Deep Sequencing Reveals Myeloma Cells in Peripheral Blood in Majority of Multiple Myeloma Patients

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Abstract

A deep-sequencing approach was used to detect and quantify myeloma cells in the peripheral blood in the vast majority of multiple myeloma patients. This study highlights the promise of a blood-based, sequencing assay for minimal residual disease that can be used to measure multiple myeloma disease burden at different time points and various disease stages.

Introduction: The evaluation of myeloma cells in multiple myeloma (MM) patients has generally been limited to the assessment of bone marrow involvement because of the sensitivity limitations of traditional minimal-residual-disease detection methods. Materials and Methods: We developed a sequencing-based method to identify myeloma cells in bone marrow (BM) and peripheral blood (PB) samples, based on their unique immunoglobulin gene rearrangements, that can detect cancer clones at levels well below 1 in 1 million leukocytes (0.0001%). In this multisite study, we used this sequencing method to determine the fraction of patients with myeloma cells in their PB at diagnosis and post-treatment time points. Results: Using this sequencing approach, we detected myeloma cells in the PB in the vast majority of MM patients (44/46, 96%). We demonstrated a clear correlation (R² = 0.57) between myeloma clone levels in paired BM and PB samples, and noted that PB clone levels were approximately 100-fold lower than levels in BM samples. The sequencing assay demonstrated a clear sensitivity advantage in the BM compartment and at least equivalent sensitivity in the PB compared with that of monoclonal-protein results. Conclusion: This study highlights the promise of a blood-based, sequencing minimal-residual-disease assay that can be used to measure MM disease burden at different time points and various disease stages.

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Introduction

Multiple myeloma (MM), a plasma cell malignancy, is the second most common hematologic cancer in the United States.1 Recent advances in the understanding of MM disease pathogenesis and the development of novel agents, such as thalidomide, lenalidomide, and bortezomib, have increased therapeutic response rates and prolonged survival in MM patients.2-8 Survival rates, which have historically ranged from 3 to 5 years, can now exceed 10 years in some patients with the advent of high-dose therapy with autologous stem cell transplantation in combination with these novel chemotherapeutic agents.2,9-10 However, there remains significant variation in survival rates of MM patients, and increasing importance has been placed on the identification of prognostic factors to inform therapeutic strategies and risk stratification in clinical trials.11,12

Previous studies have shown the prognostic relevance of circulating plasma cells in the peripheral blood of patients with MM.13-16 In patients with newly diagnosed MM, plasma cells were detected in the peripheral blood in approximately 75% of
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patients at diagnosis, and the number of plasma cells was shown to be an independent predictor of overall survival. In the setting of autologous stem cell transplantation, plasma cells were detected in the peripheral blood in approximately 40% of MM patients 2 weeks prior to stem cell harvest, and the presence of plasma cells was associated with lower survival and decreased time to progression. These studies used traditional flow-cytometric analysis, which has a sensitivity of 1 in 10,000 leukocytes (0.01%). Methods with increased sensitivity may provide additional information on the fraction of MM patients with plasma cells present in their peripheral blood at diagnosis and posttherapy and validate the prognostic importance of the presence of circulating plasma cells at various time points.

We developed a sequencing-based method to identify myeloma cells in bone marrow and peripheral blood samples, based on their unique immunoglobulin gene rearrangements. The sequencing assay can detect residual disease at levels well below 1 in 1 million leukocytes (0.00001%), which represents at least 2 orders of magnitude higher sensitivity than standard flow-cytometric methods. In this multisite study, we used the sequencing method to determine the fraction of patients with myeloma cells in their peripheral blood at diagnosis and posttreatment time points. We assessed whether the myeloma cell level in the peripheral blood correlates with the level of disease found in the bone marrow and compared the sequencing-based myeloma cell levels with traditional monoclonal protein (M protein) levels in a cohort of 60 MM patients.

