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REVIEW ARTICLE

Genetic architecture of Multiple Myeloma and its prognostic implications – An updated review

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Abstract

Multiple myeloma (MM), a clonal B-cell neoplasia, is an incurable and heterogeneous disease where survival ranges from a few months to more than 10 years. The clinical heterogeneity of MM arises from multiple genomic events that result in tumour development and progression. Recurring genomic abnormalities including cytogenetic abnormalities, gene mutations and abnormal gene expression profiles in myeloma cells have a strong prognostic power. With the advancement in technologies and the development of novel drugs, the prognostic factors and treatment paradigms of MM have been fast evolving over the past few years. Following the introduction of new high-throughput cytogenomic technologies such as array comparative genome hybridisation (aCGH) or single nucleotide polymorphism array (SNP array) and molecular techniques such as gene expression profiling (GEP) and massively parallel genomic sequencing, the prediction of survival in MM no longer solely depends on conventional cytogenetics and interphase fluorescence in situ hybridisation (iFISH) analysis findings. These new technologies enable screening for all possible chromosomal aberrations and other genomic alterations, identifying each aberration on a case-by-case basis and discovering new aberrations that are relevant in unraveling the tumor cells' complex biology. This in turn allows a better understanding of the disease complexity and heterogeneity. The objective of this review on the genetic architecture of MM is to discuss the latest developments on the cytogenetic/cytogenomic-based risk classification of MM that are currently in use and their prognostic implications.

Keywords: Multiple myeloma, cytogenetic abnormalities, iFISH, SNP array, gene expression profiling

INTRODUCTION

Multiple myeloma (MM) is a clinically heterogeneous malignant plasma cell (PC) disorder characterised by over-proliferation of monoclonal plasma cells in bone marrow leading to production of monoclonal protein and associated with organ dysfunction.[1-3] It accounts for 1-1.5%[1,4] of all cancers and approximately 13% of all haematological malignancies.[4,5] Based on Global Burden of Disease 2016 study[6], there were 138,509 incident cases of MM worldwide with an age-standardised incidence rate of 2.1 per 100,000 persons. The mortality of MM is relatively high. It accounts for 98,437 deaths globally with an age-standardised death rate of 1.5 per 100,000 persons. In 2016, MM caused 2.1 million disability-adjusted life year globally. These data clearly demonstrated a marked increase in the incidence of death as per a previous epidemiology study on MM[7] which had shown approximately 86,000 incident cases annually worldwide, accounting for about 0.8% of all new cancer cases. About 63,000 individuals die from MM annually, accounting for 0.9% of all cancer deaths.[7,8] The American Cancer Society[9] cancer statistics (2019), estimated 32,110 new MM cases and 12,960 MM deaths accounting for 1.8% of all new cancer cases, and 2.1% of cancer deaths in the United States. On the other hand, in Malaysia, based on Malaysia National Cancer (MNC) Registry Report 2007-2011, the total number of MM patients registered for the past 5 years was 744 (Male- 396, Female- 348)[10], accounting for 0.8% and 0.6% of all male and

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female cancers respectively. This incidence was in agreement with the epidemiology study by Becker[7] who reported that MM accounts for about 0.8% of all new cancer cases worldwide. Based on the MNC registry, on average there are 149 newly diagnosed MM (NDMM) cases per year in Malaysia.

Clinical Presentation

Transformation of normal plasma cells (PCs) to malignant PCs results through multiple steps.[11] The distinct stages include monoclonal gammopathy of undetermined significance (MGUS), smouldering MM (SMM), symptomatic MM and extramedullary disease or PC leukemia (PCL).[12] Majority of MM patients evolve from the asymptomatic premalignant MGUS stage which may last for over 10 years prior to the clinical diagnosis. The intermediate asymptomatic but more advanced premature stage SMM can be recognised clinically in a small percentage of patients. The typical presentation of MM includes a spectrum of symptoms that include hypercalcaemia, renal failure, anaemia and lytic bone lesions which are collectively known as CRAB features. These CRAB features are myeloma defining events (MDEs) where they signify the presence of end-organ damage. MDE consists of established CRAB features as well as three specific biomarkers [clonal bone marrow PCs ≥ 60 %, serum free light chain (FLC) ratio ≥ 100 (provided involved FLC level is ≥ 100 mg/L) and more than one focal lesion on magnetic resonance imaging (MRI)].[13] The clinical presentation of newly diagnosed MM (NDMM) is highly heterogeneous and can range from asymptomatic with only positive for biomarkers of active disease to the presence of one or more MDEs.[1] Asymptomatic patients are usually detected incidentally through abnormal blood results during routine medical check-up in which further investigations are warranted. The abnormal blood results can be presence of normocytic normochromic anaemia in full blood count, rouleaux formation in the full blood picture, elevated blood urea and creatinine that signify renal impairment in renal function test and reversal of albumin/globulin ratio in liver function test. On the other hand, the presentation of symptomatic patients can range from only bone symptoms such as bone pain, bone fracture following trivial trauma or neurological symptoms following vertebral fractures compressing on spinal cord to severe life-threatening conditions due to complications of renal failure or severe hypercalcaemia. The point to note is that different biological states of the evolution of monoclonal PCs, as well as genetic features, can be observed in NDMM patients. Some patients may have a slow progressive evolution from MGUS with a gradual development of mild anaemia, incipient evidence of bone disease and slowly emerging need for treatment. In contrast, others may be presented with frank clinical features of aggressive disease such as bone lesions and anaemia. Furthermore, in some individuals, the disease presentation may be associated with very high clonal aggressiveness, where extramedullary disease, multiple plasmacytomas and even PCL are the presenting features.[14]

