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Molecular and biologic markers of progression in monoclonal gammopathy of undetermined significance to multiple myeloma

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Abstract

Multiple myeloma (MM) is a malignant plasma cell dyscrasia localized in the bone marrow. Recent studies have shown that MM is preceded in virtually all cases by a premalignant state called monoclonal gammopathy of undetermined significance (MGUS). This review focuses on non-IgM MGUS and its progression to MM. Although certain clinical markers of MGUS progression have been identified, it currently is not possible to accurately determine individual risk of progression. This review focuses on the various biologic and molecular markers that could be used to determine the risk of MM progression. A better understanding of the pathogenesis will allow us to define the biological high-risk precursor disease and, ultimately, to develop early intervention strategies designed to delay and prevent full-blown MM.

Keywords

Myeloma; MGUS; smoldering myeloma; prognosis; molecular imaging; molecular profiling

Introduction

Multiple myeloma (MM) is a malignant neoplasm of bone marrow plasma cells. Clinically, it is characterized by skeletal lytic lesions, anemia, renal failure, and hypercalcemia (Table

I). In the United States, it is the second most common hematologic malignancy, with approximately 20 580 new cases diagnosed and about 10 580 deaths estimated in 2009 [1]. Despite significant advances in the treatment of MM, it still has a high morbidity and mortality, as reflected by an overall median survival of 4–7 years [2,3].

Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic premalignant tumor affecting approximately 3% of Caucasians over the age of 50 years, with a 1% average annual risk for progression to a malignant tumor [4–6]. Interestingly, from a population perspective, MGUS and MM show striking racial disparity patterns. For example, both disorders are 2–3-fold more common in African-Americans than in Caucasians [7]. In contrast, a few studies from Japan have reported a lower prevalence of MGUS in Asians compared to Caucasians [8–10]. Furthermore, familial clustering of MM has been described in case reports and smaller studies. Recently, a large population-based study based on the Swedish Multigenerational Registry found a two-fold increased risk for MM among 37 838 first-degree relatives of 13 896 patients with MM (compared with 151 068 first-degree relatives of 54 365 matched controls) [11]. Using a similar study design, a three-fold increased risk for both MGUS and MM was found among 14 621 first-degree relatives of 4458 patients with MGUS (compared to 58 387 first-degree relatives of 17 505 population-based controls) [12]. Taken together, these observations support a role for susceptibility genes in the causation of MGUS and MM.

Although it has been known for some time now that patients with MGUS have an increased risk of progression to MM and other related malignancies [6,7], it is only recently that two independent studies have provided convincing evidence to show that virtually all cases of MM are preceded by premalignant MGUS [13,14]. Using the prospective Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial (which includes 77 469 healthy men and women; age 55–74 years at baseline; and with annual collection of blood samples, clinical data, and prospective cancer surveillance), a total of 71 patients with MM (diagnosed during a 10-year follow-up period after baseline) were identified. Median age at MM diagnosis was 70 years; 4.3% were African-Americans. Using available stored prediagnostic serum for these 71 patients, MM was found to be consistently preceded by MGUS [13]. In parallel, another study utilized the Department of Defense Serum Repository to evaluate 30 patients with MM who underwent high-dose chemotherapy and autologous stem cell transplant for MM at the Walter Reed Army Medical Center, and who had prediagnostic serum samples available [14]. Because this study took advantage of stored samples from individuals who served in the Army, the patients were younger (median age 48 years), 97% were males, and 47% were African-Americans. Despite the fact that there were differences with regard to study characteristics, the results from the study based on the Department of Defense Serum Repository were very similar to those from the prospective PLCO Cancer Screening Trial. In fact, 27 of the 30 (90%) patients with MM had evidence of a preceding precursor state (MGUS). Regarding the three patients with no detectable MGUS preceding the diagnosis of MM, one only had prediagnostic serum available at 9.5 years before diagnosis, and two were found to have immunoglobulin D (IgD) MM (which is a MM subtype with very low levels of monoclonal immunoglobulin secretion), and with sera available only 3 and 5 years before the diagnosis. Thus, these two independent studies establish a central role for MGUS on the pathway to MM.