Materials and Methods
Clinical Samples
A total of 60 paired bone marrow and peripheral blood samples were analyzed in this study. Of these, 47 paired bone marrow and peripheral blood samples were collected according to protocols approved by the New York University Medical Center, Washington University School of Medicine, or University of California San Francisco Medical Center institutional review board. Written informed consent was obtained before specimen collection, and samples were deidentified before use in studies, and in accordance with the Declaration of Helsinki. Samples were drawn from patients at all stages of disease (newly diagnosed, during treatment, post-transplant, relapse, etc.). Baseline demographic and clinical information including age, gender, status at time of specimen collection, and M protein level were collected. The remaining 13 paired bone marrow and peripheral blood samples were purchased from a commercial source (AllCells, Emeryville, CA).

Samples were banked as cryopreserved mononuclear cells (bone marrow or blood), cryopreserved bone marrow cells separated into CD138 negative (CD138−) and CD138+ fractions following magnetic enrichment, plasma, or serum.

Flow Cytometry and Cell Sorting
On thawing of cryopreserved cells, one-third of the vial volume was washed and lysed immediately. The remainder of each vial was suspended in phosphate-buffered saline containing 2% fetal bovine serum (PBS/2FBS) and washed once before antibody labeling. Mononuclear cells were incubated with the following antibodies from BioLegend (San Diego, CA) or eBioscience (Affymetrix, Santa Clara, CA) for analysis by flow cytometry and cell sorting: anti-CD19 (clone HB19), anti-CD45 (clone HI30), anti-CD138 (clone DL-101), anti-CD38 (clone HIT2), and anti-CD27 (clone O323). Following incubation, cells were washed and suspended in PBS/2FBS containing 4’,6-diamidino-2-phenylindole (DAPI) to enable the exclusion of nonviable cells. Cells were acquired and sorted using a FACSARia (BD Biosciences, San Jose, CA). From each patient sample, normal naïve B cells (defined as CD45+, CD38−, CD19+, CD27−), normal antigen-experienced B cells, and myeloma cells (defined as CD45low, CD38+) were sorted. Sorted cells were pelletted and lysed in RLT Plus Buffer (Qiagen, Venlo, The Netherlands) for nucleic acid isolation. Analysis of flow-cytometry data was performed using FlowJo (Ashland, OR).

Minimal Residual Disease Measurements by Sequenta LymphoSIGHT™ Method
Details of the LymphoSIGHT assay (Sequenta, South San Francisco, CA) have been described elsewhere. Genomic DNA and RNA was amplified using locus-specific primer sets for IGH-VDJ, IGH-DJ, and IGH designed to allow for the amplification of all known alleles of the germline IGH and IGK sequences, as described previously. A clonotype was defined when at least 2 identical sequencing reads were obtained.

The frequency of each clonotype in a sample was determined by calculating the number of sequencing reads for each clonotype divided by the total number of passed sequencing reads in the sample. Myeloma gene rearrangements were identified using a frequency threshold of approximately 5% in bone marrow mononuclear cells (BMMC) or bone marrow CD138+ cells. In preliminary studies, the frequency of individual clonotypes among normal B-cell populations was consistently below this threshold.

The myeloma-derived sequences identified in BMMC or CD138+ cells were used as a target to assess the presence of minimal residual disease (MRD) in peripheral blood samples (ie, peripheral blood mononuclear cells, plasma or serum). For MRD quantitation, we generated multiple sequencing reads for each rearranged B cell in the reaction. For example, in cells containing an IGH rearrangement, the MRD assay was designed to achieve approximately 10× coverage per B cell. The absolute measure of the total myeloma-derived molecules present in a sample was determined, and a final MRD measurement, which is the number of myeloma-derived molecules per 1 million cell equivalents, was obtained for each sample, as described previously.

Results
Patient and Sample Characteristics
A total of 60 patients were included in this study. Baseline demographics and sample characteristics are summarized in Table 1. Samples were obtained from 47 adults diagnosed with MM at New York University Medical Center, University of California San Francisco Medical Center, or Washington University Medical Center. Baseline demographic data were collected, including age, gender, and disease status at time of specimen collection. Based on the information presented in Table 1, these samples are representative of the general patient population treated at the 3 clinical sites. Samples from 13 patients were obtained via a commercial source.
Study Schema: Sequencing the IGH and IGK Loci in Bone Marrow and Peripheral Blood Samples