Staging System

Durie-Salmon staging system (D-SSS) was first introduced in the year 1975 to stage MM.[15] The D-SSS calculates the myeloma stage by measuring five parameters namely levels of haemoglobin, levels of monoclonal immunoglobulin (Ig) (M protein) in the blood and urine, blood calcium levels, creatinine levels (which represent kidney function) and bone damage, if any. D-SSS uses these factors to classify myeloma into three stages. Stage I indicates the smallest amount of tumour cells, stage II indicates a moderate amount of tumour cells and stage III indicates the largest amount of tumour cells. Although some clinicians use this system, its value is becoming limited because of newer diagnostic methods.

A new staging system called the International Staging System (ISS) for MM has been designed in year 2005. The ISS is a simple risk stratification algorithm based on two parameters; high serum β2-microglobulin level that reflects high tumour mass and reduced renal function, and low serum albumin caused by inflammatory cytokines such as interleukin-6 secreted by the myeloma microenvironment. The ISS score identified three stage groups with different prognoses; the median overall survival (OS) was 62 months in the ISS stage I, 44 months in the ISS stage II, and 29 months in the ISS stage III groups ($P < .001$).[16] Multiple studies and clinical trials had confirmed ISS as a solid prognostic factor.[17-20]

In 2014, the International Myeloma Working Group (IMWG) proposed a prognostic model using a combination of ISS staging and cytogenetic abnormalities (CAs) to define high-risk disease.[21] However, this model was quickly replaced by another more powerful prognostic staging system. In 2015, IMWG introduced a new prognostic model that includes ISS, CAs and lactate dehydrogenase (LDH) data to effectively stratify patients with NDMM with respect to the relative risk to their survival.[22] This new algorithm is known as the revised ISS (R-ISS).

For decades, the diagnosis of MM required the presence of end-organ damage which is the CRAB features (hypercalcaemia, renal impairment, anaemia, and lytic bone lesions). The revised IMWG diagnostic criteria for MM had included three new validated biomarkers of active disease to diagnose the disease namely bone marrow clonal plasmacytosis ≥60%, serum involved/uninvolved FLC ratio ≥100, and >1 focal lesion on MRI in addition to the traditional MDEs.[23] These new biomarkers allow clinicians to accurately differentiate the subsets of patients with smouldering MM and biological malignancy. This allows early identification of patients who are at high-risk of progression to symptomatic disease (who are at imminent risk of developing CRAB features) to receive earlier efficient treatment before serious organ damage occurred, aiming to reduce significant morbidity and allow them to live longer.

Techniques used for cytogenetic and cytogenomic analysis

Conventional cytogenetic analysis (CCA) using GTG banded metaphases provides the advantage of whole genome analysis with one experiment and can identify abnormal karyotypes in about 30% of MM cases.[24] And that too more often in advanced stage disease which is more proliferative. However, due to the low number and low proliferative activity of malignant PCs in bone marrow specimens of MM patients in the early stage of the disease, detection of CAs by conventional cytogenetics is limited. Difficulty in interpreting some cryptic aberrations is also another limiting factor. With the addition of interleukin-4 to cultures, the detection rate of CA has increased by 50%.[25] These abnormal metaphases show both numerical and structural recurrent CAs many of which show complex karyotypes and which have predictive prognostic value.

The introduction of fluorescence in situ hybridisation (FISH) and microarray-based technologies has made it possible to overcome some of these drawbacks. These technologies allow the detection of target arrangements as well as chromosomal copy number changes. In FISH analysis involving interphase nuclei (iFISH), specific genomic alterations associated with MM can be studied. Similar to all other FISH-based methods, iFISH requires three steps: (1) obtaining cell suspensions (2) denaturation and hybridisation (3) microscopic visual digital analysis of hybridisation results. However, it is imperative to make sure that the iFISH test is performed on appropriate PCs which are relatively fewer in number than other cell types. To ensure this, flow cytometry is used to select positive CD138$^+$ PCs and these are sorted out using magnetic-assisted cell sorting (MACS). The sorted-out cell pellet is fixed and then dropped on slides for iFISH testing. Alternatively, these MACS-sorted CD138$^+$ cells can also be used to extract DNA to perform microarray or multiplex ligation-dependent probe amplification (MLPA) analyses. FISH is an important component of testing as some of the cryptic immunoglobulin heavy chain (IgH) locus translocations cannot be detected by standard chromosomal analysis. Especially cryptic chromosome abnormalities such as t(4;14), t(14;16) and del(13q14.3) will be easily missed in CCA and can only be detected by FISH or iFISH. Importantly, the IgH locus translocations in MM have diagnostic and prognostic significance. Another major advantage is that FISH can assess specific abnormalities not only in actively dividing cells but also in interphase nuclei.

In addition to the common IgH translocations, other genomic abnormalities namely deletion 17p, gain 1q and deletion 1p are also detected by FISH, microarray or MLPA. However, it should be noted that the IgH translocations/rearrangements which are present in 40% of myeloma cases, cannot be detected by microarray as most of these are balanced translocations. However, microarray can detect other multiple numerical and structural rearrangements that might be present in myeloma patients with IgH translocations. Some of such common examples are focal losses in 4p16.3 (which contain FGFR3 and WHSCI) and 4p15.2 which are present in association with t(4;14)(p16;q32) and also loss of 16q in association with t(14;16)(q32;q23).[26,27] FISH remains an important part of the cytogenetic workup of MM patients and hence FISH testing is often the first tier test performed in clinical laboratories.

