

## Review Article

# Risk Stratification in Multiple Myeloma

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**Received:** May 13, 2015; **Accepted:** June 25, 2015;**Published:** June 30, 2015**Abstract**

Multiple myeloma (MM) is a malignant plasma cell disorder, characterized by bone marrow infiltration with clonal plasma cells (PCs), and affects nearly 20,000 patients in United States (US) each year. Revised International Myeloma Working Group (IMWG) diagnostic criteria for MM has included biomarkers, namely, clonal bone marrow PCs  $\geq 60\%$ , serum free light chain ratio  $\geq 100$  and  $\geq 1$  focal lesion on magnetic resonance imaging (MRI) in addition to traditional CRAB (Hypercalcemia, Renal insufficiency, Anemia and Bone lesions) for defining MM. In the era of risk-adapted therapy with novel agents, importance of comprehensive risk stratification models, including a conglomerate of host factors, tumor factors and factors arising due to host-tumor interaction, is paramount. In this review, we have discussed host factors, including patient demographics and performance status, tumor factors including, albumin, C-reactive protein, lactate dehydrogenase, serum free light chain assay, complete blood count, bone marrow morphology, cytogenetics, gene expression profiling, immunophenotyping and proliferative capacity and factors related to tumor-host interaction, including  $\beta$ -2 microglobulin and renal function, which are important components of risk stratification. Furthermore, response to therapy, including impact of complete remission, early relapse and minimal residual disease after therapy have been shown to predict survival in MM. Clinical application of these components have been reflected in novel risk stratification models, including Mayo stratification of myeloma and risk adapted therapy (mSMART), IMWG and Intergroupe Francophone du Myélome (IFM), with further studies on identifying molecular characteristics of PCs in MM currently underway.

**Keywords:** Proteasome inhibition; Relapsed myeloma; Stratification**Introduction**

Multiple myeloma (MM) is a malignant plasma cell disorder, accounting for 10% of all hematologic malignancies [1]. The diagnosis of MM requires either 10% or more clonal plasma cells in bone marrow along with evidence of end-organ damage or 60% or more clonal plasma cells in the absence of end-organ damage [2]. Historically, the first case of MM was documented by Solly in 1844 in a 39 year old female presenting with fatigue and multiple fractures [3]. Cardinal clinical manifestations of MM attributable to the plasma cell clone are usually described using the acronym CRAB- Calcium elevation, Renal insufficiency, Anemia and Bone disease [4]. Estimated number of new cases of MM in 2014 in the United States (US) was 24,050 and estimated number of deaths being 11,090 [5]. The average age-adjusted incidence of MM in the US is approximately 4 per 100,000, with median age at diagnosis being 65 years [2]. The annual incidence in Europe is 4.5-6 per 100,000 with median age at diagnosis being 65-70 years [6]. In a study conducted in US on the incidence of MM in Olmsted County, Minnesota, the overall annual incidence rate was found to be pretty stable in the last 6 decades [7]. A population based study in Sweden has shown a temporal improvement in median Overall Survival (OS) from 24.3 to 56.3 months in younger ( $\leq 65$  years) patients with MM diagnosed from 1950 to 2005 [8]. Another larger study using Surveillance, Epidemiology, and End Results (SEER) database showed improvement in five-year Relative Survival rate (RS) during the time period 2004-2011 compared to 1991-2002 in all age-groups, implicating improved survival after approval of novel

proteasome inhibitor bortezomib in 2003 [9]. Among patients with relapsed MM after Stem Cell Transplantation (SCT), those relapsing after year 2000 were found to have better OS compared to those before 2000 [10]. All these studies point towards improving survival in MM with advent of novel therapeutic agents in recent decades. Since risk-adapted therapy is the standard of care in MM currently [2], the need for a comprehensive risk stratification model for prognostication and assisting with therapeutic decision-making is paramount.

MM is characterized by clinical and biological heterogeneity, with recent genetic analyses identifying subgroups with predictable prognosis across different types of treatment [11]. Various immunoglobulin gene translocations and chromosomal anomalies have been identified in addition to traditional prognostic factors like  $\beta$ -2 microglobulin, which has necessitated chromosomal studies to be conducted for front-line risk stratification and therapeutic consideration in MM [12,13]. Furthermore, data show that novel therapeutic agents like proteasome inhibitors and immunomodulators are more effective than traditional chemotherapy in patients with high-risk cytogenetics [14], advocating the use of such agents for induction prior to SCT in high-risk transplant-eligible patients [2,6]. Due to such heterogeneity in pathogenesis and response to therapy, selection of a risk-stratification tool depends upon the context of host and tumor factors, host-tumor interactions and therapeutic considerations [15]. The future of myeloma therapy lies in precision medicine due to improved understanding of MM cell biology and will require utilization of new relevant prognostic factors [16].

