallelic *CDKN2C* and *FAF1* deletions (Supplemental Fig. 2A, case #5) and one case had a heterozygous 655 Kb *FAF1* deletion without a *CDKN2C* deletion (Table 5, Fig. 3). Ten cases had deletions of *TRAF3* with 5 as heterozygous deletions and five as biallelic deletions (Supplemental Fig. 2B, case #62) and a single case had a 92.9 Kb heterozygous deletion of *TRAF2* (Table 5, Fig. 3). Twenty-eight cases had deletions of *CYLD* (40% of cohort) with 24 cases having heterozygous deletions ranging in size from 634 Kb to 90.3 Mb with the majority representing large 16q deletions and four cases will smaller biallelic deletions (Table 5, Fig. 3, Supplemental Fig. 2C, case #40). Additional alterations in *MAP3K14* were observed in four cases (three as heterozygous deletions and one as a 735 Kb gain), heterozygous deletion of *NFKB1* in seven cases, heterozygous deletion of *NFKB2* in 6 cases and a heterozygous and homozygous *BIRC2* and *BIRC3* deletions in two separate cases (Table 5, Fig. 3).

Evaluation for loss of function alterations of genes that have been associated with lenalidomide response or resistance (*CRBN, IKZF1, and IKZF3*) identified 10.0% of the cohort had either a *CRBN, IKZF1* and *IKZF3* gene alteration (Table 5, Fig. 3, Supplemental Fig. 2D, case

| MM Rearrangements | 11.14 | 14.14 | 14.16 | 14.20 | 8.14 | Hyper+IGH sep | Hyper | Other | Total abnormalities |
|-------------------|-------|-------|-------|-------|------|---------------|-------|-------|-------------------|
| T(11;14), CCND1/IGH | | | | | | | | | 17 |
| T(4;14)(p16.3;q32) FGFR3/IGH | | | | | | | | | 4 |
| T(14;16)(q22;q13) IGH/MAF | | | | | | | | | 4 |
| T(14;25)(q22;q12) IGH/MAFB | | | | | | | | | 4 |
| T(14)(q11.2;12) CCND3/IGH | | | | | | | | | 4 |
| MYC rearrangement | | | | | | | | | 4 |

**MM Copy Number Variants**

| Hyper | Trisomy 11, 11, 16, 17, 15, 17 | Trisomy 3, D3Z1 | Trisomy 4, D4Z1 | Trisomy 8, D8Z1 | Trisomy 11 or extra | Trisomy 17, D17Z1 | Trisomy 19, D19Z1 | Trisomy 17, TPS13/D17Z1 | Trisomy 19, TPS13/D17Z1 | Trisomy 13 R81/LAMP1 | Trisomy 19 R81/LAMP1 | Trisomy 19, R16/LAMP1 | Trisomy 19, R16/LAMP1 | Trisomy 19, R16/LAMP1 | Trisomy 19, R16/LAMP1 | Trisomy 19, R16/LAMP1 | Trisomy 19, R16/LAMP1 | Trisomy 19, R16/LAMP1 |
|-------|-------------------------------|-----------------|-----------------|-----------------|-------------------|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|       | 36                            | 28              | 47              | 40              | 40                | 27              | 28                | 27                | 27                | 4                 | 4                 | 4                 | 4                 | 4                 | 4                 | 4                 | 4                 | 4                 |

**Concordance**

| MPseq=FISH | Discordance | MPseq advantage | Chromosome 15 polymorphism | FISH advantage | Sample type |
|------------|-------------|-----------------|-----------------------------|----------------|-------------|
| 40         | 30          | 30              | 30                          | 30             | Plasma cells |
| 4           | 4           | 4               | 4                           | 4              | ND or RR    |
| 1           | 1           | 1               | 1                           | 1              | Risk stratification FISH |
| 1           | 1           | 1               | 1                           | 1              | Risk stratification MPseq |

**Fig. 1 Concordance between MPseq and FISH.** In bold indicates primary cytogenetic abnormalities. Cytogenetic risk applied to all cases: H: high and S:standard. ND: Newly diagnosed, RR: relapsed/refractory. For case 33, there was no FISH for MYC BAP, but detection of t(8;14) and t(6;14) was achieved using chromosome studies and CCND3/IGH rearrangement confirmed by FISH. For case 37, there was a history of trisomies 9,11,15, and IGH separation in an older sample. For cases 61 and 65 there was evidence of hyperdiploidy by FISH in older samples. Highlighted in yellow is a single case with a difference in cytogenetic risk between MPseq and FISH.
Specifically, five cases had a heterozygous deletion of \textit{CRBN} ranging in size from 457 Kb to 130.5 Mb including case #58 that had both a 7.1 Mb deletion encompassing \textit{CRBN} and a 223 Kb \textit{IKZF1} duplication with insertion of \textit{IKZF1} into 10q25.2. Two cases had a heterozygous deletion that included \textit{IKZF3} (9.1 Mb and 45.7 Mb).