Although MGUS is commonly referred to as a single entity in the literature, indeed there are two kinds of MGUS: lymphoid (or lymphoplasmacytoid) MGUS, and plasma cell MGUS. About 15–20% of MGUS cases secrete IgM, and mostly they have a lymphoid or lymphoplasmacytoid phenotype. By contrast, non-IgM (IgG > IgA > Ig light chain only > IgD > IgE) cases of MGUS mostly have a plasma cell phenotype. Typically, plasma cell MGUS can progress to MM or related plasma cell disorders, whereas lymphoid MGUS can progress to Waldenström macroglobulinemia, lymphoma, or other malignant lymphoproliferative disorders [15]. Furthermore, there is virtually no overlap of the molecular genetic events responsible for the molecular pathogenesis of the two kinds of MGUS, suggesting that they are quite distinct biological entities.

This review focuses on plasma cell MGUS, which is defined by a serum M-protein concentration of less than 3 g/dL, fewer than 10% of plasma cells in the bone marrow, and with no end organ damage (Table I). Although it mostly belongs to the plasma cell MGUS category, primary amyloidosis is an exception from the definition given above. In fact, it is characterized by pathological deposits in various tissues of monoclonal Ig light chain fragments produced by MGUS that often include only a small number of premalignant tumor cells. This review does not focus on primary amyloidosis.

Current clinical markers of progression of monoclonal gammopathy of undetermined significance to multiple myeloma

It is important to keep in mind that the vast majority of patients with MGUS will never progress to MM. Unfortunately, at this time, we lack reliable markers to predict the risk of MM progression for individual patients with MGUS. Currently, the risk of progression of MGUS is assessed by a few selected risk factors. Basically, two major models for risk stratification have been proposed: one model by the Mayo Clinic and the other by the Spanish study group.

The Mayo Clinic model (Figure 1) focuses on serum protein abnormalities. The following features are considered as adverse risk factors: non-IgG isotype, M-protein concentration > 1.5 g/dL, and an abnormal serum free light chain (FLC) ratio (normal reference 0.26–1.65) [6,15]. In the Mayo Clinic model, at 20 years of follow-up, patients with MGUS with all three risk factors on average have an absolute risk of MM progression of 58%; for MGUS patients with two, one, and none of these risk factors, the corresponding absolute risk is 37%, 21%, and 5%, respectively [Figure 1(A)] [16]. For patients with asymptomatic or smoldering MM (SMM), the risk factors for progression are serum M-protein concentration > 3 g/dL, bone marrow plasma cells > 10%, and an abnormal free light chain ratio. The cumulative risk of progression at 10 years with one, two, and three of the risk factors was 50%, 65%, and 84%, respectively [Figure 1(B)] [17].

The Spanish study group model uses multiparametric flow cytometry of bone marrow aspirates to differentiate aberrant from normal plasma cells [18] (Figures 2 and 3). Plasma cells characteristically express CD138 and intense (bright) CD38. The features of aberrant plasma cells include decreased CD38 expression, expression of CD56, and the absence of CD19 and/or CD45 (Figure 2). In 500 patients with MGUS or SMM, the ratio of

phenotypically aberrant plasma cells (aPCs) to total bone marrow plasma cells (BMPCs) at diagnosis allowed the risk stratification of patients with MGUS and SMM progression to overt MM (Figure 3). In their study, patients with MGUS and SMM with 95% aPCs/BMPCs at diagnosis had a significantly higher risk of MM progression [18]. Furthermore, on multivariate analysis, 95% aPCs/BMPCs, DNA aneuploidy, and immunoparesis were found to be independent predictors of MM progression [18]. More specifically, for patients with MGUS with no, one, or two risk factors (95% aPCs/BMPCs and DNA aneuploidy), the risk of progression at 5 years was 2%, 10%, and 46%, respectively [Figure 3(A)]. For patients with SMM (risk factors: 95% aPCs/BMPCs and immunoparesis), the corresponding numbers were 4%, 46%, and 72%, respectively [Figure 3(B)] [18].