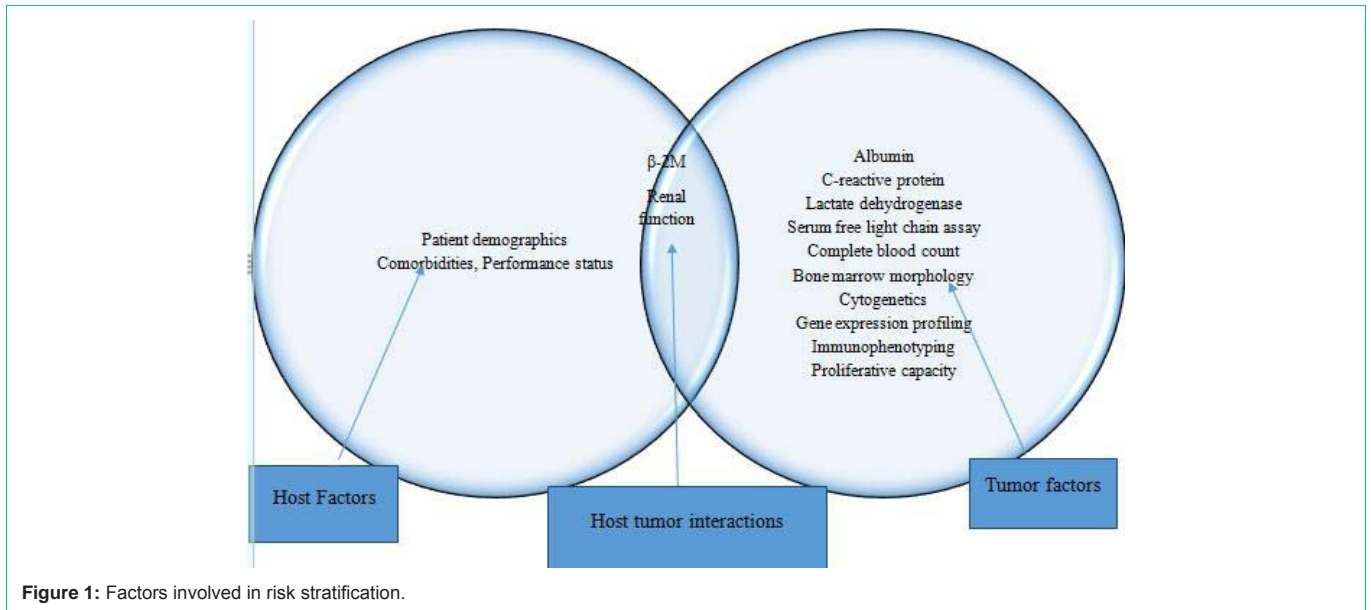


Figure 1: Factors involved in risk stratification.

This comprehensive review outlines prognostic markers inherent to patients (host factors), the tumor (myeloma-related factors) and host-tumor interaction (Figure 1). The clinical value of these markers in the era of novel therapeutic agents and transplantation has been described along with current clinical application of evidence-based strategies in prognostication and individualizing treatment.

## Host Factors

### Patient demographics

#### Age

Age is an important prognostic marker in MM [15]. Various studies have demonstrated worsening outcomes in MM with advancing age, and the etiology is considered to be multifactorial, including both biological and psychosocial factors [17]. A survival analysis of greater than 10,000 patients from the International Myeloma Working Group (IMWG) showed significantly longer median Overall Survival (OS) in younger patients (<50 years), both after conventional and high dose therapy [18]. Younger patients were found to have lesser frequency of adverse prognostic factors, including high C - reactive protein (CRP), low hemoglobin, increased serum creatinine and poor Performance Status (PS) as well as low International Staging System (ISS) and Durie-Salmon stage. A study on 2316 MM patients younger than 65 years found age >60 years to be significantly associated with shorter OS in those treated with HDT [19]. Interestingly, there was no difference in incidence of high-risk cytogenetics on further subgroup analysis, but  $\beta$ -2 microglobulin levels were higher in patients >65 years of age. Another study conducted by Nordic Myeloma Study Group (NMSG) showed prolonged survival in patients less than 60 years receiving high-dose therapy (HDT) followed by autologous SCT (ASCT) [20]. However, there is conflicting evidence on biological distinction of MM occurring in different age groups. A study on 356 previously untreated MM patients showed similar initial clinical and laboratory characteristics as well as response to therapy in all age groups [21]. Multivariate analysis in a study on MM patients >65 years of age has identified high percentage of S-phase bone marrow plasma cells to be the most powerful independent prognostic factor,

indicating the importance of proliferative activity of clonal plasma cells in this age group [22]. A few studies have shown the incidence of chromosomal abnormalities to be similar in MM patients of different age groups, indicating that difference in survival could be due to psychosocial factors and co-morbidities alone [23,24]. A study on MM patients undergoing HDT followed by ASCT did not show age as a significant prognostic marker for both overall and event-free survival [25].