\section*{Discussion}
Most clinical laboratories employ FISH analysis of CD138 enriched plasma cells as the preferred methodology in order to identify recurrent primary and secondary genomic abnormalities of prognostic and therapeutic significance in patients with PCNs. The majority of these laboratories utilize only a limited FISH panel with many focusing on high risk abnormalities defined by the revised International Staging System (R-ISS) including 1q gain, t(4;14), t(14;16) or 17p deletion. Some laboratories have incorporated the use of chromosomal microarray analysis in the detection of CNAs such as hyperdiploidy, 17p deletions and 1q gains, however microarray studies are unable to identify balanced structural rearrangements necessitating the use of other methodologies in the detection of IGH rearrangements. It has also become increasingly apparent that some FISH probes, such as those targeting \textit{MYC} rearrangements, display evidence of false negative results. In addition, FISH panels for PCNs are variable between individual laboratories, provide a limited view of the whole genome and may not always reflect genomic complexity. Given that multiple research studies and investigational trials have used NGS based techniques to identify CNAs, SNVs along with structural rearrangements, we sought to explore the feasibility of employing an NGS technique in the detection of CNAs and structural rearrangements as a FISH replacement assay within a clinical genomics laboratory.

We describe the performance and added utility of a whole genome NGS based strategy, MPseq, in comparison to the current gold standard FISH approach in the evaluation of patients with PCNs. While MPseq and FISH displayed equal performance in the ability to classify the presence or absence of a recurrent, primary cytogenetic subtype (i.e. hyperdiploidy or specific IGH

