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Review

Next Generation Sequencing in the Diagnosis of Multiple Myeloma

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Abstract

Multiple myeloma (MM) is a biologically and clinically heterogeneous plasma cell malignancy characterized by clonal expansion in the bone marrow and a highly variable clinical course. Despite major therapeutic advances, MM remains incurable, largely due to the associated genomic complexity, clonal evolution, and the emergence of treatment resistance. In this context, next-generation sequencing (NGS) has emerged as a transformative tool for improving disease characterization, risk stratification, and clinical management. This review summarizes current evidence on the application of NGS technologies, including whole-exome sequencing, whole-genome sequencing, and targeted gene panels—in the diagnosis, prognostic assessment, and monitoring of MM. Genomic profiling has consistently revealed recurrent alterations in key driver genes, copy number abnormalities, and structural variants that underlie disease heterogeneity and influence clinical outcomes. In addition to baseline characterization, NGS enables high-resolution analysis of clonal architecture and evolution, offering critical insights into mechanisms of relapse and therapeutic resistance. Importantly, NGS-based assessment of minimal residual disease (MRD) has demonstrated superior sensitivity compared with conventional techniques, providing a powerful prognostic marker and a promising tool for treatment monitoring and response-adapted strategies. Indeed, by integrating genomic and MRD data, NGS supports a more precise and dynamic approach to patient management. Overall, the growing body of evidence highlights NGS as a central component of precision medicine in multiple myeloma. Continued efforts toward standardization, validation of emerging biomarkers, and clinical implementation of sequencing-guided strategies are expected to enhance personalized treatment further and improve patient outcomes.

Keywords: multiple myeloma; high-throughput nucleotide sequencing; whole genome sequencing; exome sequencing; neoplasm; residual

1. Introduction and Etiology of Multiple Myeloma

Multiple myeloma (MM) is currently an incurable hematologic malignancy, characterized by the clonal expansion of abnormal plasma cells that produce a monoclonal immunoglobulin (Ig), commonly known as monoclonal protein or M-protein [1]. This disease is typically preceded by two premalignant stages: monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM), both of which are asymptomatic but detectable through clinical investigation. The biological mechanisms driving the transition from these precursors to overt MM in certain patients remain incompletely understood [2]. MM accounts for approximately 1% of all cancer diagnoses [3] and represents around 13–15% of hematologic malignancies, positioning it as the second most prevalent hematologic cancer after non-Hodgkin lymphoma [4]. It is notably rare in younger populations, with fewer than 2% of cases diagnosed before the age of 40 years [1].

The pathogenesis of MM is multifactorial and still not fully elucidated. A variety of risk factors have been associated with disease development, including lifestyle factors, bone marrow microenvironment, chronic immune stimulation or autoimmune conditions, a family history of MM, inherited genetic susceptibility, and the antecedent presence of MGUS [2].

2. Epidemiology of Multiple Myeloma

2.1 Incidence

The incidence of MM exhibits considerable geographical variation [4]. According to standardized incidence rates (ASIR), the highest burdens are observed in Australasia (5.8; 95% uncertainty interval [UI], 4.4–6.5), North America (5.2; 95% UI, 4.7–6.5), and Western Europe (4.6; 95% UI, 3.7–5.5) [5].

In 2016, the global burden of newly diagnosed MM cases was estimated at 138,509 (95% UI, 121,000–155,480), with an ASIR of 2.1 per 100,000 individuals (95% UI, 1.8–2.3) [3].

Two years later, data from the Global Cancer Observatory reported approximately 160,000 new cases of MM in 2018, representing 0.9% of all cancer cases. Of these, about 90,000 cases were reported in males and approximately 70,000 in females, corresponding to age-standardized incidence rates of 2.1 per 100,000 for men and 1.4 per 100,000 for women. The cumulative lifetime risk of developing MM up to the age of 74 years was 0.24% for men and 0.17% for women [6].

According to the most recent figures published by the Global Cancer Observatory (GLOBOCAN) in 2020, approximately 176,404 new MM cases were identified, representing 0.9% of all cancers. age-standardized incidence rates is 2.2 per 100,000 in males and 1.5 per 100,000 in females. The cumulative risk of developing MM by age 74 was 0.25% for men and 0.17% for women, highlighting that men are 1.5 times more likely to develop MM compared to women [7].

Overall, it is noteworthy that more than 120,000 newly diagnosed MM (NDMM) cases are reported globally each year [4].

2.2 Mortality

In 2016, MM was responsible for 98,437 deaths worldwide, corresponding to an age-standardized death rate (ASDR) of 1.5 per 100,000 individuals (95% UI, 1.3–1.7) [5].

By 2018, the global mortality from MM was estimated at roughly 106,000 cases, representing 1.1% of all cancer-related deaths. Of these, around 59,000 occurred in males and about 47,000 in females, yielding age-standardized mortality rates of 1.3 per 100,000 for men and 0.9 per 100,000 for women. The cumulative risk of mortality from multiple myeloma by the age of 74 years was estimated at 0.15% for men and 0.10% for women [6].