A scoring system based on geriatric assessment has been developed, categorizing elderly (>65 years) patients with MM into 3 groups, “fit”, “unfit” and “frail”. 18 month OS was found decrease from 92% in “fit” group to 73% in “frail” group in Cox multivariable analysis and risk of treatment discontinuation and serious adverse effects (SAEs) followed an opposite trend [26]. With increasing incidence of MM in elderly population and prevalence of elderly MM survivors on long term oral maintenance therapy with advent of novel agents, larger studies are needed to ascertain basis for biological heterogeneity of MM in different age brackets.

#### Race

The incidence of MM and its precursor Monoclonal Gammopathy of Undetermined Significance (MGUS) has been shown to be two to three-fold higher in African-Americans (AAs) compared to Caucasians [27]. A study on survival among Randomized Clinical Trial (RCT) patients in Southwest Oncology Group (SWOG) did not show any statistically significant racial disparity among MM patients [28]. Another study found AA patients tend to relapse early after ASCT in MM, mechanism of which was unclear [29]. A large population based study using SEER database, however, showed disease-specific and RS rates to be higher in blacks throughout the study period 1973-2005, but with significant survival improvement in whites compared to blacks in the same time-period [30]. Another SEER-based comparative analysis of ethnic sub-groups showed Asians having best median OS (2.7 years) and myeloma specific survival (4.1 years) and Hispanics with worst median OS (2.4 years) [31].

### Socio-economic status (SES)

Small studies with conflicting results have been performed to assess the effect of SES on myeloma survival, with no rigorous large population-based study till date [32,33]. The trend of temporal improvement in survival of Caucasians over time in various large-scale population based studies could be related to better SES and access to care [9,31]. With advent of novel agents and rising cost of myeloma therapy [34], SES and access to specialized care might become an important predictor of outcome and will require judicious allocation of resources.

### Performance status (PS)

The Eastern Co-operative Oncology Group (ECOG) PS has been shown in a univariate analysis to predict prognosis in MM, with poor PS (3 or 4) being associated with shorter median OS [35]. In a multivariate analysis, ECOG-PS has been shown to significantly predict survival in MM patients with spinal cord compression [36]. PS is a simple and inexpensive tool, which can be readily, assesses at bedside, but involves subjective assessment.

### Renal function

In a study on elderly patients enrolled in European phase III trials, the risk of death was found to be increased in patients with renal failure, defined as creatinine level  $\geq 2$  mg/dl (HR 2.02, 95% CI: 1.51-2.70;  $P < 0.001$ ) [37]. Renal impairment (RI) has been shown to be associated with increased frequency of adverse genetic factors, including del17p or t(4;14) using Fluorescence In Situ Hybridization (FISH) [38]. Bortezomib has been shown to overcome the adverse impact of RI in newly diagnosed MM patients, which could be partly due to its effectiveness in MM patients with high-risk cytogenetics [38]. RI was not found to be independently associated with inferior survival in a study on 203 consecutive MM patients treated upfront with novel agents, including thalidomide, lenalidomide and bortezomib [39]. As RI is a common presentation of MM, being more so in elderly population, novel agents might improve overall prognosis in this subgroup in future.

## Tumor-related Factors

### $\beta$ -2 microglobulin ( $\beta$ 2M)

Serum  $\beta$ 2M is a Major Histocompatibility Complex (MHC) class I subunit, which is renally excreted, used as a surrogate for tumor burden and has been shown in many studies to correlate with survival in patients with MM [40-45]. In ISS, serum  $\beta$ 2M level  $\geq 5.5$  mg/dl is used to classify MM patients as stage III and portends a median survival of 29 months [46]. A study demonstrated  $\beta$ 2M uncorrected for stage and serum creatinine level to be the most significant prognostic factor after adjustment for age [41]. If the serum  $\beta$ 2M level is corrected for the renal function, its prognostic influence in patients of renal failure is lost. Another study showed a combination of chromosome 13 abnormalities identified by FISH and  $\beta$ 2M level to be a strong prognostic factor in MM patients receiving HDT [43]. In MM patients undergoing HDT followed by ASCT,  $\beta$ 2M has been shown to predict OS and Progression-Free Survival (PFS) in univariate analysis [44]. Serum  $\beta$ 2M, however, is not predictive of long-term survival [47].