\begin{table}[h]
\centering
\begin{tabular}{|l|l|}
\hline
\textbf{Discordance Summary} & \textbf{Total} \\
\hline
FISH advantage (detected by FISH, but not MPseq) only & 7 \\
MPseq missed tetraploidy & 5 \\
MPseq missed 1q gain & 1 \\
MPseq missed trisomy 9 & 1 \\
\hline
FISH and MPseq advantage & 2 \\
FISH missed \textit{MYC}, MPseq missed trisomy 3 & 1 \\
FISH missed \textit{MYC}, MPseq missed tetraploidy & 1 \\
\hline
MPseq advantage (detected by MPseq, but not FISH) only & 15 \\
FISH missed \textit{MYC} & 13 \\
FISH missed \textit{TP53} & 2 \\
\hline
MPseq advantage and poly 15 & 2 \\
FISH missed \textit{MYC} & 2 \\
\hline
Polymorphism 15 only & 4 \\
\hline
\end{tabular}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{discordance_summary}
\caption{Discordance summary and \textit{MYC} breakpoint locations. \textbf{a} Total number of cases with evidence of MPseq advantage, FISH advantage and polymorphism of chromosome 15. \textbf{b} The location of breakpoints in the \textit{MYC} locus across all cases are depicted as vertical lines (black if the \textit{MYC} alteration was detected by FISH, light gray if it was not tested by FISH, and red if it was undetected by FISH). In cases where multiple breakpoints were found in the \textit{MYC} locus, the lines are connected by an arc. The locations of the \textit{MYC} BAP probes used for FISH detection are shown at the top (5' in red, 3' in green) and gene locations are shown in the middle (forward strand in light blue, reverse strand in pink).}
\end{figure}
| Case | FISH   | Type            | Junction | Chr Partner | Pos partner | Pos MYC | MYC Loc | Gene Pair | Primary       |
|------|--------|-----------------|----------|-------------|-------------|---------|---------|-----------|---------------|
| 2    | Complex| 2               | 11       | 26066896    | 127732671   | E       | AWO3    |           |               |
|      |        |                 | 17       | 47142366    | 127755273   |         |         |           |               |
| 3    | Balanced| 1              | 2        | 88839370    | 127770919   | R       | IGK     |           |               |
| 5    | Complex| 1              | 6        | 7913150     | 12780114    | L       | TXNDC5  |           |               |
| 6    | Balanced| 2              | 11       | 25654318    | 126537887   | E       | AWO3    |           |               |
|      |        |                 | 2        | 25075426    | 127775090   |         |         |           |               |
| 9    | Balanced| 1              | 14       | 105662404   | 12654184    | L       | IGH     |           |               |
| 16   | Tandem Dup| 1          | 8        | 97622416    | 12775097    | R       | MTDN    |           |               |
| 17   | Balanced| 1              | 4        | 63788054    | 12894292    | R       | TECRL   |           |               |
| 18   | Tandem Dup| 1          | 8        | 125269897   | 12776035    | R       | NSMCE2  |           |               |
| 19   | Small Insertion| 2       | 2        | 7926621     | 12832003    | E       | ANO3    |           |               |
|      |        |                 | 6        | 7964555     | 12823918    |         |         |           |               |
| 20   | Complex| 1              | 14       | 105577910   | 128067445   | R       | IGH     |           |               |
|      |        |                 | 8        | 128296726   | 127966701   | R       | CSMO3   |           |               |
| 21   | Complex| 2              | 8        | 115196922   | 128311995   | L       | IGK     |           |               |
| 22   | Translocation| 1          | 22       | 22896777    | 129213302   | R       | TECRL   |           |               |
|      |        |                 | 2        | 9760385     | 128244496   | R       | MTDN    |           |               |
| 23   | Small Insertion| 2       | 8        | 97631703    | 128238180   | R       | IGH     |           |               |
| 28   | Translocation| 1          | 2        | 172598535   | 128346028   | R       | PDK1    |           |               |
| 29   | Inversion| 1              | 8        | 125294077   | 127764738   | R       | NSMCE2  |           |               |
| 30   | Balanced| 1              | 1        | 117855602   | 127910156   | R       | FAM46C  |           |               |
| 33   | Translocation| 1          | 14       | 105650372   | 127085983   | L       | IGK     |           |               |
| 34   | Small Insertion| 2       | 1        | 117748520   | 128192712   | R       | FAM46C  |           |               |
|      |        |                 | 20       | 40219155    | 128324443   | R       | MAFB    |           |               |
| 35   | Small Insertion| 2       | 22       | 23049204    | 128265796   | R       | IGK     |           |               |
|      |        |                 | 22       | 22897987    | 128381540   | R       | IGK     |           |               |
| 37   | Complex| 1              | 8        | 125349863   | 12829942    | R       | NSMCE2  |           |               |
| 40   | Translocation| 1          | 14       | 105620039   | 127325949   | L       | IGK     |           |               |
| 41   | Translocation| 1          | 2        | 97966421    | 128329062   | R       | IGK     |           |               |
| 42   | Translocation| 1          | 14       | 105729139   | 128354686   | L       | IGK     |           |               |
| 45   | Small Insertion| 1          | 22       | 22914490    | 127742293   | R       | IGK     |           |               |
| 46   | Small Insertion| 2          | 6        | 7913135     | 128320871   | R       | TXNDC5  |           |               |
|      |        |                 | 6        | 7984873     | 128234759   | R       |         |           |               |
| 49   | Translocation| 1          | 2        | 88806363    | 127710025   | R       | IGK     |           |               |
| 50   | Small Insertion| 2          | 6        | 7983613     | 127815314   | E       | IGK     |           |               |
|      |        |                 | 6        | 7837483     | 127704213   | R       |         |           |               |
| 51   | Amplification| 1          | 8        | 125363400   | 127405538   | L       | NSMCE2  |           |               |
| 52   | Small Insertion| 3          | 3        | 46308943    | 127790484   | E       | CCR3    |           |               |
|      |        |                 | 3        | 46331780    | 127735500   | R       |         |           |               |
| 54   | Small Insertion| 2          | 14       | 105654852   | 127737562   | E       | IGK     |           |               |
| 55   | Small Insertion| 2          | 22       | 23019052    | 12764055    | E       | IGK     |           |               |
|      |        |                 | 22       | 22912000    | 12807000    | R       |         |           |               |
| 56   | Small Insertion| 2          | 1        | 117615769   | 12796692    | R       | FAM46C  |           |               |
| 58   | Small Insertion| 2          | 1        | 117651083   | 12830858    | E       | IGK     |           |               |
| 61   | Small Insertion| 1          | 1        | 117653222   | 128307014   | R       | FAM46C  |           |               |
| 62   | Small Insertion| 1        | 2        | 73143438    | 128285026   | R       | FCHSD2  |           |               |

For each case where a secondary alteration involving MYC was found, the relevant genomic information is provided for the junction(s). The case number is the case number. The FISH column indicates whether or not the MYC FISH test detected the secondary alteration (dark gray—detected by both FISH, light gray—detected by MPseq but not tested by FISH and red—detected by MPseq only). The type column is the type of alteration involved with MYC classified as either a balanced event, a tandem duplication, a translocation, an inversion, part of an amplification, part of a small insertion motif, a complex event, or ND where it was not possible to definitively classify the alteration. The Junction column is the number of junctions involved directly in the alteration, either 1 or 2. The Chr Partner and Pos Partner columns are the chromosome and position location (GRCh38) of the partner breakpoints that are part of alteration. The Pos MYC and MYC Loc columns give the position of the breakpoint in the MYC locus and whether the alteration is to the left, right, or encompassing (L, R, or E) the MYC gene, respectively. The Gene Pair column is the gene that is found at or near the partner breakpoint location. The Primary column is the primary alteration for the case.
Table 4  IGH, IGK, and IGL partner genes.