The presence of the myeloma clone in bone marrow and peripheral blood samples was characterized by amplification and sequencing of the IGH and IGK loci. First, BMMC or bone marrow CD138⁺ cells from a sample with a relatively high disease load were utilized to identify myeloma-specific clones based on their frequency in each sample. When BMMC from a second time point was available, DNA was isolated, and the IGH and IGK loci were amplified, sequenced, and analyzed for the presence of the myeloma clone. In a second workflow, DNA and RNA were isolated from PBMC, and IGH and IGK loci were amplified, sequenced, and analyzed for the presence of the myeloma clone. The sequence data from these two workflows provide the quantitative measure for the myeloma clonotypes in a sample. In a third workflow, normal B cells, comprising normal naive B cells (CD45⁺, CD38⁻, CD19⁺, CD27⁻) and normal antigen-experienced B cells (CD45⁺, CD38⁻, CD19⁺, CD27⁻), and cells with a myeloma immunophenotype (CD45low, CD38⁻) were sorted by flow cytometry. DNA was isolated from these sorted cells, and IGH and IGK genes were amplified and sequenced. These sorted samples were used to validate that the myeloma clones were indeed present in cells carrying an immunophenotype consistent with myeloma cells.

The unsorted and sorted sequencing data served 2 complementary purposes in this study. The unsorted sequencing data from samples with high disease load enabled the identification of myeloma clones in each patient based on a frequency threshold. Results from unsorted sequence data were also used to quantify the level of individual myeloma molecules (ie, myeloma clones) in the sample (blood or bone marrow). In contrast, sequence data from sorted populations were used to validate the myeloma disease clones. A myeloma clone was validated if its frequency was enriched in

Table 1 Patient Demographics and Baseline Characteristics

<table>
<thead>
<tr>
<th>Patient/Sample Description</th>
<th>Number</th>
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<tr>
<td>Total patients</td>
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<tr>
<td>Female</td>
<td>23</td>
</tr>
<tr>
<td>Male</td>
<td>37</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>61 (39-90)</td>
</tr>
<tr>
<td>Patients with &gt;1 time point</td>
<td>15</td>
</tr>
<tr>
<td>Sample types</td>
<td></td>
</tr>
<tr>
<td>Bone marrow CD138⁺ cells</td>
<td>31</td>
</tr>
<tr>
<td>Bone marrow mononuclear cells</td>
<td>45</td>
</tr>
<tr>
<td>Peripheral blood mononuclear cells</td>
<td>79</td>
</tr>
<tr>
<td>Plasma/serum</td>
<td>45</td>
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<tr>
<td>Status at time of specimen collection</td>
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</tr>
<tr>
<td>Newly diagnosed</td>
<td>26</td>
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<tr>
<td>Treated but not in complete response</td>
<td>14</td>
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<tr>
<td>Complete response</td>
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<tr>
<td>Relapsed</td>
<td>8</td>
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<tr>
<td>Relapsed and refractory</td>
<td>26</td>
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</table>

Figure 1 Schematic of Study Workflow

Abbreviations: MM = multiple myeloma; PBMC = peripheral blood mononuclear cell.
Detection of Myeloma Cells in Blood by Sequencing

Myeloma Clones Identified in Bone Marrow Samples

The sequencing assay was used to detect immune-cell receptor gene rearrangements, ie, IGH-VDJ, IGH-DJ, and IGK, in BMMC and bone marrow CD138⁺ cells from 60 MM patients. Of the 60 patients, 48 demonstrated a high-frequency gene rearrangement for at least 1 receptor, hereafter referred to as a “calibrating receptor.” IGH-VDJ was the most informative calibrating receptor, with IGH myeloma clones being found in 44 of 60 patients (73%) (Table 2). When the analysis was limited to MM patients with high disease load at the time of calibration, a myeloma clone was detected in 48 of 51 MM patients (94%) (Table 2). High disease load was defined as greater than 5% myeloma cells as measured by immunohistochemistry or flow cytometric analysis.