Taken together, these studies emphasize the fact that the risk of MM progression varies greatly among individuals diagnosed with MM precursor disease. Clearly, we need better markers to define high-risk (versus low-risk) MGUS/SMM and to better predict individual risk of MM progression.

Genetic abnormalities in monoclonal gammopathy of undetermined significance and multiple myeloma

Karyotypic analyses are informative for MM and sometimes SMM tumors (Figure 4). Yet, karyotypic analyses are rarely informative for MGUS because of the extremely low fraction of proliferating cells, the relatively low numbers of plasma cells in the marrow of patients with MGUS (by definition <10%; typically 3–5%), and the even lower number of monoclonal plasma cells recovered in bone marrow aspirates (often <0.5%). However, interphase fluorescence *in situ* hybridization (FISH) can detect the numbers of chromosome and chromosomal rearrangements in non-dividing cells, and consequently is a useful tool to study chromosomal aberrations in tumor cells from patients with MGUS, SMM, and MM, especially when applied to purified plasma cells [19,20] (Figure 5). Several molecular and chromosomal genetic changes have been identified in patients with MGUS and MM. These changes occur at different stages of disease progression and may serve as potential markers of disease progression (Figure 6). For MM tumors, the chromosome content identifies two major tumor groups, each constituting approximately 50% of tumors: the hyperdiploid (HRD) group (48–74 chromosomes) is associated with recurrent trisomies, particularly of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21; and the non-hyperdiploid (NHRD) group (fewer than 48 and/or more than 74 chromosomes) includes tumors with hypodiploid, pseudodiploid, or near tetraploid chromosome number. Significantly, primary translocations involving the immunoglobulin heavy chain (IgH) locus at 14q32 [e.g. t(11;14), t(4;14), t(14;16), t(6;14), and t(14;20)] are present in about 70% of NHRD tumors but only 10% of HRD tumors [21–25]. Chng *et al.* have developed an interphase FISH-based trisomy index, using a cut-off of two trisomies within a three-chromosome combination (chromosomes 9, 11, and 15) that is highly specific for hyperdiploid MM [19]. When the index was applied to 28 patients with MGUS and SMM who had normal karyotypes, 40% of the cases were identified as hyperdiploid. Using a similar approach, Chiecchio *et al.* found that 72/189 (42%) of MGUS, 70/127 (63%) of SMM, and 223/388 (57%) of MM cases were hyperdiploid [20]. For both studies the prevalence of hyperdiploidy was similar, albeit

somewhat lower in MGUS compared to MM tumors, and therefore likely occurs early in the disease evolution of most tumors (Figure 6). Chiecchio *et al.* also examined the prevalence of other specific chromosomal abnormalities in patients with MGUS, SMM, and MM. The prevalences of three primary IgH rearrangements [t(6;14), t(11;14), and t(14;16)] were found to be very similar in all three groups, suggesting that primary IgH translocations probably represent an early genetic event in myelomagenesis. This result is consistent with previous results indicating that most primary IgH rearrangements occur as a result of an aberrant IgH switch recombination event in germinal center B cells [26]. The prevalence of chromosome 13 deletion in MGUS differs in a number of studies; some have found the frequency of this deletion to be much lower in MGUS (~25%) when compared to MM (~50%) [25,27], while others have reported a similar rate of nearly 50% [28,29]. Of note, Chiecchio *et al.* [20] reported that the occurrence of chromosome 13 deletion in MGUS (25%; 47/185) vs. MM (47%; 136/395) was related to the presence of specific IgH translocations. For both MGUS and MM tumors, chromosome 13 deletion was present in approximately 90% and 70%, respectively, of tumors with t(4;14) and t(14;16). However, for tumors with t(11;14) or t(6;14), chromosome 13 deletion was present in <5% of MGUS cases but approximately 40% of MM cases. They hypothesized that in patients with MGUS with t(11;14) and t(6;14), the occurrence of chromosome 13 deletion may indicate transition to MM.