To detect copy number alterations such as copy number gains and losses throughout the genome, single nucleotide polymorphism microarray (SNP array)-based genomic profiling is increasingly used. SNP array testing on enriched PC preparations has been reported to yield abnormalities in more than 98% of MM cases.[27-29] These studies reported over 20 alterations including bi-allelic losses, copy neutral loss of heterozygosity (CN-LOH) and complex gains and losses primarily in regions not covered by FISH.[27,30] Only microarrays can detect CN-LOH and complex genomic abnormalities such as chromothripsis and chromoanasynthesis, which cluster along with high-risk factors. Currently many of these abnormalities identified do not have known predictive indications.

Treatment

Although MM is incurable, still it is highly treatable. The survival ranges from a few weeks to more than 15 years or even a cure especially in transplant-eligible patients.[1] The outcomes among patients with MM have significantly improved following the development of new anti-myeloma agents. The emergence of thalidomide, bortezomib and lenalidomide has significantly improved overall survival (OS) in MM patients. There are several classes of novel agents which were currently used for the treatment of MM, such as proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), histone deacetylase inhibitors, monoclonal antibodies, alkylators, and steroids. These novel agents have not only led to notable changes in therapeutic strategy but also significant improvements in survival. The recent approval of carfilzomib, pomalidomide, panobinostat, ixazomib, elotuzumab and daratumumab by Food and Drug Administration (FDA) USA, for the treatment of relapsed MM promises to improve outcomes further. The increased usage of autologous stem cell transplantation (ASCT) and combinations of anti-myeloma drugs have led to prolonged remission for a majority of myeloma patients.

As there is vast variation in clinical presentation and the degree of severity of each presentation, the response to treatment and survival of NDMM are also heterogenous[18] with median OS ranging from two to more than ten years.[23] Studies over the years have revealed many factors that lead to disease heterogeneity.[14,31] The prognostic biomarkers that have been identified over the years may reflect host factors and hence fitness to receive therapy, tumor-related factors which reflect tumour biology, tumour stage and disease burden as well as tumour response to treatment.[21] Generally, the factors that contribute to the disease heterogeneity can be broadly divided into two major groups namely the host factors and the tumour-related factors. The host factors include age, gender, ethnicity, comorbid illnesses and performance status of the patients. The age and existing comorbid illnesses are the most important host factors as these will define the treatment strategy, especially the eligibility for ASCT and tolerance to certain chemotherapy. It has been reported by Ludwig *et al*.[32,33] and Lenhoff *et al*.[34] that younger patients live longer despite being enriched for higher-risk genetic subtypes, presumably as a consequence of their higher tolerance ability to intensive treatment. Depending on the patient's fitness and co-morbidities, the cutoff age for transplant eligibility is usually between 65 to 70 years old. The National Comprehensive Cancer Network (NCCN) Guidelines for MM[35] have recommended different treatment options for newly diagnosed transplant-eligible and transplant-ineligible candidates, wherein these regimes were further subclassified as "preferred", "other recommended" or "useful under certain circumstances" taking into account the relative efficacy and toxicity of the regimens. The pre-existing comorbidities such as renal insufficiency and peripheral neuropathy are among the factors that can affect the choice of therapy regime selection. Elderly patients and/or frail patients are sometimes unable to tolerate a 3-drug regimen and therefore, this group of patients might be treated with only 2-drug regimen. The NCCN panel[35] recommended triplet regimes over doublet regimes as the standard of care for primary treatment of MM based on the results in several clinical trials which showed that triplet regimens provide improved response rates, depth of response, rates of progression free survival (PFS) or OS.

Tumour factors include the genetic makeup of tumour detected by CCA and iFISH analysis, tumour burden as well as tumour cell characteristics that is reflected in LDH level and β-microglobulin level where both results are essential for R-ISS staging, PC proliferation measured by the PC labeling index or CCA (an abnormal karyotype indirectly reflecting PC proliferation)[17,36], the presence of plasmablastic morphology in bone marrow morphology and immunophenotyping analysis and presence of circulating PCs.[21] The most important tumour factors are genetic aberrations and gene expression profiles (GEP)[21] (More details of these genetic architecture of MM cells are included in the following sections of this review).

Much of the clinical heterogeneity of MM is thought to arise from multiple genomic events that result in tumour development and progression.[30,37] Genetic subtypes of MM have different underlying biological features and show heterogeneity in clinical outcomes. A study conducted at the Mayo Clinic[38] found several important associations between CAs and clinical presentation. There are differences in the occurrence of renal failure and bone disease based on the underlying primary cytogenetic subtype. MM with IgH translocations, especially those involving translocation t(4;14) or t(14;16), was more commonly associated with high FLC levels and renal failure as the MDE. On the other hand, MM patients with translocation t(11;14) and t(6;14) more often presented with bone disease as the initial MDE. Studies also showed that presence of certain high-risk genetic abnormalities namely del(17p13), del(1p32), t(4;14), and 1q gains in the malignant PCs was associated with inefficient response to the lenalidomide-dexamethasone combination therapy but survival could be improved by adding a proteasome inhibitor or a monoclonal antibody to the lenalidomide-dexamethasone regime.[39-42] Bortezomib-based treatment has been shown to overcome certain adverse prognostic markers such as del(13q) resulting in better PFS and OS in patients with poor prognostic markers such as ISS stage 3, del(17p), and t(4;14).[43] The different biological subgroups in MM due to primary translocations involving genes such as MMSET [t(4;14)] and c-MAF [t(14;16)], partly explains the heterogeneous treatment outcome,[44,45] and these can be identified using iFISH and GEP.[46,47] In view of the fact that CAs in MM can affect every aspect of the disease, from the evolution of malignancy to clinical presentation, response to therapy and prognosis, several classifications have been proposed based on the identification of genomic changes that help to stratify clinically relevant genetic groups of MM patients.[37]