### Albumin

Serum albumin is an inexpensive laboratory test and provides us

with valuable information on prognosis of MM. The combination of serum albumin and  $\beta$ 2M has been shown to be the most powerful prognostic factor in ISS [46]. A review on around 1000 patients with newly diagnosed MM had shown serum albumin as an important prognostic factor on multivariate analysis [35]. Agarose gel serum protein electrophoresis (PEL), which is used universally to measure albumin as well as monoclonal (M) protein estimate, has been shown to be equally accurate in predicting survival as bromocresol green (BCG) assay in a study, highlighting the need to eliminate additional testing [48].

### C-reactive protein (CRP)

CRP is an acute phase reactant of hepatic origin and increases following cellular secretion of interleukin-6 (IL-6) [49]. CRP and alpha-1-antitrypsin (AAT) has been shown significantly correlate with survival in a univariate analysis [50]. Other studies with conflicting evidence did not find any correlation between IL-6 and survival or tumor burden in MM [51,52]. Given lack of mature data on prognostic significance of CRP, it has not been incorporated in risk stratification models of MM till date.

### Lactate dehydrogenase (LDH)

Studies have shown high LDH levels in MM to be associated with high tumor burden, occult extra-osseous disease, poor survival and overall prognosis [44,53-55]. Interestingly, poor prognosis was also noted in patients with normal LDH levels at presentation but elevated level after high-dose cytotoxic chemotherapy [54]. Study conducted on database of Greek Myeloma Study Group found high LDH to be associated with inferior OS within all ISS groups. In patients receiving novel agents, median OS of high and normal LDH group was 21 and 51 months respectively [56].

### Serum free light chain (FLC) assay

The immunoglobulin FLC is an important tool for following patients with oligosecretory MM [57]. FLC provides a rapid assessment of response to therapy, given its short half-life [58]. A study has shown higher 5-year disease specific survival in MM patients with serum FLC ratio ( $\kappa/\lambda$  or  $\lambda/\kappa$  depending on dominating monoclonal light chain) lower than median [59]. Another larger study on 790 newly diagnosed MM patients had similar findings and proposed incorporation of serum FLC ratio into ISS for better risk stratification [60]. However, serum FLC levels in MM patients treated with alkylator based therapy did not seem to correlate with 24-hour urine protein level, questioning its significance in follow-up of patients having M-protein measurable by electrophoresis [57]. A study has shown serum FLC ratio at the time of stem cell mobilization to have prognostic significance on survival endpoints following novel agent-based induction therapy and autologous SCT [61]. International Myeloma Working group (IMWG) has added normal serum FLC ratio as a criteria for stringent complete response in MM [60].

### Complete blood count (CBC)

A study has shown anemia in over 70% of MM patients on initial presentation [35]. Although hemoglobin level is included in Durie-Salmon staging system, it was dropped in ISS due to lack of prognostic significance upon multivariate analysis [46]. Anemia has been found to be associated with RI in a study on newly diagnosed MM [39].

Thrombocytopenia has been identified as an important prognostic factor on multivariate analysis in a study [35]. Although platelet count ranked highly in prognostic significance in a study, it was present only in 12% of patients, limiting its widespread use [46]. However, platelets count <130,000/ $\mu$ L was found to identify patient subset with very poor prognosis, with a hazard ratio (HR) of 1.63 on multivariate analysis, ranking next to serum  $\beta$ 2M. Absolute Lymphocyte Count (ALC) has been identified as an independent prognostic factor for OS in newly diagnosed MM patients [62]. Early ALC recovery has been shown to predict both OS and PFS in MM patients after SCT [63]. Ratio of ALC to Absolute Monocyte Count (AMC) was found to be an independent prognostic factor for OS in a Korean study, with low ALC/AMC ratio, being associated with high ISS stage [64]. CBC is an inexpensive test and can provide an incredible wealth of indirect information on prognosis of MM.

### Bone marrow morphology

Bone marrow aspiration and biopsy is required for diagnosis of MM [2]. A study has shown plasmablastic morphology to be associated with poor response rate, aggressive disease and shortened survival [65]. The plasmablastic cases in this study were found to have lower albumin levels, higher  $\beta$ 2M levels and higher percentage of bone marrow plasma cells by immunofluorescence. However, morphologic features suggested by Bartl et al. [66] have found limited space in risk stratification due to variable distribution of plasma cells in marrow.

### Cytogenetics

Cytogenetics is an essential part of initial workup for risk stratification in MM and can be performed by conventional methods, or, more commonly by techniques not based on metaphase availability, including FISH, array comparative genomic hybridization and Single Nucleotide Polymorphism (SNP) based mapping arrays [67]. Among karyotypic abnormalities, hyperdiploidy is present in about half of newly diagnosed MM patients and is usually associated with a favorable prognosis [68,69]. Hyperdiploidy usually co-exists with Immunoglobulin Heavy chain (IgH) chromosomal translocations, primarily involving chromosome 14 and has been shown in a study to even precede IgH translocation in a proportion of patients [68,70]. Non-hyperdiploidy, on the other hand, is associated with poor OS, PFS and Event Free Survival (EFS) [69,71].

