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Biological frontiers in multiple myeloma: From biomarker identification to clinical practice

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Abstract

Since the mid-1990s, the multiple myeloma (MM) treatment landscape has evolved considerably, which has led to improved patient outcomes and prolonged survival. In addition to discovering new, targeted agents or treatment regimens, the identification and validation of biomarkers has the potential to further improve patient outcomes. The International Staging System (ISS) relies on a number of biochemical parameters to stratify patients into risk categories. Other biologically relevant markers that are indicative of inherited genetic variation (e.g., single nucleotide polymorphisms) or tumor acquired genetic events (e.g., chromosomal translocations or mutations) have been studied for their prognostic potential. In patients with high-risk (HR) cytogenetics, plasma cells (PCs) undergo genetic shifts over time, which may partially explain why HR patients relapse and are so difficult to treat. Although novel agents have improved treatment outcomes, identification of markers that will enable clinicians to determine which treatment is most appropriate for HR patients following initial diagnosis represents an exciting frontier in the clinical management of MM. Biomarkers based on quantitating PCs or factors that are secreted from them (e.g., serum free light chain) may also help to risk-stratify patients with asymptomatic MM. Eventually, identification of novel biomarkers may lead to the creation of personalized treatment regimens that are optimized to target clonal PCs that express a specific oncogenomic profile. Although the future is exciting, validation will be necessary before these biologic and molecular beacons can inform decision-making processes in a routine clinical setting.

Keywords

Biomarker; cytogenetics; MGUS; multiple myeloma; smoldering multiple myeloma

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Introduction

Multiple myeloma (MM) is a hematologic disease that is characterized by the proliferation of abnormal bone marrow plasma cells (BMPCs) and immunoglobulin (Ig) or light chain overproduction, with evidence of end-organ damage. Prior to 1997, the median survival of patients with newly diagnosed MM was approximately 2.5 years (1). From 1997 to 2006, the use of high-dose anti-MM therapy, stem cell transplantation, and novel agents increased median overall survival (OS) to nearly 4 years, a 50% improvement (1). In some patients who are currently receiving such agents, improved relapse-free survival has increased to > 10 years (2), raising the potential for long term disease control and care.

Although the MM treatment landscape has improved since the mid-1990s, the intraclonal heterogeneity of malignant plasma cells (PCs), the interaction of PCs with host factors, and the bone marrow (BM) microenvironment contribute to disease progression and the molecular evolution of the disease according to “Darwinian” principles (3, 4). Taken together, these factors contribute to the generation of treatment-resistant PCs, which ultimately leads to relapsed and refractory myeloma (RRMM), the disease stage that is frequently used for drug development. However, as the nature of this disease stage continues to change as a consequence of patients receiving multiple lines of therapy, it is essential that we develop novel approaches to drug development.

If patients are to overcome treatment resistance, one approach that may improve clinical outcomes is to select treatments that are well matched to a patient's molecularly defined disease subtype (5). Another promising approach is to identify new biomarkers and tools that can identify onset or worsening of disease. According to the National Cancer Institute (NCI), a biomarker is a molecule that is found in blood and other body fluids or tissues that can serve as an indicator for a normal or abnormal process, condition, or disease (6). Herein, we will review biomarkers that have been used in MM that meet the NCI's definition of a biomarker (e.g., monoclonal [M] protein); we will also discuss molecular markers and tools that have the potential to dramatically alter how clinicians diagnose, stage, and treat patients with MM or asymptomatic “early” MM.

The emergence of clinical biomarkers in multiple myeloma

In the 1960s, MM researchers began to identify biomarkers that were independent predictors of survival, including hemoglobin, serum calcium, serum creatinine, and bone lesion severity (7, 8). In 1975, Durie and Salmon introduced a staging system that used monoclonal (M) protein, hemoglobin, calcium, and the number of bone lesions to predict MM cell tumor burden (9). In the 1980s, serum β_2-microglobulin (β_2M) was found to be a simple, yet reliable prognostic marker for disease staging; serum β_2M also enabled clinicians to predict a patient's likelihood of survival (10, 11). Subsequently, albumin (12), C-reactive protein (13), and BMPC proliferation indices (14, 15) were found to be reliable prognostic factors, but didn't come into general use. In 2005, an international consortium of researchers used serum β_2M and serum albumin to create the International Staging System (ISS), which enabled clinicians to stage patients and predict their long-term prognosis (16). This has been further refined by combining FISH data with ISS staging (17, 18).