Therefore, clinicians need to stratify patients into appropriate risk categories for optimum treatment response. In this context, tumour genetics are important in MM patients as the cytogenetic and genomic results guide treatment selection. Another main reason for risk categorisation for each patient is to inform the patient of his/her prognosis. In clinical practice, a better definition of MM genetic subgroups is essential not only to provide a framework for patient counselling but also to provide more effective personalized therapies.

Cytogenetic architecture and risk stratification of MM

The clinical heterogeneity in MM is supported by distinct molecular and cytogenetic profiles. MM is characterised by chromosomal instability and CA ranging from chromosome numbers to genetic translocation and genetic mutations. While CAs are not included in the diagnostic criteria of MM, they have been associated with malignant transformation, aggressiveness of the disease and disease progression.[37] Important prognostic information such as prediction of initial response to chemotherapy, remission duration and OS can be derived from the pattern of CAs encountered in MM patients.[48] Therefore, identification and stratification of these CA is important as they play a role in prognostication as well as monitoring of treatment. Based on the hallmark cytogenetic abnormalities, MM can be divided into hyperdiploid and non-hyperdiploid subtypes.[14,37,49] The hyperdiploid group (H-MM) is defined mainly by the gain of certain odd numbered chromosomes such as chromosomes 3, 5, 7, 9, 11, 15, 19, and 21.[50] The nonhyperdiploid group (NH-MM) is characterised by the presence of chromosomal translocations involving the common IgH locus on chromosome 14q32 region with several chromosomal partners such as chromosome 4 [t(4;14)(p16;q32)], chromosome 6 [t(6;14)(p21;q32)], chromosome 8 [t(8;14)(q24;q32)], chromosome 11 [t(11;14)(q13;q32)], chromosome 16 [t(14;16)(q32;q23)] and chromosome 20 [t(14;20)(q32;q12)].[37,49-52] Hyperdiploidy when present alone is usually associated with a favourable prognosis.[14,51] Whereas, the NH-MM is generally associated with more aggressive clinical features, shorter survival and thus, poorer prognosis.[14,50,53,54]

Following the context of evolving prognostic factors and treatment paradigms, the mSMART (Mayo Stratification of Myeloma and Risk-Adapted Therapy) risk stratification model for NDMM has been updated frequently. The mSMART 2013 guidelines[55] stratified MM into three risk categories. Based on mSMART 2013 guidelines, the high-risk group is those with t(14;16), t(14;20), del(17/17p) and high-risk signatures in GEP. The intermediate group is those patients with t(4;14), del(13), hypodiploidy and plasma cell labeling index (PCLI) ≥3%. All others including t(11;14) and t(6;14) are considered standard-risk.

The IMWG consensus in 2014 proposed a combination of ISS-genetic prognostic system as the new standard to define high-risk disease.

The parameters used are serum albumin and β-2 microglobulin for ISS staging, and iFISH for t(4;14), deletion 17p13 and 1q21 gain. A high-risk group of patients can be defined by ISS stage II/III and the presence of either t(4;14) or 17p13 while a low-risk group can be defined by age less than 55 years, ISS stage I or II and normal results for the three FISH markers for 14q32 translocations. Other abnormalities that do not fulfill high-risk and low-risk criteria are defined as standard-risk. Using this combination, high-risk patients are reported to survive less than two years despite novel therapeutic agents, standard-risk patients survive for seven years and low-risk patients survive for more than ten years. In the mSMART 2013 and 2016 schema, t(4;14) is considered to have intermediate-risk rather than high-risk as its risk can be modulated by bortezomib-based initial therapy. At that point of time, IMWG still viewed the disease as incurable and that risk-adapted therapy might be under-treating some low-risk patients. MM is unlike other hematological malignancy such as acute leukemias and Hodgkin's lymphoma where low-risk patients may get away with less intensive treatment and still be cured, whereas high-risk patients will require more intensive treatment to achieve long-term remission. Hence IMWG recommended that all myeloma patients receive the most optimal treatment tested in phase III clinical trials and currently available to achieve the best outcome.