Patients with t(4;14) and t(14;16) have a dismal prognosis, compared to patients with t(11;14) [72]. The oncogenes involved in these translocations include Fibroblast Growth Factor-3 (FGFR-3), Multiple Myeloma SET Domain (MMSET) and c-MAF [72]. Newly diagnosed MM patients with t(4;14) have been shown to have poor survival in the setting of conventional therapy, HDT and ASCT [69,71,73]. There has been conflicting evidence on the effect of bortezomib on survival in patients with t(4;14) translocation [74,75]. Long-term follow-up of 100 MM patients with t(4;14) treated with tandem transplantation identified a sub-group of patients with low  $\beta$ 2M and high hemoglobin level to have superior median OS and EFS [76], indicating its heterogeneity in MM patient population. A recent study on 1003 MM patients did not find any adverse prognostic significance of t(14;16) on multivariate analysis [77], contrary to prior studies [72,78]. Gain of chromosome 1 (+1q21) and t(14;20) has also been shown to define adverse prognosis in the context of thalidomide and conventional induction therapy, with or without ASCT [78].

Deletions of chromosome 17p and 13q are seen commonly in MM and portends a poor prognosis [72]. Chromosome 17 harbors tumor suppressor gene p53 in locus 17p13.1, deletion of which has been shown to be associated with shorter OS and PFS in MM patients undergoing HDT followed by ASCT [79]. Combining del(17p) status obtained by FISH with ISS staging has been shown to improve prognostic assessment in various studies [69,71,78,80]. Short-term induction therapy with novel agent bortezomib did not improve outcome of patients with del(17p) [74] but therapy with bortezomib-based regimen both before and after ASCT was found to reduce the adverse impact of del(17p) on PFS and OS [81]. del(17p) was not found to be an independent adverse prognostic factor in patients with Gene Expression Profiling (GEP) derived low-risk disease receiving bortezomib containing Total Therapy 3 (TT3) [82]. Monosomy and/or deletions of chromosome 13 ( $\Delta$ 13) has poor prognostic effect on MM patients treated with HDT [83]. Clinically, it leads to deletion of Rb gene, which is associated with poor PS, high creatinine, CRP and LDH levels, high percentage of BM plasma cells and advanced stage. A study has shown deletion of 13q14 identified by interphase FISH (iFISH) to be associated with increased proliferative activity (Ki-67) and shortened survival [84]. In a study comparing detection of  $\Delta$ 13 by conventional cytogenetics versus iFISH, there was no prognostic significance of abnormalities detected by iFISH alone, indicating metaphase analysis by conventional cytogenetics to be a preferred approach for testing  $\Delta$ 13 [85]. Bortezomib has been shown to overcome poor prognosis due to  $\Delta$ 13 in many studies [86-88].  $\Delta$ 13 detected by conventional cytogenetics has been included in classification of intermediate risk active MM by Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) consensus guidelines [4].

### Gene expression profiling (GEP)

To identify molecular basis of MM at a transcriptional level, Zhan et al. performed mRNA expression profiling in CD-138 positive plasma cells from MM patients undergoing HDT and tandem SCTs [89]. They introduced 7 molecular subtypes of MM: PR (proliferation), LB (low bone disease), MS (*MMSET*), HY (hyperdiploid), CD-1 (CCND1), CD-2 (CCND3) and MF (MAF/MAFB). PR and MS groups were associated with significant over-expression of 1q genes and poor survival compared with other groups. When this model was applied to relapsed MM patients enrolled in APEX phase 3 trials treated with single-agent bortezomib or high-dose dexamethasone, OS estimates of 1-year actuarial probabilities were 74% for low-risk disease versus 32% for high-risk disease [90]. Another study on newly diagnosed MM patients identified 3 additional subsets, over-expressing genes involved in Nuclear Factor (NF) kappa light-chain-enhancer (TNFAIP3 and CD40), cancer testis antigens and protein tyrosine phosphatases (PRL-3, PTPRZ1 and SOCS3) [91]. A 92-gene prognostic index (EMC-92 gene signature) was developed by GEP and validated in newly diagnosed as well as relapsed MM patients included in HOVON65/GMMG-HD4 trial [92]. Patients defined high-risk by EMC-92 gene signature had reduced OS and it remained an independent prognostic factor on multivariate analysis. In a study of Intergroupe Francophone du Myélome (IFM), patients were stratified into high and low risk based on expression of 15 genes involved in cell cycle and chromosomal stability. High-risk patients, with 3-year survival rate of 47.4%, were characterized by

over-expression of genes involved in cell cycle progression. Low-risk patients, with 3-year survival rate of 90.5%, displayed hyperdiploidy and heterogeneity in genetic signatures. GEP is a valuable tool but needs further validation in larger patient populations before incorporation into general practice and risk stratification models.

