A New Insight about Multiple Myeloma

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Abstract

Malignant myeloma (MM) is the second most common hematologic malignancy in the United States. Malignant plasma cells in multiple myeloma are usually limited to the bone marrow, although circulating malignant cells are detectable in many cases. Like normal plasma cells, the neoplastic plasma cells in myeloma secrete immunoglobulins. Because the plasma cell expansion is clonal, the malignant plasma cells secrete identical immunoglobulins, all sharing the same sequence and length (the specific sequence is unique and will vary from patient to patient). When the number of malignant plasma cells is high enough, a corresponding paraprotein can be detected as a discrete band on a diffusely stained (polyclonal) background when serum is analyzed by protein electrophoresis (serum protein electrophoresis [SPEP]). Electrophoresis has enabled early detection of monoclonal proteins in asymptomatic people. SPEP is often ordered in the workup of anemia, unexplained proteinuria, and neuropathy. The workup also often includes a bone marrow biopsy, which is examined for the level of plasma cell proliferation and light chain immunotype. Most people with an incidentally discovered paraprotein and small clonal plasma cell expansion live for years or decades with a slowly increasing monoclonal burden, but without development of overt myeloma. This state is termed monoclonal gammopathy of undetermined significance (MGUS). It is accepted that essentially all MM originates in MGUS, but most MGUS cases do not progress to MM, even after decades. MGUS is found in 5% to 10% of the general population by age 60 years; the subsequent incidence increases 1% per year Criteria for diagnosing MGUS, MM, and an intermediate state, “smoldering multiple myeloma” (SMM) have been developed by the International Myeloma Working Group (IMWG) and are set forth in the National Comprehensive Cancer Network (NCCN) Guidelines for Multiple Myeloma, version 5.2022 (at NCCN.org).

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Introduction:

Quantifying plasma cells morphologically for diagnosis is usually not a diagnostic challenge. Despite a somewhat monotonous appearance, at least 4 human plasma cell subsets are present in normal bone marrow: CD191CD38hiCD138- , CD191CD38hi CD1381, CD19CD38hiCD138- , and CD19CD38hi CD1381 [1].

The last subset is primarily limited to bone marrow, and there is evidence that this is a longlived plasma cell population [2].

The other 3 subsets occur in other tissues. Plasma cells are usually thought of as terminally differentiated cells, which seems problematic for malignant expansion; however, immunohistochemical proliferation assessment does show a modest proliferation index, and apoptosis rates seem to decrease with progression from MGUS to overt MM [2–4].

A panel of antibodies to CD19, CD27, CD38, CD45, CD56, CD81, CD117, CD138, kappa, and lambda (cytoplasmic) will identify most plasma cells. Although such thorough phenotyping is not needed for diagnosis, it is important for subsequent flow cytometric detection of minimal residual disease (MRD). Malignant plasma cells in MGUS and MM frequently show aberrant phenotypes. Next-generation flow (NGF) cytometry can detect at least 1 aberrant plasma cell of 100,000 cells. CYTOGENETICS Early cytogenetic studies showed that nearly all MM cases had one of the cytogenetic abnormalities listed in Table 1. Most MGUS cases include one of these cytogenetic changes, with translocations involving the IGH gene locus accounting for the majority; this suggests that the altered gene regulation resulting from translocations is necessary but not sufficient for overt malignancy. The translocations involving chromosomes 4, 11, and 14 do not involve fusions; rather, each brings a new gene under the control of the IGH gene promoter. The immunoglobulin genes normally undergo significant double-strand DNA (dsDNA) breaks for recombination physiologically, so the increased incidence of pathologic translocation is not surprising. Sequencing shows that the MM-associated translocations show increased Activation-Induced (cytidine) Deaminase (AID) and Apolipoprotein B mRNA Editing enzyme, Catalytic polypeptide (APOBEC) activity at the fusion site [5–7].