Later in 2015, IMWG introduced the R-ISS prognostic model that included ISS, CAs, and LDH data to effectively stratify patients with NDMM with respect to the relative risk to their survival.[22] This new and more powerful prognostic algorithm has replaced the previous IMWG prognostic model proposed in 2014. LDH level is included in this prognostic model because LDH level above the upper limit of normal not only denotes an increase in disease aggressiveness but also suggests a high proliferation rate and/or the presence of tumour mass, in particular extramedullary and extraosseous disease.[56-60] Studies performed both before the availability of novel agents such as bortezomib and lenalidomide[56] and in the era of novel agents[57], showed that high LDH levels were associated with shorter OS with median OS of high and normal LDH group being 21 and 51 months respectively.[57] In R-ISS, del(17p), t(4;14), and t(14;16) detected by iFISH were considered as high-risk CA. R-ISS stage I includes ISS stage I, no high-risk CA, and normal LDH; R-ISS stage III includes ISS stage III with high-risk CA and/or high LDH levels; R-ISS stage II includes all the remaining conditions. The combination of three different prognostic tools in the R-ISS allows a better evaluation of patient prognosis. If only one of these three factors was considered, approximately 26% of patients would have been wrongly allocated to a good-prognosis group. The study results showed an improvement in OS with novel therapies across prognostic subgroups. Patients with R-ISS stage I, II, and III had 5-year OS rates of 82%, 62%, and 40%, respectively. The survival dissection among the different groups in their study[22] was slightly better as compared to other studies. This could probably be due to their distribution of risk groups wherein, 62% of patients were in the intermediate-risk group, whereas 28% were in the low-risk group and only 10% were in the high-risk group.

In 2016, Sonneveld *et al.*[23] updated the consensus of IMWG on the treatment of high-risk cytogenetics. The IMWG consensus panel on FISH advises to test for the presence of del(17p), t(4;14), and t(14;16) routinely for high-risk MM. Any nonhyperdiploid karyotype and t(14;20) are also high-risk cytogenetics in NDMM regardless of treatment. Gain(1q) when associated with del(1p) also carried poor risk. Therefore the IMWG (2016) also recommended the incorporation of t(11;14), t(14;20), gain(1q), del(1p), del(13q), and ploidy status as an extended panel in clinical trials. Sonneveld *et al.*[23] had summarized the risk into two groups namely high-risk and standard-risk based on IMWG consensus 2016. The high-risk group included patients with t(4;14), t(14;16), t(14;20), del(17/17p), gain(1q), del(13q), nonhyperdiploid karyotype and GEP high risk signature. The standard-risk comprised all other abnormalities including t(11;14) and t(6;14). With this cytogenetic risk summary, a standard set of high-risk cytogenetic prognostic markers was outlined. This not only allows better risk stratification in trials but also allows the prognostic impact of any new prognostic markers in future studies to be compared with this standard. Hence data interpretation becomes easier and more consistent, and finally facilitates a more rapid adoption of new prognostic markers.

Vincent Rajkumar from Mayo Clinic had updated the mSMART stratification criteria in 2016[61] where patients with del(17p), t(14;16), and t(14;20) remained as high-risk MM while intermediate risk group is only those with t(4;14) translocation and gain(1q). All others are considered as a standard-risk group. The latest mSMART 3.0 classification of active MM (last reviewed in August 2018)[62] has categorized active MM patients into only two groups which are the high-risk group and standard-risk group. The high-risk group consists of those patients with presence of either high-risk genetic abnormalities, R-ISS Stage 3, high plasma cell S phase or high-risk signature in GEP. The high-risk genetic abnormalities are defined as presence of t(4;14), t(14;16), t(14;20), del 17p, p53 mutation or gain 1q detected by FISH or equivalent method. Presence of trisomies may ameliorate the effects of these high-risk genetic abnormalities if present individually. The point to note is that the previous intermediate-risk group has been incorporated into the high-risk group. The latest mSMART risk stratification also included Double Hit Myeloma (presence of any two high-risk genetic abnormalities) and Triple Hit Myeloma (presence of three or more high-risk genetic abnormalities) into the high-risk group and presence of trisomies do not ameliorate their risks. All other genetic abnormalities including trisomies, t(11;14) and t(6;14) are considered standard-risk.[62-63]

The latest mSMART 3.0 cytogenetic risk stratification for active MM[62] and IMWG 2016 had come to a consensus[23] that the presence of t(4;14), t(14;16), t(14;20), del(17/17p), gain(1q) and GEP high-risk signature are high-risk genetic abnormalities (Table 1). The mSMART 3.0 considers all other genetic abnormalities including trisomies, t(11;14) and t(6;14) as standard-risk[62], indicating that monosomy 13/ del(13q) and nonhyperdiploid karyotype are also segregated into the standard-risk group. However, the IMWG (2016) consensus had stated that del(13q) and nonhyperdiploid karyotype are high-risk cytogenetic features and all other abnormalities that are not defined as high-risk are considered standard risk. No consensus has been reached between the mSMART and IMWG for monosomy 13/del(13q) and the hypodiploid karyotype risk group.

Prognostic controversies on chromosome 13 and RB1 gene abnormalities

The association of monosomy of the RB1 gene, which is located at the long arm of chromosome 13, with poor outcomes has fluctuated in recent years. Monosomy of 13q was first identified as a poor prognostic marker[64-66], but later was found that the association with poor prognosis was due to co-segregation with del(17p) and t(4;14).[20,54,67-69] However, IMWG still holds strong on the statement that only cytogenetically detected chromosomal 13 or 13q deletion has poor risk. Based on the consensus recommendations for risk stratification in MM by the International Myeloma Workshop Consensus Panel 2 in 2011,[70] del13 or 13q detected only by FISH independently in the absence of other abnormality does not carry significantly higher risk. Sonneveld *et al*. (2016)[23] also emphasised that del(13q) when detected by karyotyping predicts impaired PFS/OS[69] although del(13q) as a single CA does not confer poor survival (71). Sonneveld *et al*'s view that the adverse impact of del(13q) detected by FISH was associated with del(17p) and t(4;14), which was supported by other studies too.[20,68,71-76] All these studies support that the detection of monosomy 13 or del(13q) by conventional cytogenetic analysis is a preferred approach for testing CA for chromosome 13 compared to iFISH approach and there is no prognostic significance of abnormalities detected by iFISH alone. Its identification by karyotyping is used as a poor prognostic marker probably because it is a surrogate of high proliferation with high tumour burden.[37,69] The abnormality in chromosome 13 by karyotyping also probably functions as a surrogate marker for hypodiploidy and IgH translocations.[49,77] FISH detection of chromosome 13 abnormalities in MM patients is important because FISH detection of del(13q) at the time of diagnosis will increase the specificity of minimal residual disease (MRD) in MM patients.