| Case | IGH partner chromosome | Putative gene target | Primary abnormality |
|------|------------------------|----------------------|---------------------|
| 5    | 1q24.3                 | BATF                 | 11;14               |
| 6    | 19p13.2                | TYK2                 | 11;14               |
| 8    | 11q14.1                | RAB39                | 11;14               |
| 9    | 1p33.3                 | PTPRU                | 11;14               |
| 10   | 20q11.21               | COMMD7               | 11;14               |
| 11   | 22q13.1                | POLR2F               | 11;14               |
| 15   | 2p24.3                 | MYCN                 | 11;14               |
| 20   | 8q24.21                | MYC                  | 4;14                |
| 30   | 5p15.33                | TERT                 | 14;20               |
| 33   | 8q24.21                | MYC                  | 6;14                |
| 34   | 7q22.1                 | Unknown              | Hyper + IGH sep     |
| 36   | Xq32.33                | MTMR1                | Hyper + IGH sep     |
| 37   | 14q24.3                | DPF3                 | Hyper + IGH sep     |
| 40   | 8q24.21                | MYC                  | Hyper + IGH sep     |
| 49   | 9p13.2                 | Pax5                 | Hyper + IGH sep     |
| 50   | 8q24.21                | MYC                  | Hyper + IGH sep     |
| 54   | 8q24.21                | MYC                  | Hyper               |

| Case | IGK partner chromosome | Putative gene target | Primary abnormality |
|------|------------------------|----------------------|---------------------|
| 3    | 8q24.21                | MYC                  | 11;14               |
| 41   | 8q24.21                | MYC                  | Hyper + IGH sep     |
| 43   | 11q13.3                | CCND1                | Hyper               |
| 46   | 8q24.21                | MYC                  | Hyper               |
| 58   | 8q24.21                | MYC                  | Hyper               |

| Case | IGL partner chromosome | Putative gene target | Primary abnormality |
|------|------------------------|----------------------|---------------------|
| 4    | 11q13.3                | CCND1                | 11;14               |
| 22   | 8q24.21                | MYC                  | 4;14                |
| 35   | 8q24.21                | MYC                  | Hyper + IGH sep     |
| 39   | 3q26.2                 | MECOM                | Hyper + IGH sep     |
| 45   | 8q24.21                | MYC                  | Hyper               |
| 50   | 17q25.1                | GRB2                 | Hyper               |
| 55   | 8q24.21                | MYC                  | Hyper               |
| 56   | 8q24.22                | ST3GAL1/NDRG1        | Hyper               |
| 61   | 8q24.22                | ST3GAL1/NDRG1        | Hyper               |
| 63   | 8q24.22                | ST3GAL1/NDRG1        | Hyper               |

Partner genes associated with IGH, IGK, and IGL showing cytogenetic location and putative target genes. Hyper: Hyperdiploidy only. Hyper+IGH sep: Hyperdiploidy with IGH separation

rerrangement), MPseq was superior compared to FISH in the characterization of rearrangement complexity, identification of secondary abnormalities, resolution of atypical FISH results and identification of novel abnormalities of prognostic significance not targeted by traditional FISH panels. Many samples chosen for this study had a high plasma cell burden (median 36% PCs) and ~33% of cases were obtained from fresh or frozen samples that did not require enrichment.

An advantage to using a whole genome NGS technique like MPseq is the ability to identify rearrangements using an unbiased approach. Other laboratories have developed and validated NGS methodologies utilizing target-enrichment approaches for PCNs allowing a custom target pull down of limited genomic regions. While these targeted approaches have reduced cost and simplified analysis workflows, a genome wide approach utilizing long-insert whole genome sequencing employed by the MMRF CoMMpass Study in their Seq-FISH analysis has demonstrated improved sensitivity with similar specificity in relation to clinical FISH testing. Although MPseq is similar to Seq-FISH with regard to a whole genome sequencing approach, a significant limitation to the current MPseq strategy is the inability to identify SNVs. This limitation can be resolved with deeper and faster sequencing, coupled with reduced sequencing costs. An integrated genomic analysis incorporating structural variation, CNAs, and SNVs together may lead to enhanced prognostication. Of practical consideration is the ~two-fold increased cost and “turn-around-time” of reporting of clinical grade testing for MPseq compared to a comprehensive FISH panel; although we anticipate over time the cost and time of reporting for NGS approaches will continue to be reduced.