According to data from 2020, approximately 117,000 deaths were attributed to MM, representing 1.2% of all cancer deaths that year. Among them, 65,197 were males and 51,880 were females, resulting in age-standardized mortality rates of 1.4 per 100,000 and 0.9 per 100,000, respectively. The lifetime risk of death from MM up to 74 years remained at 0.15% for men and 0.10% for women [8].

2.3 Risk Factors

2.3.1 Age

MM predominantly affects older individuals, with a median age at diagnosis of approximately 69 years. Among patients, 34.8% are over 75 years of age, while 9.6% are aged above 85 years [9].

The concept of clonal hematopoiesis (CH) was first introduced by Busque et al. [10] around 25 years ago. Their research demonstrated an age-associated increase in acquired skewing of X-chromosome inactivation, particularly after 60 years of age. They also identified that somatic mutations in the Tet Methylcytosine Dioxygenase 2 (*TET2*) contribute to this non-random pattern of X-chromosome inactivation. Subsequently, a variety of somatic mutations associated with aging have been detected in the blood cells of healthy individuals without evidence of hematologic malignancy. This phenomenon was initially described as "age-related clonal hematopoiesis" (ARCH) [5].

2.3.2 Sex

Globally, men are about 1.5 times more likely to develop MM compared to women [3].

2.3.3 Race

In the United States, black individuals are at approximately twice the risk of developing multiple myeloma compared to white individuals. In contrast, the variations in incidence among other racial groups are less pronounced. Specifically, Asians exhibit a significantly lower incidence rate (3.8 per 100,000) compared to non-Hispanic Whites (6.2 per 100,000), while Hispanics show a slightly higher incidence, at 6.7 cases per 100,000 population. Moreover, Marinac et al. [6] have demonstrated that the prevalence of MGUS is significantly higher among Black individuals than among Whites, particularly at younger ages.

3. Diagnosis of Multiple Myeloma

3.1 Clinical Features

MM is a hematological malignancy characterized by the uncontrolled proliferation of plasma cells, which are differentiated B lymphocytes residing in the bone marrow. Under normal conditions, B cells are responsible for producing antibodies essential for immune defense. In MM, however, neoplastic plasma cells accumulate in the bone marrow, disrupting normal hematopoiesis and leading to an overproduction of dysfunctional intact immunoglobulins or free immunoglobulin light chains.

Clinically, MM often presents with bone pain, fatigue, weakness, and anemia. Patients may also experience a heightened susceptibility to infections, renal impairment, and peripheral neuropathies.

Infection represents the leading cause of morbidity and mortality in individuals with MM, with bacterial pneumonia being the most frequent type. The risk of infection is

influenced by multiple factors, including the patient's general health status, environmental exposures, disease-related immunosuppression, and the intensity of therapeutic interventions.

The diagnosis of MM is based on a combination of laboratory investigations, urine analysis, bone marrow biopsy—interpreted according to the updated International Myeloma Working Group (IMWG) criteria—and imaging studies [9].

3.2 Laboratory Evaluation in MM

The initial approach to laboratory evaluation in suspected multiple myeloma involves a thorough blood analysis, including a complete blood count and the measurement of several key biochemical markers. These markers include serum creatinine, calcium, albumin, lactate dehydrogenase (LDH), serum free light chains, β2-microglobulin, and 24-hour urine protein electrophoresis assessed by immunofixation. Approximately 86% of patients diagnosed with multiple myeloma display detectable monoclonal proteins—abnormal immunoglobulins—in their blood.

To better evaluate organ damage resulting from elevated levels of kappa and lambda free light chains, serum-free light chain assays are performed. Establishing baseline concentrations of monoclonal proteins and free light chains is critical for determining disease burden and monitoring therapeutic response. Tracking these biomarkers over time allows for assessment of treatment efficacy. A small subset of patients, estimated at 1% to 2%, presents with "non-secretory" multiple myeloma, lacking measurable serum or urine monoclonal components.

Flow cytometry plays a pivotal role in detecting abnormal circulating B cells, particularly when a diagnosis of B-cell leukemia is considered.

In all cases where multiple myeloma is suspected, a bone marrow aspiration and biopsy are strongly recommended. Bone marrow analysis includes morphological evaluation of plasma cells, immunohistochemical quantification of CD138+ plasma cells, and further characterization by flow cytometry, fluorescence *in situ* hybridization (FISH), and conventional cytogenetics.

Urinalysis should include total protein quantification by immunofixation, alongside a 24-hour urine collection for protein electrophoresis.

FISH analyses must be capable of identifying specific cytogenetic abnormalities such as deletions in chromosomes 13 and 17p, translocations t(4;14), t(11;14), t(14;16), and amplifications involving 1q21. Detecting such losses and rearrangements is crucial for stratifying patients based on their risk profiles [3].

