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 ($R^2 = 0.57$) 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.[15] 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.[13] 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.[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] 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 HIB19), 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-diaidino-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 naive B cells (defined as $CD45^+$, $CD38^-$, $CD19^+$, $CD27^-$), normal antigen-experienced B cells, and myeloma cells (defined as $CD45^{low}$, $CD38^+$) were sorted. Sorted cells were pelleted 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.[17-19] Genomic DNA and RNA was amplified using locus-specific primer sets for *IGH-VDJ*, *IGH-DJ*, and *IGK* 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.[17]

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.