### Immunophenotyping

Myelomatous plasma cells (PCs) have been shown to express additional cell surface markers compared to normal BM PCs, which warrants further investigation into their prognostic and therapeutic significance [93]. Over-expression of CD28 by myeloma cells have been shown to be associated with tumoral expansion and treatment failure [94]. Absence of some cell surface markers, like CD45 and CD56 have been associated with plasma cell leukemia (PCL) and an aggressive subset of MM [95,96]. PCs lacking or weakly expressing CD56 have low osteolytic potential and tend to disseminate in peripheral blood. MM patients with CD45 negative PCs were shown to have worse OS on multivariate analysis [96]. Similarly, CD27 negative MM patients had worse 3-year OS in a study (50% versus 92% in CD 27 positive patients) [97]. CD 117 (c-kit), which is not expressed in normal PCs was found in 50% of MGUS, 33 % of newly diagnosed MM and 8% of relapsed MM patients [98]. However, CD117 expression was associated with better 4-year OS. CD117 negative patients with poor prognosis expressed CD221 and had t(4;14) in this study. In a prospective study on prognostic impact of immunophenotypic markers in newly diagnosed MM patients treated per GEM 2000 protocol, expression of CD19 and CD28 and lack of expression of CD117 was associated with significantly shorter OS and PFS [99]. This study proposed a risk stratification model based on simultaneous assessment of CD28 and CD117: Poor risk (CD28 positive CD117 negative), Intermediate risk (both markers negative and positive), and Good risk (CD28 negative CD117 positive). CD28 expression was associated with t(14;16) and del(17p). A splice variant of CD44, CD44v6 was found to be associated with 13q14 deletion and expressed in 43% of ISS stage II/III MM or PCL patients in a study [100]. Further immunophenotypic studies are required in large phase III trials of MM prior to treatment initiation to assess correlation with outcomes and association with critical cytogenetic abnormalities [101].

### Proliferative capacity

Plasma Cell Labelling Index (PCLI) is a surrogate for proliferative capacity of tumor cells in MM and has been shown to be an independent prognostic factor in numerous studies [44,102-105]. MM patients with high PCLI in stable plateau phase after induction regimen were found to have shorter median Time To Progression (TTP) and OS compared to high PCLI group (8 and 20 months in low PCLI versus 39 and 56 months in high PCLI groups respectively) [103]. A significant reduction in PCLI after initiation of therapy in newly diagnosed MM was an important predictor of survival, independent of  $\beta$ 2M, creatinine, serum M-spike response and baseline PCLI in a study [104]. Another study on 595 patients with MM undergoing HDT/ASCT in the Spanish GEM2000 and GEM2005 <65y trials found high PCLI (defined as  $\geq$ 1% PCs in S-phase) assessed by multiparameter flow cytometry as an independent prognostic factor of OS [44]. However, the inferior OS was overcome by treatment with bortezomib based regimens. GEP-based proliferation assessment has also been shown to be an independent prognostic factor for EFS and

OS, with higher proliferation indices being associated with +1q21 and del(13q14) and lower with hyperdiploid signatures [105]. Ki-67 is a nuclear protein expressed by dividing cells and can be used to identify proliferating cells in G1, G2, S and M phase of cell-cycle [67]. A study on Ki-67 proliferation index found its expression to increase with increasing Durie-Salmon stage and better survival in patients with Ki-67 expression less than 8% [45]. Proliferation of malignant PCs in MM, as assessed by various techniques discussed above, can be used as an important adjunct to current risk stratification models for defining prognosis and also be targeted by novel compounds in future, including tubulin polymerase and aurora kinase inhibitors [106,107].

### Imaging

Although conventional radiographs have remained a cornerstone of initial diagnostic workup in MM, advanced imaging including Computerized Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) and (99) Technetium sestamibi (MIBI) have increasingly been used for diagnostic workup, prognostication and assessing response to therapy [108].

Magnetic Resonance Imaging (MRI) is useful for detecting diffuse and focal bone marrow infiltration in the absence of osteolysis on conventional radiographs in Metastatic Bone Surveys (MBS) [109]. Various MRI patterns of the BM infiltration-normal, focal, diffuse and variegated - have been used to predict OS [108]. A study on around 600 MM patients treated with tandem ASCT showed identification of MRI-based Focal Lesions (FL) to be an independent predictor of survival [109]. A risk stratification model was created based on presence of cytogenetic abnormalities and more than 7 FL on MRI: 5-year survival was 76% in the absence of both, 61% in the presence of 1 FL on MRI and 37% in the presence of both. FL identified by MBS did not have any prognostic significance. Another study showed focal and diffuse infiltration patterns on whole body MRI after ASCT to correlate with OS [110]. Diffuse marrow infiltration on MRI portends a poor prognosis and has been shown to further stratify previously untreated MM patients with ISS stages I and II [111].