3.3 Imaging Investigation

3.3.1 Radiographic Imaging

Categorizes skeletal abnormalities in multiple myeloma into four main types: solitary bone lesions (plasmacytoma), widespread skeletal lesions (myeloma), generalized osteopenia, and sclerosing forms of myeloma.

3.3.2 Computed Tomography (CT)

Offers greater sensitivity than conventional radiography, enabling the detection of lesions with less than 5% trabecular bone loss. Moreover, CT imaging provides valuable information regarding the extent of adjacent soft tissue involvement.

3.3.3 Whole-body Magnetic Resonance Imaging (MRI)

Stands out as a highly sensitive and specific modality for the evaluation of bone marrow infiltration and skeletal disease in multiple myeloma [3].

4. Next-Generation Sequencing (NGS)

4.1 NGS Platforms and Sample Types in MM

NGS encompasses high-throughput technologies that allow simultaneous sequencing of millions to billions of DNA fragments, generating an extensive volume of genetic data in a single run [11]. Among the widely used NGS platforms are Ion Torrent and Illumina, each involving steps such as library preparation, sequencing, data analysis, and validation.

Selecting an appropriate NGS platform is a critical initial decision for any laboratory, influenced by various factors including installation costs, sequencing chemistry, read lengths, throughput capacity, instrument size, and turnaround time [12].

4.1.1 Illumina Sequencing Platform

The Illumina platform, commonly referred to as Illumina sequencing technology, represents one of the most widely adopted NGS methods, profoundly transforming genomic research. It is founded on the sequencing-by-synthesis principle, whereby DNA fragments are amplified and sequenced in parallel.

Illumina's massively parallel sequencing approach enables millions of DNA fragments to be sequenced simultaneously within a single run. The workflow includes several critical stages:

- **Library Preparation**: DNA samples are fragmented and ligated with specialized adapters that facilitate attachment to the flow cell surface.
- **Cluster Generation**: Adapter-ligated DNA fragments are immobilized onto the flow cell and undergo bridge amplification, forming clonal clusters, each composed of multiple identical copies of a single DNA molecule.
- **Sequencing**: Fluorescently labeled reversible terminators are incorporated one nucleotide at a time. After each incorporation, imaging captures the fluorescent signal, followed by chemical cleavage of the blocking group and fluorophore to allow subsequent nucleotide addition.

- **Image Processing and Base Calling**: The fluorescence images are analyzed to generate raw signal intensities, which are then translated into nucleotide sequences for each cluster.
- **Data Analysis**: Sequencing reads are aligned to a reference genome or assembled de novo. Bioinformatics pipelines are employed for tasks such as read mapping, variant detection, and structural variant analysis.

Illumina technology is renowned for its high throughput, accuracy, and data quality, making it highly suitable for a broad range of genomic applications, including whole-genome sequencing, exome sequencing, transcriptome profiling, epigenomic studies, and metagenomics. Its flexibility, scalability, and cost-efficiency have cemented its status as a leading choice for diverse genomic investigations [13,14,15].

4.1.2 Ion Torrent Sequencing Platform

The Ion Torrent sequencing platform, developed by Thermo Fisher Scientific, represents a NGS technology that leverages semiconductor-based sequencing to decode DNA or RNA sequences. This platform operates by detecting the hydrogen ions (protons) released during DNA synthesis, which enables nucleotide identification.

The sequencing process using the Ion Torrent platform follows these key steps:

- **Library Preparation**: DNA or RNA samples are first fragmented and ligated with adapter sequences, which are essential for attaching the fragments to beads or microparticles.
- **Template Preparation**: The DNA or RNA fragments undergo amplification, typically via emulsion polymerase chain reaction (PCR) or clonal amplification. Each bead or microparticle contains many copies of a single DNA or RNA fragment, forming clonal populations.
- **Sequencing**: The prepared templates are loaded onto a semiconductor chip, where each bead or microparticle is positioned in its own well or ion-sensitive field-effect transistor (ISFET). During sequencing, the addition of nucleotides triggers DNA synthesis. When a nucleotide matches the template, a hydrogen ion is released as a byproduct. This ion causes a pH change, which is detected by the ISFET.
- **Data Acquisition and Analysis**: The pH changes are translated into electrical signals that are then processed and analyzed to produce the DNA sequence. Bioinformatics tools are employed to align the sequencing data to a reference genome, identify genetic variants, and perform additional analyses [15,16,17,18].

The Ion Torrent platform offers several advantages, including high speed, simplicity, and scalability. It has found applications in diverse fields such as targeted sequencing, whole-exome sequencing, microbial genomics, cancer research, and genetic disorder investigations. Its relatively low instrument cost and flexibility have made it an attractive option for a wide range of research laboratories and applications [19].

NGS in MM relies on a variety of technological platforms, each offering specific advantages in terms of sequencing depth, turnaround time, and cost-effectiveness. The choice of platform is primarily guided by clinical and logistical considerations rather than by fundamental differences in analytical performance. As sequencing technologies continue to evolve, harmonization and standardization of workflows remain important to ensure reproducibility and clinical reliability across laboratories [20,21].