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Other types of biomarkers (e.g., serum free light chain [sFLC] ratio and cytogenetic markers) are also beginning to provide prognostic information in MM and patients with asymptomatic disease (18-20). There have been significant developments in the diagnostic platforms that are available to assess the molecular features of MM that provide extensive novel information as well as new diagnostic tests. The current emphasis is on using multi-parallel genome wide approaches, which yield huge amounts of data and offer the advantage of being able to multiplex genomes from many patients in a single run, which can reduce the cost per test. However, these technologies also raise significant issues with regard to the generation of complex datasets and how they can be reliably analyzed. These technologies will need to be robust and applicable if they are to be widely used in a routine setting. It is also appropriate to ask whether we should take a genome wide approach to biomarker development or whether we should focus on a smaller number of “MM relevant” markers that can be analyzed using simpler and potentially more robust techniques.

Cytogenetic biomarkers in multiple myeloma

Changes at the DNA level determine how a cancer cell behaves. If we can understand these molecular events, we should be able to predict the behavior of cancer. Chromosomal translocations are primary genetic events that occur early in the disease and are seen in roughly 50% of MM patients. As a consequence of the translocation events, there is overexpression of partner genes, leading to abnormal clonal PC behavior and differing clinical outcomes (17, 21-27). For example, respective four-year progression-free survival (PFS) and OS rates for patients with the t(4;14) have been estimated to be 11% and 35% versus PFS and OS rates of 32% and 60% for patients without the t(4;14) (18).

Other chromosomal translocations have been investigated for their prognostic potential. The t(14;16) is present in < 5% of patients with newly diagnosed MM and defines a group of patients who often have poor survival (17, 18, 28, 29). Although the t(14;16) is relatively rare, the *MAF* gene is overexpressed in up to 30% of MM patients, defining a greater number of patients with a unifying biological feature (4). The t(11;14) is observed in approximately 20% of patients and is associated with a favorable prognosis, higher rates of CD20 expression, lymphoplasmacytic morphology, hyposecretory disease, λ-light chain usage, and nuclear cyclin D1 (*CCND1*) expression and dysregulation (18, 21, 23, 30-32).

Hyperdiploidy of the odd-numbered chromosomes is the other major primary genetic event in MM that defines the other 50% of MM cases (Table 1) (4, 33-36). Although the exact cause of hyperdiploidy is unknown, it seems to define a group of patients with a more favorable prognosis (37). In addition to chromosomal translocations and hyperdiploidy, inherited genetic variation is an important factor in the etiology of MM. Molecular epidemiology techniques have shown that MM is associated with SNPs at the chromosomal regions 2p, 3p, 6p, 7p, 17p and 11q (4, 34, 35). However, at this point no prognostic value has been seen in association with these variables. Inherited variation can also affect response to chemotherapy and the side effect profile of a drug. To date, very few studies have been carried out to test this hypothesis, and they have not as yet defined relevant markers that can be used in this way in MM (38).

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Secondary genetic events, which are important in disease progression, also affect various molecular pathways and contribute to the biological heterogeneity of PCs (Table 2). In this context, prognostically important copy number abnormalities (including gains or deletions of whole chromosomes or interstitial copy number gains) have been identified using SNP mapping arrays (4, 18, 33, 39). Clinically relevant regions that have been identified using this approach include 1q+, 1p32-, 1p11- and 17p-. The International Myeloma Working Group (IMWG) has recommended assessing these specific cytogenetic lesions at disease presentation, as they may have prognostic value that is clinically relevant. The study of copy number change has also improved our understanding of the biology of MM, leading to the identification of 16q, a WW domain-containing oxidoreductase gene (*WWOX*), *CYLD* (a negative regulator of the NF-κB pathway), 11q- (*BIRC2* and *BIRC3*), and 14q (*TRAF3*) as being relevant recurrent events (40, 41). These findings reinforce the importance of using therapeutic approaches to target the NF-κB pathway.