Another limitation to the use of MPseq is the inability to identify rearrangements in highly repetitive regions of the genome containing constitutive heterochromatin such as those involving telomeres, centromeres, and in regions near the centromeres of chromosomes 1, 9, and 16 and in the Y chromosome. This limitation may be reflected by the inability of MPseq to identify apparent trisomies in 2 cases (cases 5 and 42) with evidence of hyperdiploidy. Case 5 displayed a gain of a structurally abnormal chromosome 3 by conventional chromosome studies. Since the centromere regions that are targeted by the FISH probes are not covered by MPseq, it is unclear whether a small gain or presence of a polymorphism of these regions are present without evidence of a bona fide trisomy or whether the trisomy was present at a subclonal level below the limit of detection by MPseq (<25% for CNAs). Polymorphisms of the acrocentric chromosome 15 have also been reported and are observed in FISH analysis of PCNs in our laboratory (data not shown). Discrepancies involving chromosome 15 are present in 6 of 70 cases in this study demonstrated by either a monosomy 15 FISH result with normal chromosome 15 s by MPseq or either a normal or monosomy 15 FISH result with trisomy 15 by...
MPseq. Since MPseq does not rely on detection of only the centromere region like FISH, analysis of copy number changes throughout the whole chromosome can be useful to interpret the presence or absence of a trisomy. On the other hand, any missed hyperdiploid cases may be of less relevance as hyperdiploidy can be detected by FISH. Risk stratification can be performed with FISH, and abnormal karyotypes may not be missed. Abnormal karyotypes should be tested by both FISH and MPseq.

After detecting abnormalities by MPseq, we performed FISH to confirm any detected abnormality. Results from MPseq were compared with FISH results to determine if any abnormalities were missed by MPseq. Of the 30 discordant cases, 19 cases had abnormalities detected by MPseq that were missed by FISH. In 5 cases, the 1q gain did not appear to be subclonal, however this sample was extracted from unsorted bone marrow with 20% clonal plasma cells, which may have contributed to this missed abnormality. Subclonal CNAs and cases with low tumor load have a risk of being missed by MPseq, a risk that also exists when performing FISH.

Approximately 66% of MYC rearrangements were found in 35% of NDMM with MYC rearrangements have been found in association with disease progression (1, 11, 43). Approximately 66% of MYC rearrangements have been found in association with non-lg partners resulting in juxtaposition to enhancer regions.
### Table 5 Abnormalities of additional genes of clinical significance

| Case | CYLD | Location | Breakpoints | Size (bp) | Primary |
|------|------|----------|-------------|-----------|---------|
| 1    | HD   | 16p13.3–16q24.3 | 0–90338345 | 90338345  | 11,14   |
| 2    | HD   | 16q11.2–16q24.3 | 64654000–90338000 | 43884000 | 11,14   |
| 4    | HD   | 16q11.2–16q24.3 | 64654000–90338000 | 43884000 | 11,14   |
| 6    | HD   | 16q11.2–16q24.3 | 64654000–90338000 | 43884000 | 11,14   |
| 8    | HD   | 16q11.2–16q24.3 | 64654000–90338000 | 43884000 | 11,14   |
| 9    | HD   | 16q11.2–16q24.3 | 50093000–89129000 | 39036000 | 11,14   |
| 15   | HD   | 16q11.2–16q24.3 | 46454000–90338000 | 43884000 | 11,14   |
| 24   | BD   | 16q12.1–16q12.1 | 50232040–50913020 | 680980  | 14,16   |
| 25   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | 14,16   |
| 28   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | 14,20   |
| 29   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | 14,20   |
| 30   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | 14,20   |
| 33   | BD   | 16q12.1–16q12.1 | 50777028–50812200 | 35172   | 6,14    |
| 35   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper + KH sep |
| 37   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper + KH sep |
| 40   | BD   | 16q12.1–16q12.2 | 50376741–52630633 | 2254092 | Hyper + KH sep |
| 41   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper + KH sep |
| 42   | HD   | 16q12.1–16q12.1 | 50193000–50827000 | 634000  | Hyper + KH sep |
| 44   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper |
| 48   | HD   | 16q12.1–16q12.2 | 50123000–55838000 | 571500  | Hyper |
| 49   | BD   | 16q12.1–16q12.1 | 50290162–51082053 | 791891  | Hyper |
| 50   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper |
| 52   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper |
| 54   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper |
| 61   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper |
| 63   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Hyper |
| 66   | HD   | 16q12.1–16q24.3 | 46454000–90338000 | 43884000 | Tetraploid |
| 67   | HD   | 16p13.3–16q24.3 | 0–90338345 | 90338345 | Monosomy 13/14 |

### Table 5 Abnormalities of additional genes of clinical significance (continued)

| Case | BIRC2 and BIRC3 | Location | Breakpoints | Size (bp) | Primary |
|------|-----------------|----------|-------------|-----------|---------|
| 6    | HD              | 11q14.1–11q22.3 | 79621665–108999346 | 29377681 | 11,14   |
| 21   | BD              | 11q22.1–11q22.2 | 101044665–102389301 | 1344636 | 4,14    |