Hyperdiploidy denotes an increased chromosome complement that is still in a near-diploid state (typically 47–57 chromosomes), and one or more of the “oddnumbered” chromosomes (3, 5, 7, 9, 11, 15, 19, or 21) are often affected in myeloma. The absence of chromosomes 13 and 17 suggests that the extra dosage of the tumor suppressors Rb (chr 13) and TP53 (chr 17) impedes myeloma development. A biological explanation for this predilection for odd-numbered chromosomes remains elusive (and is most likely “chance”). Traditional karyotyping is limited to dividing cells. Cytogenetic fluorescence in situ hybridization probes can interrogate nondividing and fixed cells. Microarrays,

combining both cytogenomicand and single nucleotide polymorphism detection formats can survey the entire genome of bulk samples at a higher genomic resolution than conventional chromosome analysis,(and detect parental disomy) but it cannot achieve cell-level resolution and cannot easily detect balanced chromosomal rearrangements. More recent cytogenetic findings include the following: 1. Subclonal translocations involving MYC are present at diagnosis in a small percentage of MGUS cases, in 25% of new cases of MM, and in 50% of advanced MM cases [8].

2. Translocation involving the lambda promoter is associated with especially poor outcome [9].

3. Chromothripsis is present in up to 35% of cases of MM [10,11]; it is a poor prognostic sign, which might, in part, result from an association with biallelic inactivation of TP53. 4. Templated insertions are found in about 20% of MM cases [6,10,12]; these are characterized by DNA segments from different areas of the genome, copied, joined, and inserted as one sequence into a chromosome. 5. Copy number aberrations (CNAs) are widespread [13].

GENOMICS Numerous gene sequence mutations had been reported in multiple myeloma before 2011, all discovered by the traditional single-gene-at-a-time approach [14], but none were dominant or category defining. The Catalogue of Somatic Mutations in Cancer (COSMIC), hosted by the Wellcome Sanger Institute, lists 6962 myeloma cases in its database (as of April 3, 2022). The database is maintained by expert curation of publications and by importing data from other databases, such as The Cancer Genome Atlas (TCGA). The prevalence of the 20 most frequently mutated genes in the COSMIC database for myeloma is given in Table 2. The genes are ranked by the percentage of positive cases. Until recently most cases were from series that studied only 1 or a few mutations. For example, after the BRAF p.V600E mutation was discovered in cancer, hundreds of studies tested for that mutation specifically, and for nothing else. As a consequence COSMIC is a good source for answering the question “Has gene variant X ever been reported,” but must be used cautiously for quantitative questions like “what is the frequency of variant X in malignancy Y. The first genomic study of myeloma was reported in 2011. Chapman and colleagues evaluated 23 myeloma cases with whole genome sequencing (WGS) and 16 additional cases with whole exome sequencing (WES). NRAS mutations were present in 9 cases, KRAS mutations in 10, TP53 mutations in 3, and CCND1 mutations in 2. Chapman and colleagues also identified likely pathogenic variants in 6 other genes, which had not been previously implicated in myeloma. The TCGA consortium generated its own data and used standardized criteria for accepting, processing, and sequencing samples. The TCGA portal (accessed March 10, 2022) showed a single project for multiple myeloma, which contrasts with 11 for acute myeloid leukemia. The most frequently identified mutations in the MM study are shown in Fig. 1. Another multiinstitutional international consortium, the ICGC (International Cancer Genetics Consortium), has no myeloma series. A comprehensive multiomic myeloma database does exist: the Clinical Outcomes in Multiple Myeloma Personal Assess Study (CoMMpass), an initiative of the

Multiple Myeloma Research Foundation. CoMMpass is a longitudinal observation study of 1150 patients with newly diagnosed myeloma at 76 institutions worldwide (accessed April 28, 2022). Each patient's clinical information is collected every 6 months for 8 years. Molecular testing includes WGS, WES, RNA sequencing (RNA-seq), and multicolor flow cytometry. Sera are analyzed for paraproteins. Data are acquired at 8 time points per patient. Both raw and high-level data are stored and available at the Genome Data Commons (https://portal.gdc.cancer.gov/). Fig. 2 shows a typical "oncogrid." Dutta and colleagues [15] summarize most of the WGS and WES studies through 2021. With highthroughput genomic sequencing (HTGS) the number of genes with probable pathogenic mutations in myeloma reached 80. None of the mutations are present in most cases. These abundant HTGS data are still being mined to address open questions. 1. Prognosis. Are any of the mutations or combinations of mutations useful for distinguishing among MGUS, SMM, and MM with respect to the risk of progression?