The use of massively parallel sequencing has identified hundreds of mutations in MM (42, 43); however, no consistently mutated gene has been identified that characterizes all occurrences of the disease. Nevertheless, these studies are in their infancy; the prognostic relevance of the multitude of exonic mutations that have been identified has yet to be determined. While potentially useful, one challenge with interpreting sequencing data is defining which mutations are so called “driver variants” that are important to disease pathogenesis rather than being “passenger variants” which are present by virtue of being carried forward by their association with other pathogenetically important genes. Driver mutations identified by this approach may be important therapeutic targets that could be used to design targeted treatment approaches.

Biomarker development

If we are to use tumor acquired genetic variants as biomarkers, it is important to consider their specificity and sensitivity for identifying the clinical outcome of interest (e.g., PFS, OS, or side effects). There is now considerable data on the use of “FISH detected” cytogenetic markers to predict clinical outcomes. What is clear from these data is that no single marker offers either good sensitivity or specificity for the prediction of either PFS or OS. If we are to alter treatment following use of a FISH-based cytogenetic approach, we will need to use a comprehensive panel of markers to define risk based on both the number and nature of the adverse markers detected. iFISH combined with the ISS is useful, but general applicability and issues with sensitivity and specificity remain. Some of these challenges can be overcome by counting the number of abnormal lesions that are present and using the number present as a way of defining risk. This approach combined with the use of a comprehensive panel of markers, including the adverse translocations t(4;14) and *MAF* together with the adverse copy number variables 1q+, 1p- and 17p- can provide very complete clinical data.

Global gene expression profiling (GEP) and prognostic signatures provide an alternative approach to FISH (44). Although GEP and prognostic signatures offer greater specificity in patients who are identified as having a poor prognosis, they lack biological relevance and remain difficult to apply in a routine clinical setting. However, GEP does provide a massive amount of data and can define the groups identified in the TC classification as well as being

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able to detect prognostic signatures. At this time, GEP cannot usefully identify the adverse groups defined by 1q+ and 17p-; these 2 subgroups with a poor prognosis still need to be detected by iFISH. The eventual clinical application and regular use of such biomarkers depends upon their clinical applicability together with the availability of effective treatments for use in specific patient subgroups.

The technology used to detect a cytogenetic biomarker is also of critical importance. Historically, metaphase cytogenetic analysis was used, but this only gave results in 18% of patients and was therefore not widely adopted. Although FISH on CD138-selected PCs is applicable and gives results in nearly 100% of patients if adequate numbers of tumor cells are obtained, this method is slow and expensive and up to now has lacked sensitivity and specificity. For predicting clinical outcomes, new genome wide technologies are useful, yet their design and application are still in their infancy. Alternative strategies that may be more applicable for detecting copy number changes include multiplex ligation-dependent probe amplification (MLPA), which can detect the clinically relevant MM-associated copy number variants at a fraction of the cost of FISH. MLPA can also deliver results in a more timely fashion while GEP can define risk status based on poor prognosis signatures. An alternative expression-based approach is to design real-time quantitative polymerase chain reaction (RQ-PCR) assays that can identify TC classification groups and prognostically relevant genes (45). If these assays are combined with MLPA copy number variant assays, this approach can provide all of the relevant prognostic variants in a set of tests that are readily applicable.

MicroRNAs (miRNAs), which are more stable than conventional RNA, can be detected in serum as well as in malignant PCs (46). In this context, most work on MM biomarkers has focused on BMPCs; little work has been done on circulating PCs or serum DNA or RNA. These areas offer considerable opportunities for future development (3, 47). Additionally, important epigenetic events—including global DNA hypomethylation and gene-specific DNA hypermethylation—are beginning to provide insights into the etiology of disease progression and could lead to the identification of clinically useful biomarkers (4, 48, 49).