### Table 5 Abnormalities of additional genes of clinical significance (continued)

| Case | TENT5C/FAM46C | Location | Breakpoints | Size (bp) | Primary |
|------|---------------|----------|-------------|-----------|---------|
| 5    | HD            | 1p22.3–1p12 | 87833886–119707445 | 31873559 | 11,14   |
| 6    | HD            | 1p36.33–1p12 | 1–119990000 | 119989999 | 11,14   |
| 9    | HD            | 1p35.3–1p12 | 29234000–119991000 | 90757000 | 11,14   |
| 15   | HD            | 1p32.3–1p12 | 51107000–119761000 | 68654000 | 11,14   |
| 21   | HD            | 1p31.1–1p12 | 77992000–119733000 | 41741000 | 4,14    |
| 23   | HD            | 1p34.2–1p12 | 42342000–121700000 | 79358000 | 4,14    |
| 24   | HD            | 1p13.3–1p12 | 110882000–120028000 | 9146000 | 14,16   |
| 28   | HD            | 1p32.1–1p12 | 58536000–119985000 | 61449000 | 14,20   |
| 29   | HD            | 1p31.1–1p12 | 75850000–118934000 | 43084000 | 14,20   |
| 30   | Translocation to MYC | 1p12 | 117855602 | N/A | 14,20 |
| 32   | HD            | 1p31.1–1p12 | 70540000–119991000 | 49487000 | 6,14    |
| 33   | HD            | 1p36.33–1p12 | 1–119982000 | 119981999 | 6,14    |
| 34   | Translocation to MYC and MAFB | 1p12 | 117611301,117746520 | N/A | Hyper + KGH sep |
### Table 5 continued

| Case | TENT5C/FAM46C Location | Breakpoints | Size (bp) | Primary |
|------|------------------------|-------------|-----------|---------|
| 35   | HD 1p33–1p12           | 49064000–119989000 | 70925000 | Hyper + IGH sep |
| 37   | HD 1p22.2–1p12         | 89819000–118483000 | 28664000 | Hyper + IGH sep |
| 40   | HD 1p13.1–1p12         | 116616000–119990000 | 3374000  | Hyper + IGH sep |
| 53   | HD 1p31.1–1p12         | 77694018–119983000 | 42288982 | Hyper |
| 56   | Translocation to MYC 1p12 | 117615759;117851083 | N/A      | Hyper |
| 58   | Translocation to IL16 1p12 | 117592488;117745524 | N/A      | Hyper |
| 60   | HD 1p22.1–1p12         | 92935000–119981000 | 27046000 | Hyper |
| 61   | Translocation to MYC 1p12 | 117653222;117665080 | N/A      | Hyper |
| 64   | HD 1p31.3–1p12         | 67860000–118597000 | 50737000 | Hyper |
| 66   | HD 1p36.33–1p12        | 1–119990000       | 119989999| Tetraploid |
| 67   | HD 1p36.33–1p12        | 1–119991000       | 119990999| Monosomy 13/14 |

| Case | CDKN2C and FAF1 Location | Breakpoints | Size (bp) | Primary |
|------|--------------------------|-------------|-----------|---------|
| 5    | BD 1p32.3–1p32.3         | 50884258–51012825 | 128567   | 11;14   |
| 6    | HD 1p36.33–1p12         | 1–119990000  | 119989999| 11;14   |
| 9    | HD 1p35.3–1p12          | 29234000–119991000 | 90757000 | 11;14   |
| 10   | HD 1p32.3–1p32.3        | 50402893–50989867 | 586974   | 11;14   |
| 15   | BD 1p32.3–1p32.3        | 50599579–51016763 | 507184   | 11;14   |
| 21   | HD 1p32.2–1p31.1        | 50276000–73879000 | 23603000 | 4;14    |
| 22   | BD 1p32.3–1p32.3        | 50924750–50971658 | 46908    | 4;14    |
| 23   | HD 1p34.2–1p12          | 42342000–12170000 | 7938000  | 4;14    |
| 24   | HD FAF1 only 1p33–1p32.3 | 49951000–50606000 | 655000   | 14;16   |
| 33   | HD 1p36.33–1p12         | 1–119982000    | 119981999| 6;14    |
| 34   | HD 1p32.3–1p12          | 50750000–117611000 | 66861000 | Hyper + IGH sep |
| 35   | HD 1p33–1p12            | 49064000–119989000 | 70925000 | Hyper + IGH sep |
| 40   | HD 1p33–1p13.3          | 49723000–109237000 | 59514000 | Hyper + IGH sep |
| 51   | HD 1p33–1p31.3          | 50018770–65125485 | 15106715 | Hyper |
| 52   | BD 1p32.3–1p32.3        | 50925212–51007221 | 82009    | Hyper |
| 58   | HD 1p32.3–1p32.3        | 50467681–107513627 | 5704946  | Hyper |
| 60   | BD 1p36.33–1p12         | 1–119990000    | 119989999| Tetraploid |
| 67   | HD 1p36.33–1p12         | 1–119991000    | 119990999| Monosomy 13/14 |