2. Prediction. Are any of the mutations or combinations of mutations useful for predicting treatment response or resistance? Methylation of an intronic enhancer leading to decreased CRBN [16] expression has been associated with loss of response to lenalidomide Mutations in PSMB5 (proteasome subunit beta type 5) explains some cases resistant to bortezomib [17].

Biallelic loss of TNFRSF17 (BCMA) explains resistance to Chimeric Antigen Receptor T (CAR-T) therapy directed at TNFRSF17 [18,19].

3. How does the myeloma genome evolve? WES or WGS data for 1 sample are enough to permit mathematical modeling, which can enumerate subclones and infer the likely order of acquisition of mutations including the putative initiating mutation [20,21] 4. Mutation signatures. On the genomic scale the pattern of alteration in nucleotide sequence, independent of the gene involved, shows characteristic patterns (like dinucleotide frequency) that are characteristic of various mutagenic processes such as exposure to UV light or exposure to melphalan [22–25].

GENE EXPRESSION PROFILES (TRANSCRIPTOMICS) The international staging system for myeloma developed by the IMWG was based only on beta-2 microglobulin and albumin levels but was updated in 2016 to include several cytogenetic aberrations: del(17p), t(4;14), and t(14;16). Numerous gene expression studies have proposed expression signatures to improve prognostication for MGUS and MM. Initially studies were done using gene expression microarrays, more recently with RNA-seq. Data for many of these studies are housed in the NCBI GEO database. The SKY92 gene expression classifier assay for myeloma risk is among the more recently advocated [26], but none have found widespread acceptance. SINGLE-CELL ANALYSIS Single-cell techniques can be used to analyze genomes, transcriptomes, epigenomes, and genome-wide chromatin occupation sites. A few studies have demonstrated combining 2 modalities. The caveat must be kept in mind that single-cell analyses are usually not complete—so statistical imputations are often necessary by looking at multiple

similar cells. With genomic and transcriptomic analysis of several thousand bulk myeloma samples already completed, it is unlikely that single-cell analysis will reveal many novel mutations or transcripts, but several important lines of investigation are opened: 1. Phylogenetics: More granular analysis of clonal and subclonal genomic structure, especially determining if mutations co-occur in a cell. 2. Cell identification: If the mutation is in an expressed coding region, then RNA-seq can potentially both detect the mutation and identify the cell type, showing how robustly the mutated transcript is expressed. Not all mutations will be in myeloma cells. 3. Robust detection of low-level mutations: This is analogous to digital display polymerase chain reaction (PCR). Each cell is like an independent PCR/ sequencing reaction chamber. A mutation in a single cell should show a variant allele frequency of 100%, 50%, or 0%. 4. Determining the transcriptional states of cell types: For example, do "exhausted" immune cell profiles predict response to immune checkpoint inhibitors. As with studies of bulk samples, single-cell data have been analyzed for prognostic and predictive factors. A listing of selected single-cell studies on MM is given in Dutta and colleagues [15].

The earliest single-cell study of MM reported an analysis of 6 patients, each carrying t(11;14) [27].

Single-cell analysis was limited to multiplexed real-time PCR, for 5 DNA targets, on CD1381 FACS (fluorescence-activated cell sorting)-sorted cells, ranging from 73 to 243 per case. Ledergor and colleagues [28] reported a single-cell transcriptomic analysis of 40 subjects spanning the clinical spectrum of MM (11 healthy controls, 7 with MGUS, 6 with SMM, 12 with MM, and 4 with primary light chain amyloidosis). The study analyzed 20,586 CD381 marrow plasma cells, with samples ranging from 56 to 1821 cells. Among the key findings are the following: 1. Minimal "heterogeneity" among controls. Only two cell expression clusters were found among plasma cells in controls. Both clusters were present in all control samples but minimally, if at all, in non-controls. This suggests only two broad physiological states (or plasma cell 'types') are present among the normal plasma cells. This is a little surprising and might only reflect the still small number of cells per sample. This finding depends critically on how "heterogeneity" is measured. The investigators define a "heterogeneity score" based on correlations of the read counts within and between clusters for a given subject. 2. Every myeloma sample showed a unique transcriptional profile. For example, in sample SMM02, one transcriptional state was defined by expression of DEFB1, a putative CCR6 ligand, whereas the other transcriptional state by expression of FRZB, a putative member of the Wnt pathway. 3. Several overexpressed genes were identified, not previously noted in MM. Overexpression was confirmed on review of other cases in the CoMMpass database..