The development of clinically relevant biomarkers is now being shaped by the regulatory framework for such testing. The Clinical Laboratory Improvement Amendments (CLIA) approach suggests that the development of biomarkers needs to be done in defined laboratories that work according to relevant standard operating procedures. This implies that biomarkers all need to be developed in the context of clinical trials where data are collected according to the principles of good clinical practice (GCP) and the biomarkers are determined in a laboratory working to CLIA standards. In the absence of such an approach, biomarkers cannot be adopted into clinical practice.

Risk-stratified treatment of MM

The identification of patient-specific cytogenetic abnormalities and patients' gene mutation status may be particularly useful in helping to direct treatment in patients with MM who possess a specific oncogenomic profile (5). It is now possible to define LR and HR disease

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subsets using GEP and FISH (50). GEP is useful in defining the molecular subtypes of MM as well as being able to define signatures of HR disease (50-53).

Based on data analyses from platforms that are derived from completed studies, we are making progress toward improving the outcomes of patients with standard-risk (SR) disease. Nevertheless, we have made little progress toward treating or managing patients with HR disease. These observations argue in favor of moving toward the design of clinical trials that specifically develop treatments for HR disease. However, before we can design such studies, the biomarkers for robustly identifying these risk groups need to be validated in order for us to screen an adequate number of patients in the molecular subgroup of interest. This clinical trial approach will require us to fully embrace modern genomic technologies that are applicable in routine diagnostic facilities.

"Clinical response" as a biomarker

Changes in BMPCs, paraprotein, and light chains form the basis of assessing patient response to therapy and could be used as an alternative treatment end point. The standard approach to using these data is with the criteria laid out in the IMWG response criteria (54). The achievement of complete response (CR) is an important end point when assessing new therapies (Table 3); it seems likely that the deeper the level of response, the better the clinical outcome will be. Indeed, the achievement of a CR with no detectable disease is the essential prerequisite for a cure. Thus, the definition of stringent CR and the criteria defining this state are of some importance. The IMWG defines stringent complete response (sCR) similarly to CR, but MM patients must also have a normal FLC ratio with no evidence of clonal BMPCs (assessed using immunohistochemistry or 2- to 4- color flow cytometry) (54). There is a consensus that is beginning to emerge on the most appropriate panel of antibodies and the strategy for detecting malignant PCs, with considerable enthusiasm being directed toward their use as a definitive clinical endpoint. Flow cytometry has considerable merit for monitoring residual disease because it offers sensitivity and does not require one to design patient/clono-specific approaches; it can also be performed in most hemato-oncology diagnostic laboratories.

A recent survey that included 30 major medical institutions in the US found that the application of multicolor flow cytometry for minimal residual disease (MRD) in MM varies greatly (55). Indeed, the definition of abnormal PCs differed substantially between institutions, with some relying on CD19 and CD45 negativity with or without CD56 positivity to determine the extent of MRD despite previous studies showing that normal PC subpopulations can be negative for CD19 and CD45 or CD56 positive (56, 57). More specific antigens such as CD27, CD81 and CD117 were used by less than half of the institutions. Even more importantly, there is considerable variation in the number of BMPCs analyzed (i.e., events) and the number of abnormal PCs that are needed to determine the presence of MRD, which affects the maximum possible sensitivity of the assay. In this survey, it was found that the maximum detection sensitivity ranged from 0.0005% to 0.02%, a 100-fold difference in sensitivity. Such data copies the establishment of standardized approaches and external quality control programs. It is important to contrast this with multiparameter flow cytometry with the application of clono-specific PCR for the same

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purpose. In most settings, the clono-specific PCR offers greater sensitivity, but this technique has been hampered by the necessity to develop clono-specific probes, which has made its general application cumbersome and expensive. This situation is now changing with the advent of massively parallel sequencing approaches, which utilize probes that can be used to tile entire IgH regions (43). This approach is sensitive, high throughput, and universally applicable.