A study on 13 patients with MM showed F-18 Fluorodeoxyglucose (FDG) PET to be able to detect medullary involvement, residual or recurrent tumor, post-therapeutic changes and response to therapy in MM [112]. Sensitivity of FDG-PET in detecting Myelomatous changes was 85% and specificity was 92% in this study. Another study tested PET/CT in newly diagnosed MM patients treated with thalidomide-based induction therapy followed by double ASCT [113]. Standardized Uptake Value (SUV) > 4.2 and Extra-Medullary Disease (EMD) at baseline was associated with shorter 4-year OS. Persistence of FDG uptake after ASCT, SUV > 4.2 and EMD were independent predictors of poor PFS on multivariate analysis. Number of FDG-avid FLs on PET/CT has been shown to correlate with high  $\beta$ 2M, CRP and LDH levels [114]. In MM patients treated with Total Therapy 3 (TT3), presence of >3 FLs on day 7 of induction therapy was indicative of inferior OS and PFS, overall and also in patients with GEP-defined high-risk disease [115]. A prospective study comparing imaging modalities in MM found MRI of spine and pelvis to be superior technique for detecting marrow involvement, but PET/CT enabled detection of myelomatous lesions in areas out of the field of view of MRI [116].

## Response to Therapy

The impact of Complete Response (CR), defined as disappearance of M-protein on immunofixation, on survival of MM has been controversial, with conflicting evidences in literature. CR has been shown to be a prognostic indicator of long-term PFS and OS in patients treated by HDT/ASCT, Total Therapy (TT) protocols and novel agents [117-121]. A study on 1175 elderly MM patients treated with novel agents showed CR to be an independent predictor of longer PFS and OS irrespective of age, ISS stage and treatment [117]. Another study on long-term survivors of MM after ASCT found CR to be an independent prognostic factor, with 12-year OS being 35% in patients who achieved CR, compared to 22%, 16% and 16% in patients achieving near CR (nCR), Very Good Partial Response (VGPR) and Partial Response (PR) respectively [120]. However, the differential significance of CR as a prognostic factor was studied in GEP-based subgroups of patients, which revealed its significance only in very high-risk subgroup [121]. A study on MM patients enrolled in SWOG phase III trials showed TTP, but not the magnitude of response, to be an independent predictor of survival [122].

In a study on relapsed MM patients after ASCT, median OS from relapse was significantly shorter in patients who had early relapse (ER),  $\leq 12$  months from ASCT (10.8 months in ER group versus 41.8 for rest,  $p < 0.0001$ ) [123]. Failure to achieve CR, along with PCLI  $\geq 1\%$  and greater than 1 treatment regimen prior to ASCT was shown in this study to independently predict early relapse on multivariate analysis. Median OS from diagnosis and from ASCT was significantly shorter in ER group as well. A Canadian study showed ER to be an independent adverse prognostic factor for OS in MM patients receiving ASCT with novel agent based induction regimen [124].

Minimal residual disease (MRD) has been shown to be have prognostic significance after therapy for MM and persistent MRD detected by multiparameter-flow-cytometry (MFC), polymerase-chain-reaction (PCR), next generation sequencing (NGS) and PET/CT has been shown to confer poor survival among patients who achieve CR [125]. In a multicenter randomized phase 3 trial, each log depletion of MRD was associated with significant improvement of OS (median OS of 1, 4, 5.9, 6.8 and  $>7.5$  years for MRD  $\geq 10\%$ ,  $<10\%$ ,  $0.1\%$  to  $<1\%$ ,  $0.01\%$  to  $<0.1\%$  and  $<0.01\%$  respectively) [126].

## Clinical Application

Several risk stratification models have been developed for prognosticating patients with MM, of which, few are used widely in clinical practice worldwide.

### ISS

ISS was developed after analysis of clinical and laboratory features of more than 10,000 previously untreated symptomatic MM patients from North America, Europe and Asia before 2002 [46]. It is fairly simple to understand and reproduce in daily clinical practice. Based on serum albumin and  $\beta 2M$  level, its stratifies patients into three stages: Stage I,  $\beta 2M$  less than 3.5 mg/L plus serum albumin  $\geq 3.5$  g/dL (median survival, 62 months); Stage II, neither stage I nor III (median survival, 44 months); and Stage III,  $\beta 2M \geq 5.5$  mg/L (median survival, 29 months). ISS staging was validated in patients receiving both conventional therapy as well as ASCT in all age groups. However,

the validity of ISS in the era of novel therapeutic agents has been questioned by some studies [127,128]. An analysis of Greek Myeloma Study Group (GMSG) found ISS to be valid in patients receiving front-line therapy with novel agents (4-year OS was 85, 61 and 26% for ISS stage I, II and III patients,  $P=0.001$ ) [127]. However, another European study found that ISS had no significant impact on survival of newly diagnosed MM patients receiving therapy with novel agents [128].