The NH-MM is characterised by a very high prevalence of IgH translocations (>85%) whereas IgH translocations are less common in the H-MM (<30%).[49,54,77-79] Chromosome 13 monosomy or partial deletion including the 13q14 region are more common in patients with nonhyperdiploid karyotype.[49,54,77,78] Among the three main recurrent IgH translocations detected in MM, only t(11;14)(q13;q32) confers a standard prognosis while both t(4;14)(p16;q32) and t(14;16)(q32;q23) confer a bad prognosis. An abnormal chromosome 13 might play a crucial role in the clonal expansion of tumours as studies have shown nearly 90% of progressive disease cases with t(4;14)(p16;q32) also harbor chromosome 13 deletion.[20,64,80-82] In general, the H-MM not only harbour lower prevalence for IgH rearrangements but also a lower prevalence of structural chromosome abnormalities.[77] A recent study by Binder *et al*.[83]

Table 1: Summary of cytogenetic risk features based on IMWG consensus 2016 and mSMART 3.0 recommendations

	IMWG consensus 2016	mSMART 3.0
High-risk	• FISH: t(4;14) t(14;16) t(14;20) del(17/17p) gain(1q) • Nonhyperdiploid karyotype • Karyotype del(13) • GEP: high-risk signature	• High-risk genetic abnormalities[a,b] t(4;14) t(14;16) t(14;20) del 17p p53 mutation gain 1q • R-ISS Stage 3 • High plasma cell S phase[c] • High-risk signature in GEP • Double Hit Myeloma: Any 2 high-risk genetic abnormalities • Triple Hit Myeloma: 3 or more high-risk genetic abnormalities
Standard-risk	• All others including: FISH: t(11;14), t(6;14)	• All others including[a]: trisomies t(11;14)[d] t(6;14)

[a]Trisomies may ameliorate, [b]By FISH or equivalent method, [c]Cut-offs vary, [d]t(11;14) may be associated with plasma cell leukaemia, IMWG- International Myeloma Working Group, mSMART- Mayo Stratification of Myeloma and Risk-Adapted Therapy, FISH- fluorescence in situ hybridisation analysis, GEP- gene expression profiling, R-ISS- Revised International Staging System. Table adapted from Sonneveld *et al.*[13], mSMART 3.0: Classification of Active MM[52]

involving 1181 NDMM patients reported a novel observation of differential effects of monosomy 13 (adverse) and partial deletion (protective) of chromosome 13q on OS. The adverse effect of monosomy 13 detected by iFISH on OS was independent and this finding was in agreement with the IMWG consensus that cytogenetically detected monosomy 13 or 13q deletion has poor prognosis risk. However, the protective effect of partial 13q deletion on OS effect needs to be validated in other studies to confirm its prognostic significance. The underlying mechanism for this protective effect is still unclear. This contrasting effect has not been observed before, probably because most previous studies had grouped the partial deletion of 13q together with monosomy 13. Therefore, no analysis was carried out for del(13q) separately from monosomy 13.

Chavan *et al.*[84] reported that biallelic inactivation of *RB1* gene that commonly results from homozygous deletion of the *RB1* gene, was associated with relapse and poor prognosis. Their study demonstrated enrichment of bi-allelic inactivation of tumour suppressor genes in high-risk cases and at relapse. Hence, bi-allelic inactivation of the *RB1* gene was identified as an independent negative prognostic marker. As mentioned earlier, previous data from multivariate analysis has shown that almost all cases with a t(4;14) in association with monosomy 13 leading to loss of *RB1*, was a prognostic marker.[14,65,85,86] In order to confirm that the negative prognostic effect of *RB1* found in their study was not due to association with t(4;14), they divided the samples based on the presence/absence of each alteration. Subsequent analyses showed that patients with either the t(4;14) or alteration of *RB1* were associated with poor prognosis. When both lesions were present together, the prognosis was worse. Bi-allelic *RB1* deletion is not commonly seen in NDMM. On the other hand, monoallelic *RB1* deletion is commonly detected in NDMM, present in up to 50% of the patients[64,65,80-82] in which 85% constitute monosomy and the remaining 15% are interstitial deletions. Therefore, based on double hit theory, the presence of monoallelic *RB1* deletion during initial presentation might be a driver mutation placing the patient at higher risk and a second hit, either mutation or deletion of the remaining *RB1* gene, might render biallelic *RB1* inactivation that is associated with poor prognosis and relapse. The adverse effect of biallelic *RB1* deletion detected by targeted sequencing on OS was independent of other genetic markers. This finding is in partial agreement with the IMWG consensus that 13q deletion has poor prognosis risk because IMWG emphasise the detection of chromosome 13 abnormalities only when detected by karyotyping. A study by Kuiper *et al*.[18] also confirmed del(13q) as a FISH marker that is consistently associated with shorter OS other than high-risk abnormalities such as t(4;14), gain(1q) and del(17p). The findings of Chavan *et al*.[84] and Kuiper *et al*.[18] in association with future supportive results might enable IMWG to reach a consensus that *RB1* gene deletion detected by iFISH alone can be prognostically significant.