Detection of Myeloma Clones in Peripheral Blood Mononuclear Cell and Cell-Free Samples

The sequencing assay was then used to detect the myeloma clones in matched peripheral blood samples. This analysis was limited to the 46 patients in which an IGH-VDJ or IGK myeloma clone was detected in the BMMC or bone marrow CD138⁺ cells. Two patients carried only an IGH-DJ myeloma clone and were excluded from this analysis because of insufficient DNA. The myeloma clone was detected in the unsorted PBMC compartment using the IGH-VDJ or IGK DNA assay in 36 of 46 patients (78%) (Table 3). This detection rate increases to 44 of 46 patients (96%) when the PBMC samples were assessed with the IGH RNA assay (Table 3), which suggests that the RNA assay may provide increased sensitivity over the DNA assay. Although only a subset of cell-free DNA samples were collected from peripheral blood for analysis, the majority of these samples (83%) also showed the presence of the myeloma clone (Table 3). When both PBMC and cell-free compartments from peripheral blood were combined, the myeloma clone was detected in 45 of 46 MM patients (98%) (Table 3).

Validation of Myeloma Clones Using Flow Cytometry

To validate the myeloma clones that were identified based on frequency alone, we used flow cytometry to assess the disease clone frequency in 3 populations sorted from peripheral blood in each individual: purified myeloma cells and 2 normal B-cell populations (Supplemental Fig 1). We predicted that myeloma clones would be present at a much lower frequency in normal B cells and at a higher frequency in sorted myeloma cells.

Myeloma clones were indeed present at a much lower frequency in both naive and antigen-experienced normal B cells (Fig. 2A and 2B). A low (nonzero) frequency of myeloma clones is expected in the sorted normal B cells because conventional fluorescence-activated cell sorting does not permit isolation of pure populations. Moreover, sort purity is lowest when target cells are at very low frequency. The few samples that were not substantially de-enriched in Figures 2A and 2B had low frequencies of normal B cells. The myeloma clone was absent in most sorted normal B cells. Specifically, the mean myeloma clone frequency was 12.5% (median 0.35%) in PBMCs compared with 0.05% (median 0%) and 0.41% (median 0%) in normal naive (CD45⁺, CD38⁻, CD19⁺, CD27⁻) and normal antigen-experienced (CD45⁺, CD38⁻, CD19⁺, CD27⁺) B-cells, respectively (Fig. 2A and 2B).

In contrast, the myeloma clone was enriched greater than 100-fold in certain patients in cells sorted for myeloma cell markers (CD45low, CD38⁺) (Fig. 2C). The extent of enrichment was lowest in patient samples with high myeloma clone frequency in unsorted cells. This is because myeloma clone frequency was high in the unsorted PBMC sample and could not be enriched further by sorting. Some myeloma clones were absent in the cells sorted for myeloma cell markers, and this can be explained by low cell numbers in the sorted PBMC sample. In summary, these results validate the assumption that the myeloma clone sequences that are detected in the peripheral blood are present in myeloma cells.

Table 2 Calibration Rates Using Bone Marrow Mononuclear Cells and Bone Marrow CD138⁺ Cells

<table>
<thead>
<tr>
<th></th>
<th>IGH VDJ</th>
<th>IGK</th>
<th>IGH DJ</th>
<th>All Receptors</th>
<th>High Disease Load</th>
</tr>
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<tbody>
<tr>
<td>Total patients analyzed</td>
<td>60</td>
<td>57</td>
<td>43</td>
<td>60</td>
<td>51</td>
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<tr>
<td>Calibrating patients</td>
<td>44</td>
<td>31</td>
<td>11</td>
<td>48</td>
<td>48</td>
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<tr>
<td>Calibration rate</td>
<td>73%</td>
<td>54%</td>
<td>26%</td>
<td>80%</td>
<td>94%</td>
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</table>