Another possible genetic marker for progression of MGUS to MM is the presence of a RAS mutation. Rasmussen *et al.* have shown that the combined prevalence of activating K- plus N-RAS mutations is much higher in malignant MM (31%) than in premalignant MGUS (5%) [30]. Combining various studies that examined the prevalence of RAS mutations in MM and MGUS, there is a similar prevalence (about 15% each) of K- and N-RAS mutations in MM, whereas K- and N-RAS mutations were detected in none and 4/57 (7%) of MGUS [30–32; M. Kuehl, unpublished data]. While the occurrence of activating RAS mutations in MGUS is neither necessary nor sufficient (at least for N-RAS) for conversion to MM, the apparently unique occurrence of K-RAS mutations in MM suggests a causal role in the transition from premalignant MGUS to malignant MM in some instances. Finally, Chesi *et al.* have reported that MYC up-regulation is a possible mechanism for progression from MGUS to MM both in the Vk+ MYC mouse model and in human tumors [33]. In further support of these findings, using gene expression profiling (GEP) data from 44 patients with MGUS and 359 with MM, the median MYC expression was found to be three-fold higher in the patients with MM (versus MGUS) despite extremely low proliferation indices in both MGUS and MM [34; M. Kuehl, unpublished data].

Taken together, although many of the abovementioned markers have been identified in patients with MGUS, and the prevalence of some markers has been reported to differ between MGUS and MM, currently it is unknown whether any markers are associated with an excess risk of progressing from MGUS to MM. Importantly, at this time no systematic study using serial samples (from the same patient over time) has been conducted to formally address the role of these genetic markers in predicting disease progression in patients with MGUS/SMM.

Gene expression profiling

An increasing number of studies have been using GEP to delineate pathogenesis and prognosis in human malignancies [35]. In MM, GEP has also been used to identify distinct molecular subgroups [36–39]. Recently, some investigators have attempted to identify GEP signatures that might predict progression of MGUS to MM [34,40]. More specifically, Zhan *et al.* [34] first identified 52 genes with differential expression levels across normal plasma cells, MGUS, and MM; of these, 41 exhibited a progressive increase in expression levels along the transition from normal plasma cells to MGUS to MM, and four exhibited a progressive reduction in expression from normal plasma cells to MGUS to MM. In their study, they analyzed the expression patterns of these genes and classified the patients into four groups which they labeled as follows: MM-like MGUS, non-MM-like MGUS, MGUS-like MM, and non-MGUS-like-MM. Based on their observations, they speculated that the patients with MM-like-MGUS may have an increased risk of progression, and that they may benefit from a closer clinical follow-up and possibly early therapeutic interventions. This is a hypothesis that has not been formally tested. Interestingly, it was also reported that the patients with MGUS-like-MM had a longer survival despite having lower rates of complete remission. However, a potential explanation of this finding might be that there was a significant contamination of non-MGUS-like MM tumor cells with monoclonal plasma cells, normal plasma cells, and even other kinds of cells. Clearly, the most important conclusion from these studies is that, currently, GEP profiles cannot clearly distinguish MGUS and MM tumor cells. Based on the abovementioned data, in our opinion, the strongest findings are that the GEP profiles of MGUS and MM tumor cells are more closely related to one another than either kind of tumor cell is to normal plasma cells.

Ria *et al.* recently reported GEP results of bone marrow endothelial cells in five patients with MM and five with MGUS [41]. They detected 22 genes that were differentially expressed in the two groups; 14 genes were down-regulated and eight genes were up-regulated in MM when compared to MGUS. Based on small numbers, the study suggested that the MM endothelial cells were functionally different from the MGUS endothelial cells, and characterized by an over-angiogenic phenotype. Due to the restricted numbers, the study did not have statistical power to assess whether different phenotypes of marrow endothelial cells in MGUS are associated with the risk of MM progression.