4.1.3 Sample Types in MM

Bone marrow remains the reference sample for molecular profiling in MM, given the high concentration of malignant plasma cells within this tissue. However, the increasing sensitivity of NGS has enabled the use of alternative sample types, including peripheral blood, plasma, and circulating tumor DNA. These less invasive approaches hold promise for longitudinal disease monitoring, particularly for assessing treatment response and detecting residual disease, although bone marrow analysis continues to be essential for comprehensive baseline characterization (see Table 1).

4.2 Clinical Applications of NGS in Multiple Myeloma

The integration of NGS into clinical practice has significantly advanced the molecular characterization and personalized management of multiple myeloma. It enables the detection of recurrent mutations in Tumor Protein P53 (*TP53*), Kirsten Rat Sarcoma Viral Oncogene Homolog (*KRAS*), Neuroblastoma RAS Viral Oncogene Homolog (*NRAS*), *DIS3* (DIS3 Homolog, Exosome Endoribonuclease and 3′-5′ Exoribonuclease), and Family With Sequence Similarity 46 Member C (*FAM46C*), as well as potentially novel drivers such as AT-Rich Interaction Domain 1A (*ARID1A*), AT-Rich Interaction Domain 2 (*ARID2*), and Ryanodine Receptor 2 (*RYR2*).

Moreover, longitudinal NGS profiling enables clinicians to track clonal evolution over time, offering insights into disease progression, treatment resistance, and relapse mechanisms. Monitoring clonal architecture not only helps assess therapeutic impact but may also identify emerging subclones that are resistant to current therapies, prompting earlier intervention or alternative treatment approaches [19,20,21,22,23].

4.3 Whole-Exome Sequencing (WES) in MM

WES has been widely applied to explore MM, providing insights into somatic mutations, clonal evolution, prognostic biomarkers, and potential therapeutic targets. In our review, 11.83%% of the selected studies specifically analyzed WES data (Table 2, Ref. [23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42]). Based on these findings, the narrative should em-

Table 1. The different types of samples used in studied articles.

Type of samples	Percentage of use
Bone Marrow (BM)	44.55%
NA	35.26%
Peripheral Blood (PB)	6.02%
BM and PB	5.46%
BM, PB and Plasma Samples	2.18%
Plasma Samples	2.18%
BM and Plasma Samples	1.09%
Serum Samples	1.09%
Plasmacytoid Dendritic Cells (PDCs)	1.09%
BM and Biopsies	0.54%
PB, BM and Formalin Fixed Paraffin Embedded Samples (FFPE)	0.54%

phasize the conceptual and clinical significance of WES in MM rather than methodological details. The key insights can be integrated into the discussion of NGS applications to strengthen the review's perspective on the translational and biological implications of WES in MM.

Several studies have explored the genetic landscape of MM using WES, consistently highlighting recurrent alterations in key genes such as *TP53*, *KRAS*, *NRAS*, *DIS3*, and *FAM46C*. For instance, recurrent alterations in *TP53*, *KRAS*, *NRAS*, *DIS3*, *FAM46C* and *IRF4* were documented by Munshi et al. [43], with frequencies ranging from 13% to 20%, while Bolli et al. [44] highlighted *KRAS* and *NRAS* (36%), *TP53* (28%), *DIS3* (20%), *FAM46C* (16%), and *TRAF3* (12%) as key contributors to MM pathogenesis.

Manier et al. [45] analyzed cfDNA from 45 MM patients and observed recurrent variants in *KRAS*, *NRAS*, *TP53*, *DIS3*, and *FAM46C*, demonstrating the potential of WES to capture disease heterogeneity. In a larger cohort, Miller et al. [38] reported that *KRAS* and *NRAS* variants were associated with longer progression-free survival, whereas *TP53* variants correlated with poorer outcomes, reinforcing their prognostic significance. Additional studies by Walker et al. [39], and Munshi et al. [41] corroborated these findings, consistently identifying *TP53*, *KRAS* and *NRAS* as key drivers of aggressive molecular subtypes, with frequent involvement of *DIS3*, *FAM46C* and *CYLD* [39,41].

High-throughput approaches have further illustrated the utility of WES in clinical genomics. Zielinska et al. [46] successfully identified known variants in *TP53*, *KRAS*, *NRAS*, and *BRAF* (B-Raf Proto-Oncogene, Serine/Threonine Kinase) using the NextSeq 500 platform. Bolli et al. [47] also demonstrated that WES, when combined with matched germline DNA, could reveal both somatic variants and chromosomal alterations, providing a more comprehensive genomic profile than conventional methods.