### mSMART

mSMART is a risk stratification model based on consensus recommendations from Mayo Clinic myeloma physicians and uses a combination of conventional metaphase cytogenetics, PCLI, FISH and GEP [4]. It stratifies patients into three groups: High risk, with del(17p), t(14;16), t(14;20) and high risk GEP signature; Intermediate risk, with t(4;14), del(13), hypodiploidy and PCLI  $\geq 3\%$  and Standard risk, which includes t(11;14), t(6;14) among others. The median OS in years is 3, 4-5 and 8-10 in high, intermediate and standard risk patients respectively. Bortezomib based regimens are recommended for patients with t(4; 14) For high risk patients, this model advocates use of bortezomib-lenalidomide-dexamethasone as induction therapy before ASCT, based on current evidence.

### IMWG

IMWG advocates use of ISS staging in conjunction with FISH for t(4; 14), deletion 17p13 and 1q21 gain for risk stratification in MM [129]. High risk group is defined as ISS stage II/III and the presence of either t(4; 14) or 17p13 and low risk group is defined as age  $< 55$  years, ISS stage I/II and normal results for the three FISH markers. The median OS in high, standard and low risk patients was 2, 7 and  $>10$  years respectively in this model. Testing for gain of 1q or del(1p) has been advocated by European Myeloma Network (EMN) in addition to above-mentioned cytogenetic abnormalities [130].

### Intergroupe francophone du myélome

Data from Intergroupe Francophone du Myélome (IFM) trials were used to create a prognostic model to evaluate risk of death related to MM progression within 2 years of treatment initiation [131]. Three independent prognostic variables identified including LDH, ISS III and adverse cytogenetics [t(4;14) and/or del(17p)], were used to create a score ranging from 0-3. The odds ratio estimates for MM progression-related death were 1, 2.9, 5.7 and 24.0 for scores of 0, 1, 2 and 3 respectively. This prognostic index was validated in three other European trials and was found to segregate patients receiving bortezomib-based induction therapy in these trials into four categories based on scores 0-3.

A comparison of new risk stratification models, including mSMART, IMWG and IFM have been presented in Table 1.

## Conclusion

As biological heterogeneity of MM is increasingly being recognized, cytogenetics and molecular abnormalities will be used widely for risk stratification in future [129]. High response of novel agents in patients with high-risk cytogenetics has led to increased acceptance of risk-adapted therapy and improved survival in recent decades. A study on prospective analysis of GEP in CD138 positive PCs is currently ongoing for classification and risk stratification

**Table 1:** Overview of novel risk stratification models for multiple myeloma.

Prognostic model	Variables	Risk categories	Median OS/ Risk of progression-related death	Reference
mSMART	Cytogenetics (FISH), GEP and PCLI	<b>High risk:</b> del(17p), t(14;16) and t(14;20) on FISH and high-risk signature on GEP <b>Intermediate risk:</b> t(4;14) on FISH, cytogenetic deletion 13, hypodiploidy and PCLI $\geq 3\%$ <b>Standard risk:</b> All others including t(11;14) and t(6;14) on FISH	Median OS: 3, 4-5 and 8-10 years in high, intermediate and standard risk respectively	[4]
IMWF	ISS (I/II/III) and cytogenetics (FISH)	<b>High risk:</b> ISS II/III and t(4;14) or 17p13 deletion <b>Low risk:</b> ISS I/II and absence of t(4;14), 17p13 deletion and +1q21 and age <55 years <b>Standard risk:</b> Others	Median OS 2, 7 and >10 years in high, standard and low-risk respectively	[128]
IFM	LDH, ISS (III) and cytogenetics (FISH)	<b>Scores 0-3</b> (higher score indicating poor prognostic subgroup), with 1 point each for high LDH, ISS III and adverse cytogenetics [t(4;14) and/or del (17p)]	Odds ratio estimate for MM progression-related death 2 years from treatment initiation: 1, 2.9, 5.7 and 24.0 for scores 0, 1, 2 and 3 respectively	[130]

of MM (NCT01619358). Another study on identifying molecular characteristics of MM by FISH, SNP, GEP and mRNA expression profiling is being conducted with secondary outcome of predicting OS and EFS (NCT00639054). Further studies are required to unravel cytogenetic correlation of GEP and immunophenotypic signatures and development of new models which are prospectively tested in the context of novel agents.

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