Taken together, importantly, one has to keep in mind that GEP analysis of MGUS has inherent problems. First, the percentage of plasma cells is low (by definition <10%), so there is significant contamination with other kinds of cells despite selection of CD138+ cells on magnetic beads. Second, in patients with MGUS—unlike in those with MM—monoclonal plasma cells are likely to be significantly contaminated with normal plasma cells (due to the relatively low percentage of monoclonal plasma cells in MGUS). Until we have better processing methods and better assays, one has to be very cautious when interpreting GEP analyses of plasma cells selected by CD138 expression from patients with MGUS.

MicroRNA profiling

MicroRNAs (miRNAs) are small (about 22 nucleotides long), single-stranded RNA molecules that regulate gene expression [42,43]. It is now known that miRNAs can act as tumor suppressors or protooncogenes [44], and that they are misregulated in most human cancers. Subsequently, several studies have shown that miRNAs could potentially be used as markers of malignant disease in a wide variety of cancers [45]. Pichiorri *et al.* described that a characteristic miRNA signature differentiates plasma cells of patients with MGUS from healthy plasma cells [46]. With respect to normal CD138+ plasma cells, they found 41 miRNAs to be up-regulated and seven to be down-regulated in MGUS. The most up-regulated miRNAs in MGUS were miR-21, miR-181a, known to have a role in B and T cell differentiation [47], and the oncogenic cluster miR-106b~25, in particular miR-93, miR-106b, and miR-25. Also, analysis of PCs in patients with MM and myeloma cell lines revealed up-regulation of 60 and down-regulation of 36 miRNAs when compared to CD138+ healthy controls. Similar to the signature observed in MGUS, miR-21 and the miR-106a~92 cluster were found to be up-regulated in subjects with MM and MM cell lines. However, unlike MGUS, miR-32 and the cluster miR-17~92 (in particular miR-19a and b) were significantly up-regulated only in MM samples, thus suggesting a possible role in the progression of MGUS to MM. These results suggest the possibility of using the miRNA expression pattern as a potential biomarker of disease progression in patients with MGUS. As stated above, in the future there is a need for prospective studies using serial samples (from the same patient over time) to address the role of miRNAs in predicting MM progression.

Gene promoter methylation

DNA methylation is an epigenetic process of methylating specific CpG dinucleotides in the genome. These 'CpG islands' are usually associated with the promoter region, and their methylation usually is inhibitory for gene expression. Aberrant gene promoter methylation is therefore a potential mechanism for silencing tumor suppressor genes [48]. Methylation specific polymerase chain reaction (MS-PCR) followed by DNA sequencing can identify the methylated CpG dinucleotides.

Takahashi *et al.* have shown that the methylation index (MI) in patients with MM is comparable to other hematologic malignancies but is significantly higher than in patients with MGUS [49]. A restricted number of studies have been conducted to assess the role of promoter methylation in patients with MGUS and MM. Based on small numbers, P16 (which is a member of the INK4 family of tumor suppressor genes involved in the INK4/cyclin D1/RB cell cycle pathway); E-cadherin (which mediates Ca²⁺-dependent intercellular adhesion); DAPK (which is a pro-apoptotic serine/threonine kinase); hMLH-1 (which is involved in DNA repair); and SOCS-1 (which is part of the JAK-STAT pathway) have been shown to be methylated with increased frequency in MM when compared to patients with MGUS in prior studies [50–53]. However, based on small numbers, there are considerable differences in terms of the genes that are methylated in the various studies. For example, one study found that there is significant difference in the p16 methylation status in MGUS and MM [50], while others have shown that there is no such difference [51–53]. Clearly,

additional studies, especially with serial samples from the same individual, are needed to confirm these findings and to assess their role in relation to the risk of MM progression.