As discussed earlier, based on the genetic abnormalities that MM patients harbour, the disease can be distinguished into two major ploidy groups namely H-MM and NH-MM.[37,49] The total chromosome number for H-MM ranges from ≥47 chromosomes to <75 chromosomes resulting from the gaining of the odd chromosomes 3, 5, 7, 9, 11, 15, 19 and 21. In general, hyperdiploidy do not confer poor prognosis.[23,51] The favourable prognostic value of H-MM was attributed to the presence of trisomy 3,[23,87] trisomy 5[23,26,42,87] and trisomy 21.[87] Few studies[26,87,88] have shown an ameliorating effect of concomitant trisomies in patients with newly diagnosed high-risk MM while some did not.[89,90] Chretien et al.[87] reported that trisomy 3 and 5 had protective effect while trisomy of chromosome 21 was associated with worsening survival. Some H-MM patients have a more aggressive variant of the disease.[52,91] Few of our unusual MM cases with complex karyotypes showed very poor prognosis and adverse disease outcomes.[92,93] Chng *et al*.[94] reported that within the H-MM, GEP can identify four recurrent groups with distinct clinical and biological associations namely NF-kB/anti-apoptosis signature, an interleukin-6/HGF signature, a cancer testis antigen signature and 'other' in which a subgroup showed very bad prognosis and a subgroup responded particularly well to bortezomib. Meanwhile, NH-MM that is frequently associated with IgH translocations (14q32), is further divided into three subgroups: hypodiploid (≤44 chromosomes), pseudodiploid (45–46 chromosomes) and near tetraploid (>75 chromosomes).[54] Near tetraploid is believed to be originated from the doubling of the hypodiploid and pseudodiploid karyotypes.[77,78,95] As explained previously, NH-MM also harbours a higher prevalence of other structural chromosome abnormalities other than the IgH translocations[54,77] as compared to the H-MM group. Among the NH-MM, the hypodiploid subgroup is hypothesised to have a more advanced clonal state of NH-MM as this subgroup showed an enrichment of abnormalities associated with poor outcome and progression as compared with other NH-MM subgroups.[95] Therefore, all H-MM when not in association with other genetic abnormalities generally do not confer poor prognosis while NH-MM is associated with worse prognosis and shorter survival than H-MM.[54,77,78,95]

Risk stratification of MM based on cytogenomic and gene expression profiling data

With the advancement in technologies, the prediction of survival in MM, which previously solely depended on conventional cytogenetics and iFISH findings, has moved to include molecular classification. The introduction of new high-throughput molecular technologies such as array CGH or SNP array, GEP and massively parallel whole genomic sequencing (WGS) are enabling screening for all possible chromosomal aberrations, identifying each aberration on a case-by-case basis and discovering new aberrations that are of relevance in unravelling the complex biology of the tumour cells. Better understanding of the disease complexity will then answer some of the survival heterogeneity that are not able to be explained solely by the cytogenetics risk classification.

Array CGH (aCGH) is a powerful method to screen for copy-number abnormalities.[17,26,52,95] Using the molecular classification of aCGH profiles, Carrasco *et al*.[52] reported that H-MM is a genetically heterogeneous disease and that a poor prognosis subset of H-MM patients can be identified by the presence of ch(1q) gain and/or ch13 loss. Another study from the Intergroupe Francophone du Myèlome (IFM) using SNP array[87] reported that gain of chromosome 19 was associated with prolonged survival. Their finding was in agreement with Merz *et al*.[90] who reported that some trisomies impact survival regardless of ploidy status, especially of gain 19q13 in patients with del(17p). High-resolution aCGH and microarray profiling technologies were used in GEP as well. Nair *et al*.[96] reported that genes residing on chromosome 1q21 contribute critically to the high-risk designation in the GEP70 model and suggested that clonal evolution in myeloma can be traced to a copy number–dependent increased expression of genes within the 1q21 amplicon. The higher the copy number of 1q21amp at diagnosis, the higher the risk of death.[86] The proportion of cells with amplification of 1q21 was always noted to be increasingly present at relapse, suggesting that cells with more copies of 1q21 have a resistant phenotype.[86,97]

As mentioned earlier, in addition to cytogenetic and cytogenomic data, GEP also plays a role in the disease prognosis. GEP, a form of transcriptomic analysis that reflects the biology of MM in individual patients,[19] is not only capable of providing additional prognostic information but also allowing clinicians to further refine the current cytogenetic and molecular-based risk stratification model. GEP is able to explain a larger amount of variance in survival compared to ISS and cytogenetics. Studies have shown that some patients with molecularly and cytogenetically defined high-risk diseases do not receive the same benefit from certain approaches as the low-risk patients and need alternative therapies.[98,99] GEP is a useful tool and may be helpful in selected patients. However, currently it is still not routinely used in clinical practice during diagnostic workout. GEP allows the identification of high- and low-risk gene signatures as well as differentially expressed genes which are useful for prognostication[23], estimation of disease aggressiveness and individualised treatment.[35]

The IMWG risk stratification recommendations are more widely accepted as compared to mSMART risk stratification because recommendations by IMWG are based on larger randomized trials as compared to mSMART. Up to date, IMWG still recommends individualized treatment according to genomic characteristics of the disease rather than the risk-adapted therapeutic approach recommended by mSMART. Most of the risk-adapted therapy recommendations by mSMART are based on retrospective studies and have not been validated in prospective studies. The main reason why IMWG proposed that all patients should be treated with the same approaches, independent of their individual risk, was to avoid the risk of under-treating some low-risk patients. The second reason was due to the lack of predictive markers that can be used to provide information specifically about different drugs or regimens and the likelihood of good response and the outcome with them.[21] The predictive markers are useful for individualising treatment, whereas the prognostic markers are useful in risk stratification to know the outcome.