Collectively, these studies highlight the critical involvement of *KRAS*, *NRAS*, *BRAF*, *TP53*, *DIS3* and *FAM46C* variants in MM, emphasizing their potential as therapeutic targets and prognostic biomarkers. In SMM, variants in *TP53*, *BRAF*, *DIS3* and Ataxia Telangiectasia Mutated (*ATM*) have been linked to high-risk features, supporting early identification of patients who may benefit from timely intervention [33].

4.4 Assessment of MRD in MM by NGS

The World Health Organization (WHO) defines MRD as "the presence of residual disease at levels below the threshold of detection by conventional morphological and cytogenetic techniques" [48]. In MM, MRD assessment has become a critical component of patient management, providing valuable prognostic information, guiding treatment decisions, and helping predict the risk of disease relapse [2,13].

MRD testing typically involves examining bone marrow samples for clonal plasma cells using highly sensitive techniques such as flow cytometry, PCR, or NGS. The main goal is to detect residual disease at very low levels, ideally indicating the absence of detectable cancer cells. Achieving MRD negativity is consistently associated with improved clinical outcomes, including prolonged progression-free survival (PFS) and overall survival (OS), compared to MRD-positive patients. Although MRD testing is not yet universally available, rapid advances in technology and growing understanding of MM biology suggest it will become a standard practice in clinical care.

Types of MRD Tests:

Several approaches are currently used to detect and quantify MRD in MM:

• **NGS-based MRD**: This method employs high-throughput DNA sequencing to identify and quantify residual cancer cells in blood or bone marrow. Its high sensitivity allows detection of very low levels of disease, making it a powerful tool for monitoring treatment response and predicting outcomes [14,15].

• **Flow cytometry-based MRD**: Flow cytometry analyzes blood or bone marrow samples to detect residual myeloma cells. It is efficient, relatively simple, and widely used for ongoing disease monitoring [16,17,18].

Table 2. Multiple myeloma genetic investigations using WES.

	Study	Year of the study	Country	Number of patients used in the study	Type of patients	Type of samples used
1	[23]	2021	India	62	-	-
2	[24]	2021	India	71	NDMM	PCs
3	[25]	2021	Finland	8	-	BM
4	[26]	2021	USA (New York)	1154	-	-
5	[27]	2020	Ireland	291	RRMM	BM
6	[28]	2020	USA (Florida)	196	-	-
7	[29]	2019	Italy	42	RRMM	-
8	[30]	2019	Italy	40	RRMM	-
9	[31]	2018	USA (Arkansas)	1141	-	-
10	[32]	2018	USA (Boston)	629, 1144, 205	MM, NDMM, NDMM	PB
11	[33]	2017	USA (Boston)	186	-	-
12	[34]	2017	Australia (Adelaide)	10	-	-
13	[35]	2017	USA (New York)	19	-	-
14	[36]	2017	Belgium (Liège)	10	-	BM
15	[37]	2016	UK (Southampton)	25	-	-
16	[38]	2016	-	1000	NDMM	-
17	[39]	2016	USA (Little Rock)	2161	-	-
18	[40]	2016	USA (Little Rock)	33	-	-
19	[41]	2015	USA (Boston)	29	-	-
20	[42]	2012	UK (London)	-	-	BM

WES, whole-exome sequencing; MM, multiple myeloma; NDMM, newly diagnosed MM; PCs, plasma cells; RRMM, Relapsed/Refractory Multiple Myeloma; SMM, smoldering multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance.

• **Droplet digital PCR (ddPCR)**: Unlike traditional quantitative PCR (qPCR), ddPCR allows direct and highly precise quantification of target DNA sequences without requiring a standard curve. Studies indicate that ddPCR offers sensitivity, accuracy, and reproducibility comparable to qPCR, representing a promising alternative for MRD evaluation [19].

• **PCR-based MRD**: PCR amplifies DNA sequences specific to myeloma cells, enabling detection of very small amounts of residual disease. PCR-based methods are highly sensitive and specific, supporting treatment monitoring and prognosis prediction [20,21].

The choice of MRD testing method depends on factors such as disease stage, technology availability, and individual patient considerations.

Among the various methods available, several studies in our review utilized the LymphoTrack® immunoglobulin heavy chain (IGH) panel to assess MRD by detecting clonal IGH gene rearrangements in patients' blood or bone marrow. This approach allows for precise monitoring of residual cancer cells: DNA is extracted from patient samples and sequenced using NGS, and the obtained sequences are compared with baseline samples to identify any remaining malignant clones. Quantifying MRD in this way provides insights into treatment response and disease progression over time.

Comparative studies have also evaluated the performance of NGS versus next-generation flow cytometry (NGF) for MRD detection in MM. For instance, Medina et al. [49] reported that NGS and NGF achieved similar sensitivity, detecting MRD in 92% and 89% of cases, respectively. NGS was able to identify MRD at lower levels, with a detection limit of 0.01% compared to 0.1% for NGF. These findings highlight the effectiveness of both approaches, while suggesting that NGS may offer advantages in detecting minimal residual disease at very low levels. Nonetheless, further research is needed to optimize MRD assessment and fully integrate it into treatment decision-making for MM patients.