Predictive biomarkers

In recent years, it has become evident that patients with the t(4;14) have more favorable outcomes when they are treated with proteasome inhibitors. A group at the Mayo Clinic recently discussed the concept of "risk-adapted therapy" (5) whereby patients with high-risk disease (i.e., patients with the t(14;16), t(14;20), and/or del17p) may be better candidates for triplet combination therapy (e.g., bortezomib-lenalidomide-dexamethasone) compared to patients with intermediate- or standard-risk disease. Although triplet combinations may have a less favorable side effect profile relative to "single-agent" therapies, triplet combination regimens are more appropriate for patients with high-risk disease because median overall survival in that cohort is only 3 years compared to 4-5 years and 8-10 years for patients with intermediate-risk and standard-risk disease, respectively. In the absence of using cytogenetic markers to risk-stratify transplant-eligible and transplant-ineligible patients, it would be difficult to determine which treatment regimen is most appropriate for patients with multiple myeloma.

Nevertheless, the t(4;14) cannot be considered to be a true prognostic biomarker because many patients who lack this translocation also respond and have good clinical outcomes following proteasome inhibitor therapy. Moving forward, information from genome wide sequencing studies is leading to the identification of frequent pathogenetically important mutations in MM, including those involved in the ERK signal transduction pathway. These biomarkers include *NRAS*, *KRAS*, and *BRAF* (4). In recent years, BRAF-V600E has emerged as one of the most promising ERK mutations that can be targeted, validating the concept of targeted treatment. For example, one MM patient with extramedullary disease and the BRAF-V600E mutation responded well to low doses of vemurafenib, a mutation-specific *BRAF* inhibitor (58), making it a true predictive biomarker in the absence of which a response was not observed.

One potential issue with use of targeted therapy is the presence of intraclonal heterogeneity (ICH) relating to subclonal variability. ICH is the essential substrate for clonal evolution according to the principles of Darwinian evolution (4). In recent years, three patterns of clonal evolution have been identified: relapse can be genetically stable; it can linearly evolve with several new genetic variants at relapse; or it can come from a clone that wasn't present at the time of diagnosis (59). Paired sample analysis of 28 MM patients revealed that patients with SR cytogenetics typically have PC clones that are genetically stable (59). In contrast, patients with HR cytogenetics have PC clones that undergo many more genetic modifications over time.

The presence of ICH has important implications for the development of biomarkers. Although it was once acceptable to determine whether a molecular target was present, it is

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now essential to obtain an idea of the size of the sub-clone carrying the target. The size of the clone has important therapeutic implications for targeted treatment because completely eradicating a clone present only 5% of the time would have little importance clinically, whereas in a clone present 90% of the time, there would be a significant clinical response.

The presence of ICH also has an effect on the assessment and treatment of relapse. Traditionally, clinical data were used to define prognosis, response to therapy, duration of prior response, rate of relapse, the presence of extramedullary or blastic disease, performance status, and the toxicity of prior treatment. In the era of molecular medicine, cytogenetics, focal lesions, tidal clone status (i.e., same clone, new mutant, or prior clone), GEP signature, and methylation status are beginning to provide clinicians with more information about the clinical behavior of the disease at relapse.

Biomarkers in MGUS and smoldering multiple myeloma

In the 1970s, Kyle and Greipp began to use the phrases “monoclonal gammopathy of undetermined significance (MGUS)” and “smoldering multiple myeloma (SMM)” to describe premalignant PC disorders that are not associated with end organ damage or treatment (60, 61). Currently, asymptomatic MM is an active area of biomarker research where the challenge is to predict those patients who are at HR of disease progression for whom treatment intervention is essential to prevent the emergence of significant end organ damage. The use of many of the molecular markers described above is difficult because the molecular features of MM that require treatment are often present in patients with asymptomatic disease (62, 63), meaning that alternative approaches to predict risk status are required.