| Case | MAP3K14 Location | Breakpoints | Size (bp) | Primary |
|------|----------------|-------------|-----------|---------|
| 2    | HD 17q21.31–17q21.32 | 44943000–47142000 | 2199000  | 11;14   |
| 36   | HD 17q21.31–17q21.31 | 44583000–45982000 | 1399000  | Hyper + IGH sep |
| 44   | HD 17p13.3–17q21.31  | 1–45734287    | 45734286 | Hyper |
| 45   | Gain 17q21.31–17q21.31 | 45191000–45926000 | 735000   | Hyper |

Blood Cancer Journal
### Case NFKB1 or NFKB2 Location Breakpoints Size (bp) Primary

| Case | NFKB1 or NFKB2 | Location | Breakpoints | Size (bp) | Primary |
|------|----------------|----------|-------------|-----------|---------|
| 6    | HD NFKB1       | 4q13.2–4q26 | 65930271–115942883 | 50012612 | 11;14   |
| 23   | HD NFKB1       | 4p14–4q35.2 | 36402000–189875000 | 153473000 | 4;14    |
| 33   | HD NFKB1       | 4p16.3–4q35.2 | 1–190214555 | 190214554 | 6;14    |
| 34   | HD NFKB2       | 10q24.1–10q26.3 | 9705000–133797422 | 36792422 | Hyper + IGH sep |
| 40   | HD NFKB1       | 4p16.3–4q35.2 | 1–190214555 | 190214554 | Hyper + IGH sep |
| 44   | HD NFKB1       | 4q13.3–4q31.3 | 73221000–150520000 | 77299000 | Hyper    |
| 46   | HD NFKB2       | 10q24.32–10q24.33 | 101899000–103362000 | 1463000 | Hyper    |
| 47   | HD NFKB2       | 10q24.32–10q25.1 | 102399071–104266176 | 1867105 | Hyper    |
| 51   | HD NFKB1       | 4p16.3–4q35.2 | 1–190214555 | 190214554 | Hyper    |
| 65   | HD NFKB2       | 10q24.32–10q25.1 | 102399071–104266176 | 1867105 | Hyper    |
| 66   | HD NFKB1       | 4p16.3–4q35.2 | 1–190214555 | 190214554 | Hyper    |
| 66   | HD NFKB2       | 10q24.32–10q24.33 | 101899000–103362000 | 1463000 | Hyper    |
| 57   | HD NFKB1       | 4p16.3–4q35.2 | 1–190214555 | 190214554 | Hyper    |

### Abnormalities of genes of known clinical significance in MM
Large gains of chromosome material are not indicated

**HD** heterozygous deletion, **BD** biallelic deletion indicated in bold, cytogenetic band and location in GRCh38

### Case TRAF2 or TRAF3 Location Breakpoints Size (bp) Primary

| Case | TRAF2 or TRAF3 | Location | Breakpoints | Size (bp) | Primary |
|------|----------------|----------|-------------|-----------|---------|
| 8    | HD TRAF3       | 1q22.3–1q32.33 | 56254000–104990000 | 48736000 | 11;14   |
| 23   | HD TRAF3       | 1q11.2–1q32.33 | 19958000–105864169 | 85906169 | 4;14    |
| 32   | BD TRAF3       | 1q32.32–1q32.32 | 102754161–102809688 | 55527 | 6;14    |
| 37   | HD TRAF3       | 1q24.3–1q32.33 | 77344000–105590563 | 28246563 | Hyper + IGH sep |
| 44   | HD TRAF3       | 1q21.1–1q32.33 | 39707000–107043718 | 67336718 | Hyper    |
| 47   | HD TRAF3       | 1q32.32–1q32.32 | 102845410–102902550 | 57140 | Hyper    |
| 52   | BD TRAF3       | 1q32.32–1q32.32 | 102722216–102790013 | 67797 | Hyper    |
| 60   | BD TRAF3       | 1q32.32–1q32.32 | 102741220–10288391 | 14171 | Hyper    |
| 62   | BD TRAF3       | 1q32.31–1q32.32 | 102680377–102913538 | 233181 | Hyper    |
| 67   | BD TRAF3       | 1q32.32–1q32.32 | 102841855–102878463 | 36608 | Monosomy 13/14 |
| 67   | HD TRAF2       | 9q34.3–9q34.3 | 136828276–136921241 | 92965 | Monosomy 13/14 |