In this review, only prognostic implications of the cytogenetic derangements of MM in clinical settings are discussed in detail. The power of cytogenomics to unravel the prognostic subcategories of the disease also are covered in this review. We do not intend to discuss the prognostic markers that are still under research and not in clinical use yet such as gene expression signature, mutation analysis of other key driver genes of cell survival and proliferation involved in the development and progression of MM.[30] As for examples, RAS mutation, nuclear factor-B-activating mutations, other deregulation factors for the cyclin-dependent pathways regulators and genes that are responsible for the progression of normal PCs to MM cells via immune-mediated pathways[8] as well as the roles of epigenetic such as methylation and microRNAs (miRNAs) in the pathogenesis of MM are beyond the scope of this review.

Future perspectives

The treatment of MM is becoming increasingly challenging for healthcare professionals despite new therapeutic agents in clinical development. Understanding the factors that affect disease heterogeneity is crucial to refine the current risk stratification model for NDMM. Currently used risk stratification methods in MM are suboptimal. A more accurate prognostic model may lead to the development of treatment plans that are specifically aimed at improving the survival of high-risk MM patients. MM shows tumour clonal heterogeneity at the time of diagnosis with differential sensitivity to different drugs leading to clonal selection and evolution. Hence targeting of a wide range of targets including tiny subclones is required for successful treatment. Therefore, monitoring gene changes of the tumour cell population under the pressure of treatment selection to evaluate efficacy is highly recommended. An interesting area might be the complex genomic abnormalities identified through microarray-based studies. These complex abnormalities need to be investigated further to identify markers that could be used to follow residual disease and may be of added value to treatment. In the future, the high-risk group can be selected accurately for more intensive treatments in terms of dose-dense chemotherapy and prolonged therapeutic schemas. Incorporation of signatures based on gene expression into current widely used prognostic models: clinical-based prognostic system (β-2-microglobulin and albumin in ISS) and biology-based prognostic markers (iFISH-based cytogenetics) as routine MM workout shall be able to augment the prediction of the high-risk population.[18,19] A better prognostic model to increase accuracy in outcome prediction in MM is needed for better patient counselling, adaptation of treatment according to individual risk status and eventually more effective personalized therapies using patient-specific markers as a directional key.

Another option could be selection of treatment based on the co-existence of subclones, dynamic evolution of the disease and gene mutational profile. So far, the information obtained on a spectrum of mutants and whether drugs that target these mutational changes will provide a meaningful or lasting response in MM patients are not sufficient yet to define their role and place in the personalised treatment of MM. Integrative genomics using new approaches such as GEP and WGS may provide robust predictive markers in the near future to identify genetically distinct subclones, potentially with different levels of drug resistance and thus allow the selection of drug that is most effective for each subtype and stage of disease.[100] Patients can benefit from treatment with entirely novel drugs targeting the tumour cells with low toxicities for normal cells and avoiding the chemotherapy-associated risk. Targeted therapy guided by the mutation of gene expression profile could be an attractive treatment option for personalized medicine. Sequencing based methods are replacing array based methods. Newer high throughput technologies are being incorporated into research to improve upon the tremendous progress already made in MM. Next generation sequencing (NGS) methods are providing additional diagnostic and prognostic markers which might prove to be relevant for genome-guided targeted therapies in the future. The meaning of large amount of data being generated through high throughput technologies are delineated using bioinformatics methodologies. New markers such as mutation splicing events, non-coding RNA, and microRNA are being incorporated as part of integrative oncogenomic efforts to improve both predictive and prognostic markers. It is anticipated that the integration of gene mutational profile, splicing events, gene expression profile along with ISS and cytogenetics may be used to improve MM classification that may become a standard of care in MM patients in the near future.

CONCLUSION

Over the years, with the advancement of technologies, the understanding on genetic architecture of MM has been improved leading to the development of more accurate risk stratification of MM. The genetic architecture of MM can be broadly divided into a hyperdiploid group, characterised by trisomies of odd number chromosomes and a non-hyperdiploid group characterised by IGH translocations involving chromosome 14q32 region to various partner chromosomes. These genetic subtypes have different underlying biological features and show heterogeneity in clinical outcomes. Up to date, CCA and FISH/iFISH analyses are still the mainstay methods in the identification of high-risk genetic features at diagnosis which allows MM patients to be stratified into the new risk-adapted treatment strategies. However, genomic technologies such as array CGH, GEP and NGS are gaining importance in the risk stratification and identifying new prognostic markers allowing additional and better definition of MM genetic subgroups. Identification of predictive markers that could be used to decide treatment options as well as markers that could be used to follow residual disease and may be of added value to treatment. Once the high-risk group can be selected accurately, they could be subjected to more intensive treatments in terms of dose-dense chemotherapy and prolonged therapeutic schemas. It is hoped that, in the near future, more and more prognostic and predictive markers can be identified and thus provide more effective personalized therapies for MM patients.

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