In 2003, the IMWG used laboratory and clinical markers (e.g., serum and urine M protein, clonal BMPCs, and sFLC) to define patients with asymptomatic MM where treatment was not indicated (64). The sFLC ratio is one of the most promising biomarkers in asymptomatic MM; it has been used as a prognostic indicator in patients with MGUS (65), SMM (66-68), and newly diagnosed MM (69), but it is not without controversy. The Spanish Programa Español de Tratamientos en Hematología (PETHEMA) and Mayo Clinic groups both used sFLC ratios, circulating PCs, and PC proliferation rates to create two models that classify asymptomatic patients as being at low-, intermediate-, or HR of disease progression (66, 70). A group at the NCI used the Spanish PETHEMA Model (70) and the Mayo Clinic Model (66) to categorize 77 patients with SMM as being at low-risk (LR), intermediate-risk, or HR of developing active MM (71). In the NCI's study, concordance between the PETHEMA and Mayo Clinic Models was low (28.6%); there was also significant discordance between how each model classified patients' risk status. Thus, although characterization of PC immunophenotype and measuring sFLC ratios both have prognostic potential, they lack specificity and do not have enough positive predictive value to be useful in determining when treatment should be initiated in patients with asymptomatic disease.

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Imaging tools for "early" myeloma

Given that nearly all patients with symptomatic MM develop osteolytic lesions, clinicians use BM aspirates and trephine samples to monitor bone morphology throughout the course of disease progression. Although these tools are useful, there can be variability in the distribution of BMPCs throughout the body, increasing the likelihood of heterogeneous sample recovery and sampling error, particularly in patients with asymptomatic disease. Given the importance of understanding the underlying biology of bone in MM, clinicians use x-rays to complete skeletal surveys, a technique that is currently the gold standard for evaluating severity of bone disease. Despite the utility of skeletal surveys, magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET)/CT scanning are becoming increasingly important tools for assessment of disease severity. MRI is a useful noninvasive technique for imaging the spine, soft tissue, and infiltration of BMPCs and is particularly useful for patients with SMM because it can detect diffuse lesions, the presence of which is an adverse prognostic factor for PFS (72). In addition, detection of seven or more focal lesions with MRI is considered to be an adverse prognostic factor in patients with MM (73). If MRI is unavailable, CT may be helpful for assessing the extent of damage to the spine or soft tissues. Nevertheless, its use should be limited because CT can expose patients to nearly three times more radiation than conventional MRI. Lastly, fusion of CT and fluorodeoxyglucose (FDG) PET images can be very useful for evaluation of patients with symptomatic disease and has the potential to be useful in patients with MGUS or SMM (74). In the future, functional imaging techniques that are used in combination with biomarker data are likely to contribute to the management of patients with "early" MM and the development of novel MRD assays.

Target Modulation

Cytotoxic chemotherapy, immunomodulatory agents, and proteasome inhibitors have been highly effective and universally applicable in MM. Going forward, the next generation of targeted treatments will depend not only on the presence of a target, but also on their ability to modulate their target. This will be especially important during the early phases of clinical development of targeted treatments, where target modulation should be considered to be an important endpoint. In MM, it is, perhaps, not optimal to use circulating lymphocytes as targets to assess target modulation because of differences in cellular biology and tissue penetration. Consequently, access to and characterization of BMPCs is important and can be achieved using immunohistochemistry on BM trephines or flow cytometry on aspirate samples. Examples include the assessment of antiapoptotic proteins to predict a patient's response to agents that target these pathways, the demonstration of down-regulation of pERK, or evidence of changes in histone methylation states following evaluation of *MMSET* inhibitors.

Summary and Future Directions

Over the past 40 years, biomarkers such as M-protein and serum β_2M have contributed innumerable insights into our present understanding of MM. Today, many cytogenetic markers are beginning to provide information about the severity of disease; these markers

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are also beginning to inform clinicians regarding which anti-MM treatment regimen is most appropriate for a particular patient. It is clear that rapid technological advances are changing the way biomarkers are perceived as well as their clinical relevance (Figure 1). In the near future, diagnostic tests based on massively parallel sequencing approaches will enable detection of recurrent molecular abnormalities as well as actionable mutations that are rarely observed but which could utilize treatments that are used in other disease settings. Massively parallel sequencing approaches will also enable clinicians to monitor disease longitudinally, which may provide opportunities for early intervention before clonal PC expansion and disease complexity render existing treatments ineffective. Finally, combining functional imaging techniques with assays that utilize biomarkers has the potential to change clinical practice in patients with "early" myeloma and patients with symptomatic disease.