Table 3 Detection of Myeloma Clone in Peripheral Blood Samples

<table>
<thead>
<tr>
<th></th>
<th>Peripheral Blood Mononuclear Cells</th>
<th>Plasma/Serum</th>
<th>Peripheral Blood Mononuclear Cells and Plasma/Serum</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>DNA</td>
<td>DNA or RNA</td>
<td>DNA</td>
</tr>
<tr>
<td>Total patients analyzed</td>
<td>46</td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td>Number of patients positive for myeloma clone</td>
<td>36</td>
<td>44</td>
<td>15</td>
</tr>
<tr>
<td>Rate of detection</td>
<td>78%</td>
<td>96%</td>
<td>83%</td>
</tr>
</tbody>
</table>
Myeloma Clone Frequencies in Sorted Normal B Cells and Myeloma Cells. Myeloma Clone Frequencies are very low in Sorted Normal B Cells and enriched in Myeloma Cells Sorted From Blood. (A) The Plot Shows Myeloma Clone Frequencies in Peripheral Blood Mononuclear Cells (PBMCs) and Corresponding Normal Naive B Cells (CD27^-) Sorted From PBMCs From Multiple Myeloma (MM) Patients. (B) The Plot Shows Myeloma Clone Frequencies in PBMCs and Corresponding Normal Antigen-Experienced B Cells (CD27^+) Sorted From PBMCs From MM Patients. (C) The Plot Shows Myeloma Clone Frequencies in PBMCs and Corresponding Myeloma Cells (CD45^low, CD38^+) Sorted From PBMCs.
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Direct Relationship Between Levels of Myeloma Clones in Blood and Bone Marrow

We compared myeloma clone levels in paired, unsorted PBMC and BMMC samples to determine whether a quantitative correlation existed between the 2 compartments. Patients included in this analysis met 2 criteria. First, paired, unsorted BMMC and PBMC samples were available for each patient included in the correlation analysis. Second, myeloma clones were detected in the paired PBMC and BMMC samples using the IGH-VDJ DNA or IGK assay. Thirty-three patients met these criteria and were included in the correlation analysis, with 2 patients having samples from multiple time points. DNA levels were compared using the IGH-VDJ or IGK assay, and a direct correlation was found between myeloma clone levels in the peripheral blood and bone marrow ($R^2 = 0.57$) (Fig. 3). Importantly, the peripheral blood myeloma clone levels were approximately 100-fold lower than levels in paired bone marrow samples.

Comparison Between Sequencing and M Protein Levels

We analyzed the concordance between myeloma clone levels obtained by sequencing and disease levels measured using serum protein electrophoresis (M protein), an established measure of disease load in MM patients. This analysis was limited to IGH-VDJ or IGK calibrating patients with unsorted BMMC (24 patients) or PBMC (35 patients), in which a corresponding M protein value was available. More than 1 time point was included for 13 patients. Importantly, the sequencing assay was performed without knowledge of the results of M protein testing.

In the bone marrow compartment, the 2 methods gave concordant results in 24 of 27 samples (89%) (Fig. 4A). In 3 of 27 samples (11%), the myeloma cell level was positive by sequencing, but undetectable by M protein (Fig. 4A). In peripheral blood samples, results were concordant in 49 of 52 samples (94%): 45 (86%) were positive and 4 (8%) were negative by both methods (Fig. 4B). In 6 of the 49 positive concordant samples, the myeloma clone was detected by sequencing RNA isolated from PBMCs (Fig. 4B). In 2 of the positive, concordant samples, the sequencing assay identified the myeloma clone in the cell-free compartment (Fig. 4B). Results were discordant between the 2 methods in 3 of 52 samples (6%) (Fig. 4B). The myeloma clone level was positive by sequencing but negative by the M protein assay in 2 of the 3 discordant samples (Fig. 4B). In the other discordant sample, the myeloma clone level...
was positive by M protein assay but undetectable by sequencing (Fig. 4B). The DNA analyzed by the sequencing test in this sample corresponded to < 275,000 input cells. Because the sensitivity of the sequencing assay is limited by the number of input cells, the myeloma clone may have been detected by the sequencing assay if more starting material had been provided.

**Discussion**

The evaluation of myeloma cells in MM patients has generally been limited to the assessment of bone marrow involvement because of the sensitivity limitations of traditional MRD-detection methods.21-26 Reports using flow cytometry have shown that plasma cells can be detected in the blood in a fraction of MM patients, and that the presence and level of circulating plasma cells have prognostic implications.13-15,27 Molecular studies using allele-specific oligonucleotide (ASO) polymerase chain reaction (PCR) have also identified low levels of clonal cells in the blood of some MM patients.27 These reports hint at the blood involvement in some MM patients. We hypothesized that a tool with improved sensitivity and specificity could be used to determine whether myeloma cells are present in the peripheral blood of MM patients and evaluate the extent of this phenomenon. In this study, we employed a sequencing-based approach to demonstrate that circulating myeloma clones are present in the blood in the vast majority of MM patients. Furthermore, we validated that these circulating myeloma clones are present in cells that have an immunophenotype that is consistent with a myeloma cell. Our findings demonstrate that circulating myeloma cells are present in the blood in almost all MM patients.