a. LAMP5: a lysosome-associated membrane protein b. WFDC2: an endopeptidase inhibitor c. CDR1: a short intronless gene on the X chromosome. 4. Most genes overexpressed in multiple myeloma do not change posttreatment. Five subjects were sampled before and after treatment; 2 were complete responders. 5. Circulating malignant plasma cells showed the same transcriptional profiles

as the corresponding marrow aspirates. Cohen and colleagues [29] used single-cell RNA-seq (scRNA-seq) in conjunction with a prospective multicenter single-arm clinical trial to determine safety and efficacy of a 4-drug combination (daratumumab 1 carfilzomib 1 lenalidomide 1 dexamethasone) in patients who had either failed to respond to bortezomib induction or relapsed early (primary refractory MM [PRMM]). The molecular study followed 41 patients longitudinally: at baseline, cycle 4 (month 3), and cycle 10 (month 9). Only 4 subjects had completed the regimen; 15 were ongoing, and 22 discontinued. As controls, samples from 15 patients with "newly" diagnosed MM (NDMM) and 11 healthy controls were used. These controls were from an earlier study (Ledergor and colleagues [28]), which raises some concern over suitability, given the well-known sensitivity of gene expression to preanalytical variables and batch effects, but this seems unlikely to affect the main findings [30,31].

Overall there were 97 samples from 67 subjects. Plasma cells from bone marrow were collected by FACS sorting on CD38. Satisfactory quality was seen in 51,297 cells (@500 cells/sample). The key conclusions are: 1. Every MM case showed a distinctive transcriptional state 2. There was no significant change in expression of driver genes, over time or treatment. 3. Three gene expression signatures (modules) distinguished NDMM from PRMM samples and split PRMM into 2 subsets. 4. Expression of the PPIA gene identified a new highrisk MM marker. The investigators studied the PPIA gene, peptidylprolyl isomerase A, in detail, deleting it with CRISPr in a human myeloma cell line. The PPIA / cell line was sensitive to carfilzomib (CFZ). Similarly, fresh MM cells showed synergistic sensitivity to treatment with cyclosporin A and CFZ. Interestingly, cyclosporin was noted to have an effect on myeloma cells in the 1990s but fell out of favor [32].

Several extensive single-cell transcriptome surveys of normal bone marrow, mouse and human, made no mention of plasma cells [33–35].

The single-cell studies of myeloma described earlier were limited to CD381 plasma cells. The bone marrow has many cell types; several surely influence dormancy or progression of myeloma. scRNA-seq studies of other bone marrow populations in MM have been reported. Zavidij and colleagues [36] looked at CD451CD138- cells, analyzing 19,000 cells from 23 patients (including MGUS, lowrisk SMM, high-risk SMM, and MM) and 9 healthy controls. The key findings (some of which could have also been made from flow) are: 1. Significant enrichment in NK, T, and CD161 cells in MM 2. Decreased plasmacytoid dendritic cells, granzyme K1 lymphocytes, and CD141 monocytes in MM. 3. These alterations were observed in some MGUS samples These studies were extended to show that granzyme K (GZMK)1 T cells were associated with significantly longer progression-free survival (PFS) in treated patients with SMM [37].

de Jong and colleagues [38] investigated the nonhematopoietic cell stroma of bone marrow in 13 patients with NDMM and 7 healthy controls. Flow cytometric sorting excluded CD451 (hematopoietic), CD2351 and CD711 (erythroid), and CD381 (plasma) cells. The resulting niche

accounted for 0.002% of the total aspirated cells. From this population, they sequenced transcriptomes of 19,983 cells from subjects with MM and 7038 cells from controls. The analysis of MM and control cells together generated 5 mesenchymal stromal cell clusters, MSC1 to MSC5, as well as 1 cluster for endothelial cells, 1 (SELP1) for selectin-P-positive cells, and 1 for osteolineage cells. The clusters MSC1 and MSC2 were markedly enriched in the MM cases (Fig. 3), and the gene sets defining these clusters were enriched in genes involved in inflammation, particularly genes mediating tumor necrosis factor-alpha signaling through nuclear factor-kB, well-known MM cell survival factors IL6 and LIF, and multiple chemokines. MSC1 and MSC2 were prominent in all the MM cases, regardless of the cytogenetic abnormalities, and nearly absent in control subjects. The osteolinage cluster, identified with markers including RUNX2 and SP7, contained few cells. The investigators note an often neglected fact, which is that bone marrow aspirates are often not fully representative of the intact bone marrow.