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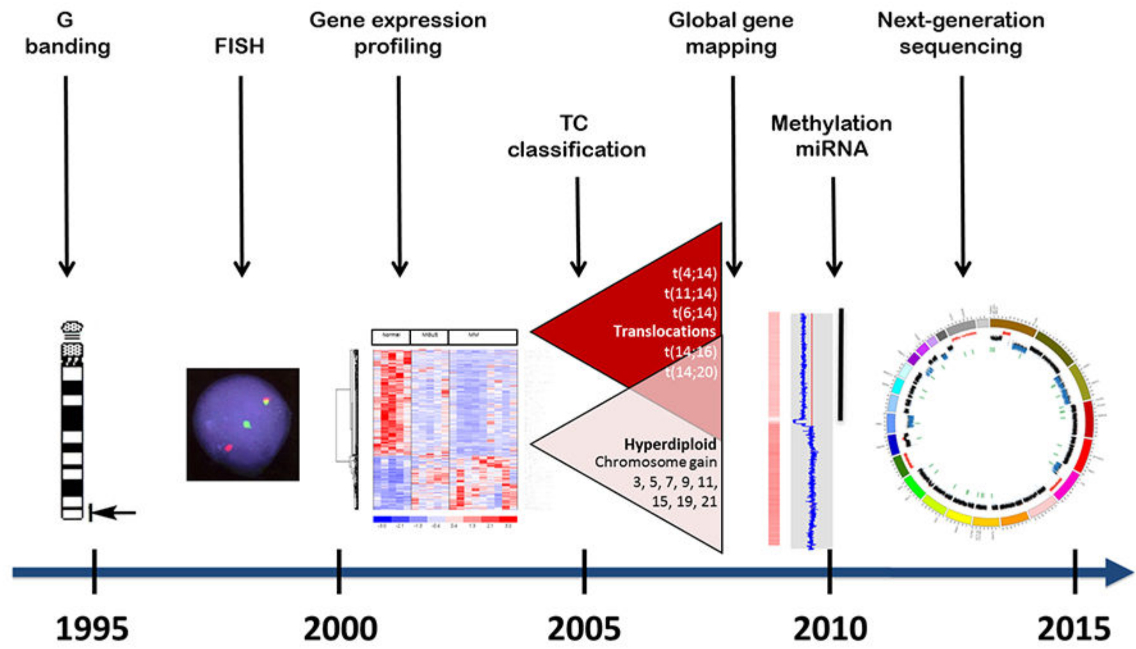

Figure 1. Technological advances in detecting biomarkers in multiple myeloma

FISH, fluorescence in situ hybridization; miRNA, micro RNA; TC, transporter classification.

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Table 1

Inherited Variation and Primary Genetic Events in Multiple Myeloma

Inherited Variation			
Single-nucleotide polymorphisms (SNPs)			
Chromosome	**Gene(s)**	**Primary tool(s)**	**Reference(s)**
2p23.3	*DTNB* and *DNMT3A*	GWAS	(34, 35)
3p22.1	*ULK4* and *TRAK1*		
7p15.3	*DNAH11* and *CDCA7L*		
Primary Genetic Events			
IGH@ translocations			
Translocation	**Gene(s)**	**Primary tool(s)**	**Reference(s)**
t(4;14) at 4p16.3	*FGFR3* and *MMSET*	Karyotype analysis (cytogenetics), FISH, GEP, GWAS, RT-PCR or sequencing	(25)
t(6;14) at 6p21	*CCND3*		(21, 23)
t(11;14)(p21;q11)	*CCND1*		(21, 23, 27)
t(14;16)(q32;q23)	*MAF*		(24)
t(14;20)(q32;q12)	*MAFB*		(17, 26)
Hyperdiploidy (chromosomal trisomy)			
Chromosome(s)	**Gene(s)**	**Primary tool(s)**	**Reference(s)**
3, 5, 7, 9, 11, 15, 19, and 21		GEP and FISH	(41)