We used a sequencing-based method known as the Lympho-SIGHT platform to identify myeloma clones and assess whether these clones were present in the marrow and peripheral blood compartments in MM patients. This method employs consensus primers and high-throughput sequencing to amplify and sequence all rearranged immunoglobulin gene segments present in a myeloma clone. Similar to other PCR-based platforms for MRD analysis, the sequencing method utilizes a high disease load sample for initial identification of the myeloma clone. In this cohort, the sequencing method detected a myeloma clone in 94% (48/51) of MM patients with high disease load (plasma cells > 5%) at the time of calibration, which demonstrates the high applicability of the technique. The sequencing assay may be capable of identifying a greater proportion of myeloma rearrangements compared with standard PCR-based techniques, because of optimized primer sets for amplification of the *IGH* and *IGK* loci. Importantly, the technology currently relies on the collection of samples at times of high disease load, such as initial diagnosis or relapse, for the initial identification of the myeloma clone. In contrast, other MRD-detection techniques, such as flow-cytometric methods, do not require initial identification of the myeloma clone prior to subsequent analysis in follow-up samples.

Following the identification of a myeloma clone in a diagnostic or high disease load sample, the sequencing platform can be used for MRD assessment, or quantitation of the myeloma clone based on its unique *IGH* or *IGK* gene rearrangement, in follow-up samples with unprecedented sensitivity and specificity. The sequencing method is quantitative at frequencies above $10^{-5}$, and the lower limit of detection is below $10^{-6}$. As shown in previous studies, the assay can detect cancer clones at levels well below 1 in 1 million leukocytes (0.0001%);17 which represents at least 2 orders of magnitude higher sensitivity than standard flow-cytometric methods.20 The enhanced sensitivity of the sequencing method, therefore, enables the detection of circulating myeloma cells in the blood compartment with greater depth than previous studies. Using this method, we demonstrate the presence of myeloma cells in the peripheral blood in the majority of MM patients (44/46, 96%).

This analysis uses both DNA- and RNA-based assays to detect the myeloma clone in the peripheral blood of MM patients. Whereas the DNA assay provides a quantitative measure of MRD in MM samples, the RNA assay allows for the sensitive detection of multiple copies of the MM clone. MM clones were detected in the PBMC and cell-free compartments using the DNA assay in 37 of 46 MM patients (80%). When both the DNA and RNA assays were used, the detection rate in the PBMC and cell-free compartments increased to 45 of 46 MM patients (98%). Thus, the increased sensitivity afforded by the RNA assay complements the highly quantitative nature of the DNA assay.

We demonstrated a clear correlation between the myeloma clone levels in paired bone marrow and peripheral blood samples. Specifically, the peripheral blood myeloma clone levels were approximately 100-fold lower than levels in paired bone marrow samples. The substantial correlation ($R^2 = 0.57$) between clone levels in the marrow and blood compartments suggests that blood samples with sufficient input cells could be used as a measure of disease burden in bone marrow. Flow-cytometry studies have shown that the direct measurement of bone marrow disease is highly predictive of outcome in MM.35 Thus, a blood-based sequencing assay, which reflects bone marrow disease levels, has potential utility as a prognostic biomarker for MM disease load and clinical outcome.

Biomarkers for MM disease burden, such as M protein and free light chain assays, are currently used to monitor patients during therapy and posttherapy. We compared the myeloma clone levels in marrow and blood with M protein levels, an established measure of MM disease load. The sequencing assay demonstrated a clear sensitivity advantage in the marrow compartment and at least equivalent sensitivity in the peripheral blood compared with M protein results. Samples with more input cells will likely enhance the sensitivity of the blood-based MRD assay. In conclusion, these studies highlight the promise of a blood-based, sequencing MRD assay that can be used to measure MM disease burden at different time points and various disease stages.