Perhaps the most intriguing finding in this study is that after 4 cycles of therapy, including cases in which MM cells were no longer detected, the MSC population structure remained abnormal. A commentary by Sklavenitis-Pisotfidis [39] and colleagues compared these results with analysis of MSCs from 2 bulk studies, one showing no effect of successful treatment on the MSC population and the other showing changes. (MACRO) SPATIAL HETEROGENEITY Rasche and colleagues (2017) [40] looked at 42 newly diagnosed patients and 11 treated patients with MM. Paired lesions were analyzed—a standard iliac crest biopsy and an image-guided needle core biopsy of a focal lesion elsewhere [40].

Five NDMM cases had 4 to 5 focal lesions biopsied. Cytogenetic microarray studies showed on average 3 different CNAs between the diagnostic and the additional focal lesion (range 1–28 differences). Classic cytogenetic translocations were stable, but in 2 cases hyperdiploid status was not shared. WES showed significant divergences in 75% of the subjects. Rasche and colleagues [40] relate their findings to theories of cancer evolution. The investigators note that MM primarily grows in the marrow, where free movement among sites through the circulation is assumed, and that their expectation was that when a fitter clone arises, it will sweep to dominance everywhere. Rasche and colleagues (2022) [41] extended the work by Rasche and colleagues (2017) [40], looking at 144 samples collected over 14 years from 25 patients, each with multiple focal lesions. The investigators describe 3 patterns of evolution: (1) the original dominant clone persists after treatment, (2) preexisting subclones compete after relapse, and (3) distinct subclones persist at different sites. These patterns cover most of the a priori possibilities. More interestingly, they observed that numerically minor subclones can persist for more than 10 years and then expand at relapse. Merz and colleagues (2022) [42] looked at 24 sites in 10 patients, and did an scRNA-seq analysis, looking at 148,630 cells. Here, too, WES showed limited variation but scRNA-seq showed wide variation. If 2 sites show divergent genomic or transcriptomic profiles, it could blunt the effectiveness of prognostic/ predictive

signatures based on the diagnostic bone marrow sample alone. As a practical matter, additional focal lesions are rarely biopsied. Profiling both the standard biopsy and circulating malignant plasma cells could hint at the presence of such a divergence. SINGLE-CELL SPATIAL GENOMICS Single-cell genomics and transcriptomics reveal nothing about relationship among cells. In single-cell spatial genomics DNA or RNA is used to construct nextgeneration sequencing (NGS) libraries on a cell-bycell basis from all the cells within a tissue section. The geographic location of the cell is registered in an image and correlated with the known unique barcode predeposited at that position of the slide [43–45].

Knowing each transcriptome's location enables exploration of cell-cell interactions. Application to MM might be difficult because the usual sample is a calcified bone core. Ethylenediaminetetraacetic acid incubation can render a bone marrow core susceptible to sectioning, but the effect of incubation on the transcriptome might be substantial. Treatment The dismal overall survival (OS) rate began to improve in the 1990s with the introduction of high-dose melphalan, a cytotoxic (and mutagenic) alkylating agent, as an induction agent for subsequent stem cell transplantation [22,46].