Ha et al. (2022) [50] have highlighted the high sensitivity and clinical relevance of NGS-based approaches for detecting MRD in MM. this study demonstrated that Ig gene clonality analysis using NGS was both highly sensitive and specific, with MRD negativity associated with improved PFS and OS. This method also detected MRD in a larger proportion of patients compared to standard flow cytometry, proving particularly useful for those with low MRD levels.

Similarly, Ho et al. (2018) [51] evaluated an NGS-based assay for MRD detection in plasma cell neoplasms, reporting a high success rate (95.7%) for clonal characterization in MM patients. The assay detected MRD with 89.5% sensitivity and showed strong concordance with flow cytometry (kappa = 0.68), indicating its reliability as an alternative method for MRD assessment.

In a cohort of Korean MM patients, Kim et al. (2019) [52] applied NGS to analyze IGH and immunoglobulin kappa light chain (IGK) rearrangements alongside somatic

hypermutation (SHM) status. The approach achieved a high clonality detection rate (91.5%) in high-risk MM patients, and SHM analysis complemented rearrangement analysis when the latter was inconclusive. Clonality assessment by NGS demonstrated strong agreement with conventional methods such as PCR and flow cytometry, reinforcing its utility for reliable clonality detection.

Rustad et al. (2020) [53] also reported that NGS detected MRD with 95.2% sensitivity and 100% specificity in bone marrow samples, showing near-perfect concordance with standard flow cytometry (kappa = 0.85). The study further highlighted the potential of NGS to track clonal evolution in MM, illustrating its value for both MRD monitoring and understanding disease dynamics.

Collectively, these studies demonstrate that NGS-based assays provide a highly sensitive and reliable tool for MRD detection and clonality assessment in MM, with strong concordance to conventional methods and clear clinical implications for prognosis and treatment monitoring.

Cho et al. (2022) [15] compared the performance of fragment analysis (FA) and NGS for evaluating the prognostic value of MRD in MM. Both techniques confirmed that MRD negativity was associated with improved PFS and OS. However, NGS demonstrated higher sensitivity than FA and showed a stronger association with favorable clinical outcomes. These findings suggest that NGS may be a more effective method for MRD detection in MM, with MRD negativity by NGS reliably predicting improved prognosis regardless of treatment type.

Other studies have similarly evaluated NGS against alternative techniques. Yao et al. (2021) [54] analyzed 50 MM patients who achieved complete or partial responses and compared MRD detection by NGS and real-time qPCR. NGS identified MRD in 90% of patients (45/50) compared to 86% by qPCR (43/50), with a high concordance between methods (correlation coefficient 0.92). NGS also showed higher specificity, with no false-positive results, and demonstrated superior sensitivity in detecting low-level disease, highlighting its advantage for monitoring residual disease and clonal evolution.

Earlier, Yao et al. (2019) [55] developed a standardized NGS-based MRD assay and evaluated its sensitivity and specificity in 40 MM patients. The assay achieved a sensitivity of 0.001% and specificity of 100%, detecting MRD in 55% of patients versus 40% using traditional methods such as flow cytometry or ASO-qPCR (Allele-Specific Oligonucleotide Quantitative Polymerase Chain Reaction). The assay was also reproducible and offered a rapid turnaround time, supporting its reliability for clinical MRD monitoring.

Building on this, Yao et al. (2021) [54] introduced an upgraded version of the standardized NGS-based MRD assay and evaluated it in 166 MM patients. The upgraded assay maintained 0.001% sensitivity and 100% specificity, detecting MRD in 62% of patients compared to 31% with traditional methods. It demonstrated reproducibility, fast turnaround, and potential utility in guiding treatment decisions and predicting clinical outcomes.

Collectively, these studies illustrate that NGS-based MRD assays provide highly sensitive, specific, and reproducible tools for MRD detection in MM, outperforming conventional techniques and offering clear prognostic and clinical value.

4.5 WGS (Whole-Genome Sequencing) and Targeted Panels in MM

WGS enables a comprehensive analysis of an individual's complete genetic code, capturing mutations and DNA variations across the genome. In MM, WGS has provided valuable insights into somatic mutations and chromosomal aberrations that are relevant for prognosis and disease progression. Such genomic information can inform personalized treatment strategies and improve patient outcomes.

Complementing WGS, targeted gene panels focus on a selected set of genes known to be associated with MM or other hematologic malignancies. These panels typically include genes involved in oncogenesis, DNA repair, cell cycle regulation, and other pathways critical to cancer development and progression, allowing for efficient molecular characterization of patients.

Large cohort studies have illustrated the translational value of these approaches. For instance, Walker et al. [39,56] analyzed 2161 MM patients using WGS, WES, and RNA sequencing to develop a clinical classification strategy that segments MM into therapeutically meaningful subgroups. Five major translocation groups with prognostic significance were identified—t(4;14), t(6;14), t(11;14), t(14;16), and t(14;20)—while smaller translocations and mutational subgroups require the sensitivity of WGS for accurate detection [39].