**Conclusion**

The assessment of residual tumor cells persisting after therapy, or MRD, is a central component of accurate disease prognosis and monitoring in many hematologic malignancies. The prognostic value of MRD has been clearly established in the chronic and acute leukemias and lymphomas.36-37 As a result, molecular MRD analysis is currently used for risk stratification, as well as assessment of therapy-induced reduction in tumor burden and regrowth after chemotherapy, in these indications.38 Recent studies have suggested that MRD assessment may also play a role in the MM treatment paradigm. Numerous reports have shown that molecular MRD status is predictive of progression-free
Detection of Myeloma Cells in Blood by Sequencing and overall survival in MM patients.23,24,26 These studies have primarily assessed the bone marrow for the presence of MRD, due in part to the sensitivity limitations of traditional MRD analysis techniques.

In this study, we employed a novel sequencing-based approach to demonstrate that circulating myeloma clones are present in the blood in the vast majority of MM patients. Moreover, we demonstrated a clear correlation between the quantitative myeloma clone levels in paired bone marrow and peripheral blood samples. These results suggest that a sequencing-based MRD assay can be used to directly measure MM disease burden from peripheral blood samples taken during and following treatment. A sensitive, blood-based MRD test for MM patients would offer a clear practical advantage over current assays that require marrow collection. The sensitivity limits and prognostic value of this sequencing-based approach will be the subject of future studies.

Clinical Practice Points

- Previous studies have shown the prognostic relevance of circulating plasma cells at the time of diagnosis in the peripheral blood of patients with MM.15-16 These studies were performed using traditional flow-cytometric methods, which are characterized by limited assay sensitivity. Techniques that are more sensitive may provide additional information on the fraction of MM patients with plasma cells present in their peripheral blood at diagnosis and posttherapy and validate the prognostic importance of the presence of circulating plasma cells at various time points.

- We used a sequencing-based method to identify myeloma cells in bone marrow and peripheral blood samples, based on their unique immunoglobulin gene rearrangements.17-19 The sequencing assay can detect residual disease at levels well below 1 in 1 million leukocytes (0.0001%), which represents at least 2 orders of magnitude higher sensitivity than standard flow-cytometric methods.20

- Using this novel sequencing platform, we demonstrated that circulating myeloma clones are present in the blood in the vast majority of MM patients. Moreover, we observed a clear correlation between the quantitative myeloma clone levels in paired bone marrow and peripheral blood samples. These studies highlight the promise of a blood-based, sequencing MRD assay that can be used to measure MM disease burden at different time points and various disease stages.

Acknowledgments

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Author Contributions

Mark Klinger performed the sequencing assays and analyzed and interpreted data; Jeesun Park, Jacob Paasch, Mark Fiala, and Denise O’Dea provided M protein and other clinical data; Ravi Vij, Amitabha Mazumder, Thomas Martin, and Jeffrey Wolf led the clinical studies under which the samples were collected; Jeffrey Wolf, Ravi Vi, Amitabha Mazumder, and Malek Faham designed the research, analyzed and interpreted data, and wrote the manuscript with the input of all other authors. All authors approved the final version of the manuscript.

Disclosure

Malek Faham and Mark Klinger are employees of and stockholders in Sequenta, Inc. Ravi Vij, Amitabha Mazumder, Denise O’Dea, Jacob Paasch, Thomas Martin, Li Weng, Jeesun Park, Mark Fiala, and Jeffrey Wolf have stated that they have no conflicts of interest.

Supplemental Data

Supplemental figures accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clnl.2013.09.013.

References


Figure S1  Cell Surface Expression on Peripheral Blood Mononuclear Cells in a Normal Healthy Donor and a Patient With Myeloma.  

(A) Cell Surface Expression of CD45 and CD38 on Peripheral Blood Mononuclear Cells (PBMCs) From a Normal Healthy Donor (Left) and a Myeloma Patient (Right). Myeloma Cells (Defined as CD45low, CD38hi) are Indicated With a Gate at the Lower Right of Each Plot; CD45hi, CD38lo Cells are Indicated With a Gate in Upper Left of Each Plot.  

(B) Cell Surface Expression of CD19 and CD27 for CD45lo, CD38hi Cells From PBMCs From a Normal Healthy Donor (Left) and a Myeloma Patient (Right). Normal Naive (CD19hi, CD27lo) and Antigen-Experienced (CD19lo, CD27hi) B Cells are Indicated in Left and Right Gate of Each Plot, Respectively. Units in each plot are in fluorescence.