When possible, autologous stem cells are used to avoid graft-versus-host disease. Multiple additional novel agents are now available and have demonstrated dramatic improvements in PFS and OS, leaving high-dose therapy with autologous stem cell transplant reserved for refractory cases. Therapeutic Monoclonal Antibodies (Rx-mAbs) Ironically, myeloma, the paradigmatic disorder of cells producing monoclonal immunoglobulins, is one of the earliest malignancies to be successfully treated with monoclonal antibodies (mAbs). Most new therapeutic mAbs are IgG1 kappa immunoglobulins, developed in transgenic mice, which have had a human immunoglobulin locus engineered into their genome. Daratumumab Daratumumab is directed against CD38, an abundant surface marker on plasma cells. CD38 is also present on many immune cell types and on red blood cells (RBC). CD38 can function as an enzyme, a cyclic ADP ribose hydrolase, but is probably multifunctional. Daratumumab binding to MM cells causes apoptosis by uncertain means. CD38 expression can downregulate, blunting efficacy.

Isatuximab Isatuximab is directed against CD38, but at an epitope different from that targeted by daratumumab [47].

Elotuzumab Elotuzumab is directed against the SLAMF7 receptor (CD319), a surface receptor, of uncertain function, at a high level on plasma cells [16].

Laboratory pitfall with therapeutic monoclonals Therapeutic human monoclonals create a problem for monitoring the response of MM to treatment. Initially the response is monitored by following the patient's endogenous monoclonal level by SPEP. Human therapeutic mAbs can be detected on SPEP as monoclonals; they are "true" false-positives. Each mAb has a characteristic mobility, but this does

not always permit distinguishing the therapeutic from the endogenous monoclonal protein on SPEP. Mass spectrometric analysis can distinguish endogenous monoclonal immunoglobulin from multiple Rx-mAbs in a single assay [48,49].

OTHER THERAPIES Proteasome inhibitors and immunomodulatory drugs have each effected a dramatic improvement in clinical course for myeloma [50–54].

The modes of action, involving proteolysis inhibition and ubiquitination, presage the development of ProTacs, an approach generalizable to multiple cancer types [55–58].

Bortezomib carries a boron molecule that it donates to the 26S complex, shutting down proteolysis. Inhibition of proteolysis affects a wide array of proteins— cyclins, proapoptotic factors, and inhibitors of apoptosis, among them. The net effect seems to be increased apoptosis. The mechanism of action of immunomodulatory agents such as lenalidomide has been worked out in detail: it interacts with the ubiquitin E3 ligase cereblon and targets this enzyme to degrade the Ikaros transcription factors IKZF1 and IKZF3. CAR-T cells directed at myeloma are in several clinical trials [59–61].

The most common target is TNFRSF17. Examples of resistance, with biallelic deletion of both gene copies, have already been reported. Other targets are SLAMF7, CD38, and CD138 [62].

Other therapies in development include panobinostat, a pan-HDAC inhibitor [52]; selinexor, an exportin inhibitor [63]; venetoclax, a Bcl-2 inhibitor [64]; and immune checkpoint inhibitors. MINIMAL RESIDUAL DISEASE With the current 3- and 4-drug regimens and agents for maintenance, a complete remission is attainable in up to 80% of patients; unfortunately the majority are not durable, with a 50% ten-year OS [65,66].

More sensitive assays, to monitor for emergence of malignant plasma cells following completion of treatment, might better distinguish patients who will have durable responses from those who will have relapse. If a patient is identified as MRD positive early in treatment, it raises the possibility of intensifying or changing the treatment, but the merits of such changes in a given setting are usually untested. It is customary to monitor serum/urine for the diagnostic paraprotein following treatment, but because of the long half-life of immunoglobulins, the level does not decline rapidly; it is also not a sensitive test. For a paraprotein to be detected over background by SPEP typically 5% of plasma cells must be clonal. Serum free light chains show a more rapid decline than do intact monoclonal immunoglobulins, but most myeloma cases do not express a clonal excess of light chains. Nevertheless, moderately sensitive detection of the circulating clonotypic immunoglobulin is feasible by mass spectrometry [67,68].

NGF cytometric and NGS assays of bone marrow are the recommended modalities for sensitive detection of residual malignant plasma cells. Numerous trials have shown that MRD negativity of

bone marrow aspirates predicts significantly longer PFS and OS. The IMWG has published criteria for detecting/reporting MRD [69].

It requires a method able to detect a malignant plasma cell with a minimum sensitivity of 1 in 100,000 cells to be designated MRD(). Because of stochasticity in detecting a single cell, a reliable test cannot assay just 100,000 cells; rather it must assay 106 6 cells [66].