Similarly, Ashby et al. (2018) [31] applied WGS and WES to a cohort of 1141 newly diagnosed MM patients to investigate hyperhaploidy and biallelic inactivation of *TP53*, which are associated with poor prognosis. This study highlights the capacity of genome-wide sequencing to uncover high-risk genetic features that may guide clinical decision-making (Table 3, Ref [26,31,33,39,41,42,57,58,59,60,61,62,63,64]).

NGS has important implications for guiding treatment strategies in MM. For example, Raab et al. (2020) [57] investigated the efficacy of combined *BRAF* and Mitogen-Activated Protein Kinase Kinase (*MEK*) inhibition in patients with relapsed/refractory MM harboring activating BRAF V600E mutations. Using WGS and phospho-IHC (Phospho-specific Immunohistochemistry), the study demonstrated that phospho-IHC effectively revealed pharmacodynamic suppression of BRAF/MEK signaling during therapy and restoration at relapse, providing insight into therapeutic response and resistance mechanisms.

Table 3. Studies that have used WGS for Diagnosis of MM.

Study	Year	Country	Number of cases	Type of patients	Type of samples	Type of NGS investigation
[26]	2021	USA (New York)	1154	-	-	WGS and WES
[57]	2020	Germany	15	RRMM	-	Exploratory biomarker assessments include cytogenetics, genomic analysis (WGS, RNAseq) and phospho-IHC
[58]	2019	USA (New York)	154	-	BM	WGS with myTYPE panel
[59]	2018	USA (New York)	-	-	BM	WGS using myTYPE panel
[31]	2018	USA (Arkansas)	1141	-	-	WGS, WES, and targeted panel sequencing
[60]	2018	USA (Little Rock)	439	NDMM	-	WGS, WES or targeted panel (TP) modalities
[61]	2017	USA (St. Louis)	995	-	-	WGS (were identified using custom Seq-FISH software on long-insert whole genome sequencing data.)
[33]	2017	USA (Boston)	186	-	-	WES and WGS libraries were constructed with Agilent SureSelect XT2 library prep kit
[39]	2016	USA (Little Rock)	2161	-	-	WGS, WES, targeted panel sequencing, expression data from RNA-Seq and Gene Expression array
[62]	2016	USA (New York)	-	-	-	WGS
[41]	2015	USA (Boston)	29	-	-	WGS (22 patients) WES (17 patients)
[63]	2014	USA (Phoenix)	-	-	-	WGS
[64]	2012	UK (London)	13	MM, SMM	BM	WGS
[42]	2012	UK (London)	-	-	BM	WGS, WES, SNVs

WGS, whole genome sequencing; NGS, next-generation sequencing; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization; SNVs, single-nucleotide variants.

Table 4. Studies using the myTYPE panel for clonotype detection and MRD assessment in MM.

Study	Year	Country	Number of cases	Type of samples	Type of NGS investigations
[65]	2022	USA (New York)	159	-	NGS MRD assay with myTYPE panel
[66]	2020	USA (New York)	74	-	NGS-based assay with myTYPE panel
[58]	2019	USA (New York)	154	BM	WGS with myTYPE panel
[59]	2018	USA (New York)	-	BM	WGS using myTYPE panel
[67]	2018	USA (New York)	147	BM	NGS assay based on the myTYPE panel
[68]	2018	Norway	177	BM	- LymphoTrack® VDJ assay - NGS based myTYPE panel assay

VDJ, variable, diversity, and joining gene segments.

Relapse and treatment resistance in MM are increasingly attributed to cancer stem cell (CSC)–like populations, which survive therapy and regenerate the malignant clone. These cells, enriched in CD138– fractions but also present among CD138+ plasma cells, display stemness features including quiescence, self-renewal, and enhanced aldehyde dehydrogenase (ALDH) activity. Their resistance is mediated by ATP-binding cassette (ABC) transporter–driven drug efflux, activation of developmental signaling pathways (Wnt, Hedgehog, Notch, PI3K/Akt/mTOR), and support from the bone marrow microenvironment. CSC-like populations contribute to resistance against proteasome inhibitors, immunomodulatory agents, and corticosteroids, acting as reservoirs for relapse and clonal persistence.

NGS provides a powerful tool to characterize these resistant subclones at high resolution, capturing the genetic and epigenetic alterations underlying CSC biology and treatment evasion. By profiling minor subpopulations within the tumor, NGS can detect low-frequency variants associated with drug efflux, pathway activation, and metabolic adaptation that may expand under therapeutic pressure. Longitudinal sequencing further enables the tracking of clonal evolution, revealing how resistant progenitor-like cells, such as pre-plasmablasts or Xbp1-deficient clones, emerge during therapy and drive relapse. Integrating NGS into clinical practice offers the potential to anticipate resistance, stratify patients based on subclonal risk, and guide the rational use of agents targeting CSC-related pathways, ultimately improving treatment durability and patient outcomes [57].