Molecular imaging

Based on current diagnostic criteria, the absence of end organ damage including skeletal lesions is a prerequisite for diagnosis of MGUS and SMM. However, the presence or absence of end organ damage is also dependent on the type of laboratory and imaging studies used. The presence of lytic lesions indicates a diagnosis of MM. Table II lists the conventional imaging studies that are currently used in the management of MM. The standard of care to screen for bone lesions in these patients is a whole-body skeletal survey. However, with the advent of newer molecular imaging techniques including positron emission tomography/computed tomography (PET/CT) using novel MM-specific tracers, in the future it will likely become easier to identify lytic lesions at an earlier stage. Consequently, early detection of lytic lesions by the use of molecular imaging techniques may well enable us to identify patients with precursor disease who are at an increased risk of developing full-blown MM.

Fluorodeoxyglucose (FDG)-PET/CT is a functional imaging study that has been used in a variety of clinical settings. For example, FDG-PET/CT can detect bone marrow/myelomatous involvement with a quite high sensitivity and specificity (Figure 7). At our institution, the radiation exposure for FDG PET/CT is about 1.6 REM. In a patient with MM, a single examination with FDG-PET/CT can detect and distinguish between intramedullary and extramedullary lesions [54]. A small study of 49 patients with plasma cell malignancies (six patients with MGUS) described increased sensitivity in detecting bone lesions that were otherwise undetectable by conventional imaging techniques, including CT and magnetic resonance imaging (MRI) [55]. In this study, two out of the six patients with MGUS were found to have positive FDG-PET/CT; however, both these patients also had a separate malignancy in addition to MGUS (one patient had a thyroid cancer and the other had an intestinal tumor). It is therefore unclear whether the FDG-PET/CT positivity was related to MGUS or to the other malignancies.

^{18}F -fluoride (^{18}F) is a positron emitting, bone seeking radiotracer that was originally developed for clinical use in the 1960s; however, it fell out of favor due to production logistics and the availability of cameras to generate satisfactory images [56]. This radiotracer is the most sensitive agent for detection of skeletal metastases, when compared to $^{99\text{m}}\text{Tc}$ -methyl diphosphonate ($^{99\text{m}}\text{Tc}$ -MDP) bone scintigraphy, and it has the advantage of detecting both osteoblastic and osteolytic activity [57]. The ^{18}F uptake occurs, as expected, at the margins of the lytic lesions. This can be nicely correlated with focal bone abnormalities on the fused CT. Thus, ^{18}F PET/CT appears to be a promising modality for identifying sites of osteolysis in MGUS/MM. To our knowledge, at this time, no studies have been conducted to evaluate the efficacy of ^{18}F PET/CT in identifying early skeletal lesions in patients with MGUS.

One hypothesis to explain the transition from premalignant to malignant states is that there is an 'angiogenic switch' that results from cumulative genetic damage leading to an imbalance

of pro- and anti-angiogenic factors, creating an adequate microenvironment for a tumor to grow [58]. Dynamic contrast-enhanced MRI (DCE-MRI) is a non-invasive imaging technique that can be used to derive quantitative parameters that reflect microcirculatory structure and function in imaged tissues. The process of angiogenesis has been shown to be of great importance for development, growth, and prognosis in solid as well as hematologic malignancies [59,60]. In MM, increased angiogenesis results in changes of microcirculation in bone marrow. Hillengass *et al.* recently examined 222 patients (60 patients with MGUS, 65 patients with asymptomatic MM, and 75 patients with newly diagnosed symptomatic MM) and 22 healthy controls with DCE-MRI of the lumbar spine [61]. They found a gradual increase of microcirculation from early stages of disease to full-blown MM. More interestingly, they found that in patients with MGUS and a low-intensity pattern of microcirculation, there was lower bone marrow plasmacytosis. One patient with MGUS/SMM with a pathologic DCE-MRI pattern developed a progression to MM 6 months after the DCE-MRI. The study identified a subset of patients with MGUS with an abnormal microcirculation pattern; these patterns correlated with increased plasmacytosis in the bone marrow. Future studies are needed to assess whether DCE-MRI can be used as a method to predict MM progression in patients with MGUS.

Conclusions and future directions

Recent studies have shown that MM is consistently preceded by a precursor state (MGUS). Despite this fact, little is known about the molecular and biologic markers that help identify patients with MGUS with a high risk of progression.