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Table 2
Secondary Genetic Events in Multiple Myeloma

Secondary Genetic Events			
Secondary translocations			
Chromosome(s)	**Gene(s)**	**Tool(s)**	**Reference(s)**
t(8;14)	*MYC*	FISH	(75)
Gains			
Chromosome	**Gene(s)**	**Tool(s)**	**Reference(s)**
1q	*CKS1B* and *ANP32E*	GEP	(41)
12p	*LTBR*		
17q		Comparative genomic hybridization (CGH)	(76)
Deletions			
Chromosome	**Gene(s)**	**Tool(s)**	**Reference(s)**
1p	*CDKN2C*, *FAF1* and *FAM46C*	GEP, GM and FISH	(41, 77)
6q			(41)
8p	*TRAIL-R1* and *TRAIL-R2*		(41, 78)
11q	*BIRC2* and *BIRC3*		(79, 80)
13	*RB1* and *DIS3*		(41)
14q	*TRAF3*		(79, 80)
16q	*CYLD* and *WWOX*		(40, 41)
17p	*TP53*		(41, 81)
Epigenetic events			
Global hypomethylation (MGUS to MM) and gene-specific hypermethylation (MM to PC leukemia)		Genome-wide methylation arrays	(49)

Table 3

Partial List of Response Criteria from The European Group for Blood and Marrow Transplantation (EBMT) and the International Myeloma Working Group (IMWG)

Parameter	EBMT Response Criteria (82)	IMWG Uniform Response Criteria[a] (54)
sCR	• Not defined	Patient meets all of the following criteria: • CR as defined • Normal free light chain ratio • Absence of clonal PCs by immunohistochemistry or 2- to 4-color flow cytometry
CR	Presence of all of the following: • Absence of M-protein in serum and urine, measured by immunofixation, maintained for 6 weeks • <5% bone marrow plasma cells – If absence of M-protein is sustained for 6 weeks, it is not necessary to repeat the bone marrow exam except in patients with nonsecretory MM • No increase in size or number of lytic bone lesions • Disappearance of soft tissue plasmacytomas	Patient meets all of the following criteria: • Negative immunofixation of serum and urine • Disappearance of any soft tissue plasmacytomas • 5% PCs in bone marrow
Immunophenotypic CR	• Not defined	Patient meets all of the following criteria: • sCR as defined • Absence of phenotypically aberrant PCs (clonal) in bone marrow with a minimum of 1 million total BM cells analyzed by multiparametric flow cytometry (with >4 colors)
Molecular CR	• Not defined	Patient meets all of the following criteria: • CR as defined • Negative allele-specific oligonucleotide polymerase chain reaction (sensitivity 10^{-5})
VGPR	• Not defined	Patient meets one of the following criteria: • Serum and urine M-component detectable by immunofixation but not on electrophoresis • 90% reduction in serum M-component plus urine M-component <100 mg/24 h
PR	Patient meets all of the following criteria: • 50% reduction of serum M-protein for 6 weeks • Reduction in 24-hour urinary light chain excretion either by 90% or to <200 mg for 6 weeks	Patient meets all of the following criteria: • 50% reduction of serum M-protein and reduction in 24 h urinary M-protein by 90% or to <200 mg/24 h • If serum and urine M-protein can't be measured, a 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria

Parameter	EBMT Response Criteria (82)	IMWG Uniform Response Criteria[a] (54)
	• Nonsecretory MM only: 50% reduction of plasma cells in BM aspirate for 6 weeks • 50% reduction in the size of soft tissue plasmacytomas • No increase in size or number of lytic bone lesions	• If serum and urine M-protein can't be measured, and serum free light assay also can't be measured, 50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was 30% • In addition to the above listed criteria, if present at baseline, a 50% reduction in the size of soft tissue plasmacytomas is also required

[a]CR, sCR, VGPR, and PR require 2 consecutive assessments to be made at any time before beginning any new therapy. If radiographic studies were performed, these categories also require no known evidence of progressive or new bone lesions.

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