Several studies have consistently identified recurrently mutated genes in MM, including TP53, KRAS, NRAS, and BRAF, highlighting their critical role in disease pathogenesis and prognosis. Overall, the application of

WGS in the diagnosis and management of MM has demonstrated considerable potential. As sequencing technologies continue to evolve, WGS is expected to play an increasingly central role in guiding personalized treatment strategies and improving clinical outcomes in this complex disease.

Several studies performed between 2018 and 2022 evaluated the clinical utility of the myTYPE panel in the diagnosis and molecular characterization of MM (Table 4, Ref. [58,59,65,66,67,68]). In their initial study published in *Blood* (2018), the authors compared the myTYPE panel with the Adaptive NGS V(D)J assay and demonstrated that clonotype detection was limited in samples with low DNA input [67]. A subsequent study published in *Clinical Cancer Research* (2022), conducted at Memorial Sloan Kettering Cancer Center, further confirmed these findings, showing that the myTYPE panel achieved significantly higher V(D)J clonotype detection rates compared with Adaptive NGS-based MRD assays in both univariate and multivariate analyses [65]. Together, these studies highlight the robustness of the myTYPE panel for clonotype detection in routine clinical samples.

Complementary evidence was provided by studies from Yellapantula et al. [58,59], published in *Blood* in 2018 and 2019, which combined WGS and the myTYPE panel for genomic profiling in MM. The 2018 study demonstrated complete concordance between myTYPE and WGS in detecting key copy number alterations, including deletions of 1p, 13q, 16q, and 17p, as well as gains of 1q and 11q [59]. When compared with FISH, the myTYPE panel additionally identified t(11;14) translocations and detected certain deletions (13q− and 1p−) not captured by FISH, underscoring its potential added diagnostic value while also highlighting the need for validation in larger clinical cohorts.

More broadly, multiple studies have shown that NGS-based assays achieve high success rates in clonal characterization and MRD detection in plasma cell neoplasms [51,68]. Other reports demonstrated that NGS can reach sensitivities of 10^{-6} or lower, surpassing conventional PCR- and flow cytometry–based approaches [55]. Comparative analyses have further confirmed that NGS offers superior sensitivity and specificity for MRD detection compared with flow cytometry and PCR-based methods [15,49].

Collectively, these findings support NGS-based assays, including targeted panels such as myTYPE, as highly accurate and reliable tools for molecular profiling and MRD assessment in multiple myeloma. Their integration into clinical practice holds significant potential for improving risk stratification, guiding treatment decisions, and predicting patient outcomes.

5. Conclusion

This review highlights the central role of NGS in advancing the molecular and cytogenetic understanding of MM across both newly diagnosed and treated patient populations. By integrating data from genome-wide and targeted sequencing approaches, current evidence consistently reveals the marked genomic heterogeneity and clonal complexity that underlie MM pathogenesis and disease evolution.

Recurrent alterations in key genes such as TP53, KRAS, NRAS, DIS3, and FAM46C confirm their pivotal role in prognosis and disease biology, while the identification of additional candidate driver genes, including ARID1A, ARID2, and RYR2, further expands the molecular landscape of MM. These findings reinforce the importance of comprehensive genomic profiling for risk stratification, prognostic assessment, and the identification of actionable therapeutic targets.

Beyond baseline characterization, NGS has emerged as a highly sensitive and reliable tool for MRD assessment, enabling the detection of residual clonal populations at levels below the reach of conventional techniques. The growing body of evidence supporting NGS-based MRD as a strong predictor of relapse and clinical outcome underscores its potential to refine treatment monitoring and guide therapeutic decision-making.

Collectively, these advances illustrate how the complementary use of targeted panels, whole-exome sequencing, and genome-wide approaches can provide a more integrated and clinically meaningful view of MM biology. As sequencing technologies continue to evolve, future efforts should focus on standardization, validation of emerging biomarkers, and the prospective evaluation of sequencing-informed strategies to fully realize the impact of precision medicine in MM management.

Author Contributions

Conceptualization: IEF, HI, LB and MEE; methodology: LB; validation: MAb and MAh; formal analysis: RB, AO, SN and HEM; resources: IEF and HI; writing—original draft preparation: IEF, HI, SN and AO; writing—review and editing: IEF, HI and HE; visualization: KO; supervision: MEE and LB; project administration: MEE and LB. AW actively contributed to the research of the diagnostic section, the creation and revision of the tables, and the overall language editing of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

As this work is a literature review, it did not involve human participants or animals, and therefore ethical approval and consent to participate were not required.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

The authors declare that artificial intelligence–assisted technologies (ChatGPT, OpenAI, 2025) were used to support the drafting and editing of the manuscript. The authors carefully reviewed, verified, and approved all content generated with AI assistance and take full responsibility for the final version of the work.

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