### Case # CRBN or IKZF1 or IKZF3 Location Breakpoints Size (bp) Primary

| Case # | CRBN or IKZF1 or IKZF3 | Location | Breakpoints | Size (bp) | Primary |
|--------|------------------------|----------|-------------|-----------|---------|
| 8      | HD IKZF3               | 17q12–17q21.31 | 35371000–44480000 | 9109000 | 11;14   |
| 17     | HD CRBN                | 3p26.3–3p26.2 | 2738159–3194829 | 456670 | 4;14    |
| 22     | HD CRBN                | 3p26.3–3q22.1 | 1–130531000 | 130530999 | 4;14    |
| 28     | HD CRBN                | 3p26.3–3p25.2 | 1–12659000 | 12658999 | 14;20   |
| 41     | HD CRBN                | 3p26.3–3p24.1 | 1–28213000 | 28212999 | Hyper + IGH sep |
| 44     | HD IKZF3               | 17p13.3–17q21.31 | 1–45734287 | 45734286 | Hyper    |
| 58     | HD CRBN                | 3p26.3–3p26.1 | 1–7110000 | 7109999 | Hyper    |
| 58     | IKZF1 Gain + insertion to 10q25.2 | 17p12.2 | 50207542–50430511 | 222969 | Hyper    |

Abnormalities of genes of known clinical significance in MM

HD heterozygous deletion, BD biallelic deletion indicated in bold, cytogenetic band and location in GRCh38
sequences promoting aberrant MYC gene expression, which may be targeted by BRD4 inhibitors in MM. Identification of MYC rearrangements using a break-apart probe strategy resulted in a 50.0% false negative rate in our patient cohort. Whether these false negative insertion cases have the same prognostic implication as other MYC rearrangements remains unknown.

Two cases had deletions of the TP53 gene region that were not identified by FISH. For cases 4 and 21, MPseq identified a deletion of TP53 (5.6 Mb in case 4, 2.7 Mb in case 21). Interestingly, for case 21, MPseq also identified a translocation involving TP53 (to 4q32.1). Both cases were scored as having two copies of TP53 by FISH and represent false negative results due to the location of the deletion in relation to the FISH footprint in case 4 and the TP53 translocation in combination with the deletion in case 21. Although these cases had missed high risk abnormalities, the mSMART risk did not change since those cases also had additional high risk abnormalities [1q gain for cases 4 and t(4;14) for case 21]. For case 4, a separate NGS assay analyzing SNVs identified a pathogenic TP53 mutation [Chr17:GRCh37:g.7577111 G>T; NM_001126113.2 (TP53):c.827 C>A; p.Ala276Asp] located in the DNA-binding domain and in vitro functional data predicts that this variant results in non-functional p53 protein. TP53 mutations in combination with 17p deletions are associated with double hit MM with reduced overall, progression-free and relapse-free survival. Therefore, this missed TP53 deletion fails to identify the presence of a likely double hit MM in patient 4. The combination of a rearrangement and deletion also likely represents a double hit MM abnormality in case 21.

The CCND1/IGH dual color, dual fusion probe set is used to identify CCND1/IGH rearrangements. However, three copies of CCND1 in the absence of IGH fusion can indicate trisomy 11 or non-IGH CCND1 rearrangements. MPseq identified three CCND1 rearrangements including case 4 (IGL/CCND1), case 43 (IGK/CCND1) described more fully in Peterson, et al, and case 57 (BRINP3/CCND1). FISH also identified amplification of CCND1 in case 4, three copies of CCND1 in case 43 and a normal signal pattern for CCND1 in case 57. The CCND1 rearrangement identified in case 57 was a complex translocation between 1q31.1 and 11q13.3 consisting of four junctions and deletions of ~100 kb at both ends. Through this complex event, CCND1 is brought into close proximity to the 3’ end of BRINP3, while the balancing set of junctions brings the 5’ end of BRINP3 near 11q24.3. Additionally, the derivative chromosome containing CCND1 has been copied. Overall this would result in three copies of CCND1, two of which have been translocated near the 3’ end of BRINP3. This case demonstrates how MPseq is able to determine complex rearrangements involving important genes without prior knowledge of the junction partner location.

Although immunoglobulin lambda rearrangements have been recently reported in association with poor prognosis, light chain rearrangements are typically not evaluated in the diagnostic work up of MM in most clinical genomics laboratories. Using MPseq data, we identify 10 cases (14.3% of entire cohort) with IGL rearrangements and five of these cases with standard risk cytogenetic results. IGL rearrangements and other focal deletions of clinical significance are typically not evaluated by FISH. Given the high rate of false-negative MYC rearrangements and inability to appreciate all abnormalities of clinical significance, we demonstrate that MPseq has increased clinical value compared to FISH in characterizing genomic abnormalities in PCs.
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