From a clinical standpoint, the identification of appropriate biologic and molecular markers is crucial to identify patients with MGUS who have a high risk for progression. This will aid in determining a closer clinical follow-up of such high-risk patients and possible early therapeutic interventions. It may also be possible to use some or all of these markers to determine the choice of therapy and response to therapy in patients with full-blown MM. Eventually, these markers can aid clinicians in determining the prognosis, treatment, and survival in patients with MGUS/MM.

From a research perspective, the development of better biologic and molecular markers will improve our understanding of the molecular pathways involved in disease progression in patients with MGUS/MM. This will help us not just in understanding the biology of plasma cell dyscrasia but also in identifying newer therapeutic targets and novel treatments for the treatment of these diseases. For example, it is known that all cancer cells carry mutations. Some of these mutations confer a selective clonal growth advantage to the cells and thereby act as ‘drivers’ of myelomagenesis. With the completion of human genome sequencing it is now possible to identify all somatic mutations in individual cancer genomes [62–64]. Future studies looking at the genome sequences in a patient with MGUS who subsequently develops MM may reveal important information about the pathogenetic pathways involved in disease progression and also identify novel markers of progression.

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Figure 1.

The Mayo Clinic risk stratification model for progression of MGUS and SMM. (A) Risk factors for progression of MGUS are (1) elevated serum M-spike (>1.5 g/dL), (2) non-IgG MGUS, and (3) abnormal free light chain ratio (<0.26 or >1.65). The corresponding absolute risk of progression at 20 years of follow-up for patients with 3, 2, 1, and 0 of these risk factors is 58%, 37%, 21%, and 5%, respectively. Reproduced from *Blood* by S. Vincent Rajkumar and others. Copyright 2005 by American Society of Hematology (ASH).

Reproduced with permission of American Society of Hematology (ASH) in *Blood* via Copyright Clearance Center. (B) Risk factors for progression of SMM are (1) elevated serum M-spike (>3 g/dL), (2) bone marrow plasma cells $>10\%$, and (3) abnormal free light chain ratio (<0.125 or >8). The cumulative risk of progression at 10 years of follow-up with 1, 2, and 3 of the risk factors is 50%, 65%, and 84%, respectively. Reproduced from *Blood* by Angela Dispenzieri and others. Copyright 2008 by American Society of Hematology (ASH). Reproduced with permission of American Society of Hematology (ASH) in *Blood* via Copyright Clearance Center.

Figure 2.

Flow cytometric immunophenotyping of plasma cells from the bone marrow aspirate of a patient with SMM. CD138 (+) CD38 bright (+) plasma cells are identified (gated in red) with intermediate side scatter (SSC) properties (A, B). Both abnormal and normal plasma cells are present. Abnormal plasma cells (highlighted by gray ovals) exhibit abnormally diminished CD19, CD45, and CD27 expression, with aberrant expression of CD56 and CD117 (C–F), and monoclonal cytoplasmic kappa light chain expression (G, H).

Figure 3.

The Spanish PETHEMA study group risk stratification model for progression of MGUS and SMM. (A) The model for MGUS on the basis of the percentage of immunophenotypically aberrant PCs within the BMPC compartment (<95% aberrant PCs, score of 0; 95%, score of 1) and DNA index: aneuploid (score of 1) or diploid (score of 0). The risk of progression at 5 years with scores of 0, 1, and 2 is 2%, 10%, and 46%, respectively. (B) The model for SMM was built on the basis of the percentage of immunophenotypically aberrant PCs within the BMPC compartment (<95% aberrant PCs, score of 0; 95%, score of 1) and the presence (score of 1) or absence (score of 0) of immunoparesis. For patients with SMM, the risk of progression at 5 years with scores of 0, 1, and 2 is 4%, 46%, and 72%, respectively. Reproduced from Perez-Persona et al., *Blood* 2007;110:2586–2592 [18].

ISCN: 46,XY,t(1;8)(p13;q24.1),t(6;14)(p21.1;q32)

Figure 4.

