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# A Next-Generation Sequencing Strategy for Evaluating the Most Common Genetic Abnormalities in Multiple Myeloma

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Identification and characterization of genetic alterations are essential for diagnosis of multiple myeloma and may guide therapeutic decisions. Currently, genomic analysis of myeloma to cover the diverse range of alterations with prognostic impact requires fluorescence *in situ* hybridization (FISH), single nucleotide polymorphism arrays, and sequencing techniques, which are costly and labor intensive and require large numbers of plasma cells. To overcome these limitations, we designed a targeted-capture next-generation sequencing approach for one-step identification of *IGH* translocations, V(D)J clonal rearrangements, the IgH isotype, and somatic mutations to rapidly identify risk groups and specific targetable molecular lesions. Forty-eight newly diagnosed myeloma patients were tested with the panel, which included *IGH* and six genes that are recurrently mutated in myeloma: *NRAS*, *KRAS*, *HRAS*, *TP53*, *MYC*, and *BRAF*. We identified 14 of 17 *IGH* translocations previously detected by FISH and three confirmed translocations not detected by FISH, with the additional advantage of breakpoint identification, which can be used as a target for evaluating minimal residual disease. IgH subclass and V(D)J rearrangements were identified in 77% and 65% of patients, respectively. Mutation analysis revealed the presence of missense protein-coding alterations in at least one of the evaluating genes in 16 of 48 patients (33%). This method may represent a time- and cost-effective diagnostic method for the molecular characterization of multiple myeloma. *(J Mol Diagn 2016, ■: 1–8; http://dx.doi.org/10.1016/j.jmoldx.2016.08.004)*

Q10 Multiple myeloma (MM) is a highly heterogeneous disease at the genetic and clinical outcome levels. Several research groups have determined the value of chromosomal translocations involving the *IGH* locus and copy number variations in the definition of risk subgroups.[1–4] Recently, next-generation sequencing (NGS) studies using whole-genome and whole-exome approaches have yielded new insights into the pathogenesis and evolution of the disease.[5–8] This has led to the identification of key activated or inactivated survival pathways that are potentially

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druggable by novel targeted approaches.[9,10] Therefore, to help in the diagnosis, staging, risk stratification, and management of MM, it is important to fully characterize the genetic and molecular profile of the main clone, including identifying recurrent translocations, copy number abnormalities, and nonsynonymous point mutations of genes that could be candidates for targeted therapy (eg, in the *RAS/MAPK* pathway). So far, fluorescence *in situ* hybridization (FISH) of purified plasma cells along with conventional cytogenetics when sufficient sample is available are routinely used to detect the chromosomal abnormalities of prognostic value in MM. At best, conventional cytogenetics provides genetic information in 53% of MM patients,[11] and FISH studies can find *IGH* translocations in approximately 40% to 50% of MM cases.[12] In addition, FISH studies are restricted to abnormalities covered by the specific probe used in the analysis.[13] Comparative genomic hybridization and single nucleotide polymorphism arrays have proved to be powerful methods for detecting copy number alterations. However, these techniques cannot identify gene mutations, and their simultaneous use is not a cost-effective alternative. Clonal rearrangements of V(D)J, DJ, and switching genes, whose assessment is required for monitoring future minimal residual disease (MRD), are usually characterized by PCR and Sanger sequencing or NGS. Finally, representative mutations of relevant genes (eg, *TP53*) have to be detected by targeted gene sequencing or whole-genome/exome sequencing. However, this fragmented strategy has important disadvantages in terms of costs, applicability, laboriousness, and data handling.[14]

The rapid development of NGS technologies has resulted in different strategies being available for DNA sequencing. NGS target-enrichment strategies would allow the simultaneous detection of *IGH* translocations, IGH clonal rearrangements, copy number variations, and somatic mutations to rapidly classify myeloma patients into clinically relevant molecular subgroups. This would also include the identification of V(D)J usage, translocation breakpoints, and specific sequences generated during isotype switching. All such events produce monoclonal sequences that are patient and tumor specific,[15] which convert them into highly interesting targets for MRD monitoring in MM. In addition to detecting the main genetic abnormalities, this strategy enables the definition of tumor subclones, revealing intraclonal tumor heterogeneity that may have important therapeutic implications,[6,10,16] and might prove to be of clinical significance. Moreover, targeted-sequence capture and NGS may overcome the limitations associated with whole-genome and whole-exome sequencing, such as the relatively high cost and time-consuming nature of the analysis.

This background prompted us to design an NGS-based strategy to detect *IGH* translocations, V(D)J rearrangements, and the most recurrent gene mutations in a series of MM patients with the ultimate purpose of standardizing this approach for diagnosing and monitoring MM patients.

## Materials and Methods

### Patients and Clinical Characteristics

Samples from 48 newly diagnosed MM patients enrolled in Spanish PETHEMA/GEM (Programa para el Estudio y la Terapéutica de las Hemopatías Malignas/Grupo Español de Mieloma) trials (GEM05MENOS65 and GEM05MAS65) were studied. Plasma cells were isolated from bone marrow samples using $CD138^+$ autoMACS sorting (Miltenyi Biotec, Auburn, CA), and DNA was extracted using a commercial kit (Qiagen Inc., Valencia, CA). The clinical and biological characteristics of the patients are listed in Table 1, showing a conventional series of MM patients. [T1]

### Targeted NGS Strategy

We designed a custom pull-down panel that covered the *IGH* gene (including constant genes, switching and enhancer regions, JHs, and VHs) and six additional selected genes: *NRAS*, *KRAS*, *HRAS*, *TP53*, *MYC*, and *BRAF*. Details of the coordinates are described in Supplemental Table S1 (human reference genome GRCh37/hg19, downloaded from UCSC, *http://hgdownload.cse.ucsc.edu/goldenPath/hg19/chromosomes*, last accessed November 2013). Probes were purchased from the Roche NimbleGen SeqCap EZ Choice library application (Roche NimbleGen Inc.,

**Table 1** Clinical and Biological Characteristics of the 48 Study Patients

| Characteristic | Value |
|---|---|
| Age, median years (range) | 63 (46–78) |
| Sex, *n* M/F | 26/22 |
| Hemoglobin, means ± SD g/dL | 11.3 ± 1.9 |
| Bone marrow tumor plasma cells, means ± SD % | 13.7 ± 12.1 |
| Creatinine, means ± SD mg/dL | 1.0 ± 0.3 |
| Albumin, means ± SD g/dL | 3.7 ± 0.7 |
| Calcium, means ± SD mg/dL | 9.6 ± 1.2 |
| $\beta_2$-Microglobulin, means ± SD mg/L | 4.1 ± 2.9 |
| Elevated lactate dehydrogenase, % | 10 |
| Type of myeloma, % | |
| IgG | 74 |
| IgA | 17 |
| Bence Jones | 9 |
| ISS, % | |
| I | 41 |
| II | 38 |
| III | 21 |
| Plasma cells in S-phase ≥2%, % | 6 |
| DNA index, % | |
| >1 (hyperdiploid) | 71 |
| ≤1 (nonhyperdiploid) | 29 |





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