Multiple studies show that an MRD cutoff of 1 cell in 1 million leads to even better PFS and OS. A suitable NGF assay, with validation approved by the IMWG, is the EuroFlow assay. The assay is designed to detect 10 antigens, using 8 fluorophores, in a 2-tube assay: CD138, CD27, CD38, CD56, CD117, CD45, CD19, CD81, c-kappa, c-lambda. It should be noted that this is done on total aspirate, and not on CD1381-selected cells. The EuroFlow protocol calls for testing 107 cells to detect 2 106 cells with a 20- cell cutoff [70].

MRD by NGS is designed to detect the myeloma clone-specific rearrangement of the IGH gene; this requires a baseline sample from the time of diagnosis. The format is similar to the traditional assay for assessing B-cell clonality. Several primer pools targeting the FR1-3 regions of VH and JH portions of IGH are used to amplify V-D-J rearrangements by PCR. The NGS assay uses similar primers, but oligonucleotide adapters are ligated to the ends of the target fragments for construction of an NGS library and include unique molecular barcode sequences for pooled sample sequencing. The sensitivity identifying any expanded clone, as in a diagnostic sample, with NGS-based clonality studies is comparable to traditional PCR-based fragment-size analysis methods (eg, EuroClonality/BIOMED-2-based analysis). The sensitivity for detecting a specific sequence (clone) is much higher, and suitable for MRD. At present (March 30, 2022) the ClonoSeq assay is the only FDA-approved NGS-based assay for detection of MRD in chronic lymphocytic leukemia (CLL), MM, and B-cell acute lymphoblastic leukemia. This assay has been extensively validated and shown to be able to detect 1 malignant plasma cell out of 10^6 cells. Other commercial NGS-based clonality/MRD offerings are available (eg, LymphoTrack, InVivoScribe, Inc., San Diego, CA); Oncomine Clonality Assay, Thermo Fisher Scientific, Waltham , MA) and described in published validations, including one showing comparability to NGF [71].

MRD by NGS has a comparative drawback compared with NGF—it requires analysis of a patient-specific “positive” (baseline) sample, usually from the time of diagnosis. At present this is not routinely provided prospectively. Stored aspirate slides may be acceptable. NGF assays have the comparative drawback that the sample must be analyzed promptly, compounded by the need for many sites to send the sample to a reference laboratory. IMWG guidelines recommend MRD testing of unfractionated bone marrow aspirates. The optimal time to first test for MRD and optimal interval for monitoring is unsettled. At present, the IMWG guidelines recommend assessing MRD status over the disease course,

rather than at a single timepoint after complete response (CR) is achieved, because this may provide a more robust evaluation of disease control [72].

Traditionally the first pull of a bone marrow aspirate is reserved for morphologic analysis, and the last for molecular testing. The trauma of biopsy induces hemorrhage, increasing with every aspiration. MRD testing must be done on the "first pull" [72].

Two other groups have offered informative consensus opinions [72,73].

The clinical utility of monitoring MM by iliac crest biopsy is well established, but for many patients, frail from age or cytopenias, it is not a trivial procedure. MM involvement in the bone marrow can be spotty, complicating reliance on the biopsy. MRD testing has been applied to circulating myeloma cells in blood and circulating free nucleic acids released into blood from myeloma cells. MRD analysis of blood would avoid the problem with single bone marrow aspirates not being representative and could, potentially, also detect mutated genomes shed from other sites of involvement. NGF and NGS assays can detect circulating myeloma cells in a moderate number of putative CR cases but are not as frequently positive as the bone marrow biopsies [74,75]; this could still have a role. Blood can be monitored more frequently; if positive, it might obviate bone marrow biopsy. SUMMARY Noncoding RNAs, both long and short, have been relatively underinvestigated, but genomic and transcriptomic analyses of myeloma would seem exhaustive, with more than 80 putative "driver" genes identified. Even so, there has been little clinical impact of genomics on diagnosis, prognosis, or therapy selection. As of 2022 the NCCN guidelines for myeloma (version 5.2022) only takes note of cytogenetics at diagnosis. A modest number of mutations and methylations have been associated with therapy resistance. In MRD, NGS and NGF play a critical role.Meanwhile, novel therapies have continued to markedly improve clinical response including OS.

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