G-banded karyotype from a patient with smoldering myeloma showing two balanced translocations. One involves the entire short arm of chromosome 1 and the distal long arm of chromosome 8, with a break in band 8q24.1, presumably in c-myc. The second involves the short arm of chromosome 6 and the distal long arm of chromosome 14, with a break in band 14q32, presumably in IgH. (Image kindly contributed by Diane C. Arthur, MD, Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland.)

Figure 5.

Interphase FISH analyses of MM. (A) Fusion signals for t(4;14)(p16;q32). IgH probes in green and MMSET/FGFR3 probes in red. Two fusion signals indicated by yellow arrowheads likely identify der(4) and der(14) but could represent two copies of der(4). (B) Extra copies of three chromosomes in a hyperdiploid tumor. Three copies of chromosome 5 (green) and chromosome 9 (circled) and four copies of chromosome 15 (red). (C) Two copies of chromosome 17 (green) and deletion of one copy of P53 (red). (D) Loss of one copy of chromosome 13/13q. In all four panels, the cytoplasm is blue due to immunostaining of Ig-kappa or Iglambda expressed by the tumor plasma cells. Reproduced with permission from Swerdlow et al., *World Health Organization classification of tumors of haematopoietic and lymphoid tissues*. Lyon: IARC; 2008.

Figure 6.

Stages in myelomagenesis: molecular and genetic changes in the evolution from MGUS to MM. Genetic changes in plasma cells tumors can be categorized into four groups based on the timing of the acquisition. Early changes occur in pre-germinal B cells prior to the occurrence of MGUS, and these include primary IgH translocations, cyclin D dysregulation, hyperdiploidy, and deletion 13 in a subset of patients. Intermediate changes might occur during the transition from MGUS to MM and include K-RAS and FGFR3 mutations, MYC up-regulation, and deletion 13 in a subset of patients. MYC rearrangements involving immunoglobulin loci, and also p53, p18, and Rb inactivation are late events. Secondary translocations and NFκB activating mutations do not seem to occur at a specific stage of the disease. Del, deletion; 1°, primary; 2°, secondary; IgH, immunoglobulin H.

Figure 7.

FDG-PET/CT imaging in a 65-year-old gentleman with SMM. Patient had no lesions on skeletal survey. Left panel shows a CT scan of the patient's right lower extremity with a lytic lesion in the right distal femur. Centre panel shows PET image which demonstrates increased 18F-FDG uptake at the corresponding region. 18F-FDG-PET also shows two additional areas of increased uptake likely representing early lytic changes. Right panel is a composite image derived from the CT scan and FDG PET images.

Table I.

Criteria for diagnosis of MGUS, SMM, and MM.

Characteristic	MGUS	SMM	MM
Serum M-protein	<3 g/dL	>3 g/dL	Any level
Bone marrow PCs	<10%	>10%	Any level
Clinical presentation	Asymptomatic with no end organ damage except for amyloidosis in some patients	Asymptomatic with no end organ damage except for amyloidosis in some patients	End organ damage: 1. Lytic lesions 2. Anemia 3. Hypercalcemia 4. Renal failure

MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; SMM, smoldering MM; PCs, plasma cells.

Table II.

Comparison of standard imaging modalities in MM.

Modality	Radiological detection of				Advantages	Disadvantages
	Osteolytic lesions	Bone marrow focal lesions	Extramedullary disease			
Metastatic bone survey	Yes	No	No	Widely available Historical standard		Poor sensitivity Poor interobserver reliability Poor tolerance by patients (multiple positions, long duration)
Computed tomography	Yes	No	No	Very sensitive for osteolytic lesions No repositioning Rapid test Better assessment for pathologic fracture risk		Relatively high radiation dose
MRI	No	Yes	No	Very sensitive for bone marrow focal lesions No radiation exposure		High cost Not widely available Long duration of exam Limited fields of view
PET/CT	Yes	Yes	Yes	Widely available		High cost Limited sensitivity in spine

MM, multiple myeloma; MRI, magnetic resonance imaging; PET/CT, positron emission tomography/computed tomography.