Investigative Tools for Diagnosis and Management

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Recent advances in genomics and proteomics have advanced our understanding of myeloma pathogenesis, recognized novel mediators of disease process, and identified new therapeutic targets. These developments have provided newer diagnostic tools for myeloma, improved monitoring of the disease status and allowed for molecular classification of the disease. The recent advances in investigative techniques that have helped refine the diagnostic work up in myeloma includes use of serum free light chains, especially in oligosecretory myeloma, patients with renal disease and with amyloidosis; use of MRI and PET scan in diagnosis and managing bone disease; and use of cytogenetics and fluorescent in situ hybridization (FISH) technique to determine prognosis. Newer risk stratification protocols have included international staging systems as well as FISH-detected chromosomal changes, specifically t(4;14), t(14;16), and del 17p. These improved predictive risk stratification models are guiding treatment algorithms. As the novel therapies are able to attain complete responses in a significant number of patients, the response categories are also being redefined. Immunophenotypic identification of clonal plasma cells, inclusion of free light chain response and molecular markers of disease now allow us to define stringent complete responses. Recent studies show the increasing importance of attaining complete remission to extended overall survival. The ongoing oncogenomic studies including high-throughput expression profiling, high-density single nucleotide polymorphism (SNP)–arrays and array based comparative hybridization (aCGH) have been utilized to not only understand myeloma pathobiology, but for gene discovery, identification of biomarkers, and delineation of patient subgroups to incorporate them into therapeutic strategies and to eventually provide optimal individualized therapy.

Monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) are the two most common plasma cell disorders characterized by presence of clonal plasma cells and excess of monoclonal immunoglobulin. The traditional diagnostic work-up includes confirmation of clonal plasma cell expansion, evaluation for any associated end-organ damage, and then determination of prognostic variables that may help determine therapeutic intervention as well as long-term outcome (**Table 1**).

Table 1. Patient evaluation.

Diagnostic evaluation
Presence and characterization of monoclonal protein
Serum protein electrophoresis and quantitative immunoglobulin
24-hour urine: total protein & Bence Jones protein
Immunofixation of urine and serum
Serum free light chain and ratio
Bone marrow aspirate and biopsy
Histology
Immunophenotyping – clonality
End organ damage evaluation
CBC with differential
Chemistry panel: serum creatinine and calcium
Skeletal survey
Prognostic evaluation
Albumin
β2 microglobulin
LDH
C-reactive protein
Bone marrow cytogenetics and FISH
Specialized studies for selected patients
Abdominal fat pad or rectal biopsy for amyloid
Solitary lytic lesion biopsy
Serum viscosity if IgM or high IgA levels or serum M-component > 7 g/dL
Immunofixation for IgD or IgE in select cases
MRI with STIR images in oligosecretory disease

Diagnostic Evaluation

Detection of monoclonal protein

The initial laboratory evaluation to detect and quantitate the clonal cellular expansion includes serum protein electrophoresis as well as 24-hour urine protein electrophoresis including immunofixation and quantitation of serum immunoglobulins. IgG subtype is observed in 70%, IgA subtype in 20% and IgM in less than 1% of patients. In an additional 5% to 10% of patients monoclonal light chain only is associated with suppression of all of the three major classes of immunoglobulins. In rare cases (<1%) with suppressed immunoglobulins and a small M peak on serum protein electrophoresis, a less common IgD or IgE myeloma may be suspected. Less than 1%, of patients have non-

secretory myeloma. Suppression of uninvolved immunoglobulins (e.g., IgM and IgG in IgA myeloma) is present in the majority of the patients at diagnosis. Despite a higher initial response rate, inferior survival is observed with IgA myeloma; currently there is no difference in therapeutic approach between the different types of myeloma. Occurrence of biclonal or triclonal myeloma is extremely rare. The idiotype in myeloma does not change with evolution of the disease; however, occasionally myeloma cells may lose production of heavy chain and progress as light chain–only disease or, in rare cases, as non-secretory disease.[1] Occasional transient detection of isotype switch and appearance of abnormal protein bands after high-dose therapy are associated with improved survival, are considered related with normal immunoglobulin recovery and do not represent change in myeloma idiotype.[2] The level of monoclonal proteins required for diagnosis of MGUS versus myeloma is described in **Table 2**.

Table 2. Diagnostic criteria for multiple myeloma and monoclonal gammopathy of unknown significance (MGUS).[34]

Monoclonal gammopathy of undetermined significance
- M-protein in serum ≤ 30 g/L
- Bone marrow clonal plasma cells < 10%
- No evidence of other B-cell proliferative disorders
- No myeloma related organ or tissue impairment (no end organ damage, including bone lesions)*#

Smoldering myeloma
- M-protein in serum ≥ 30 g/L and/or
- Bone marrow clonal plasma cells ≥ 10%
- No related organ or tissue impairment (no end organ damage, including bone lesions) or symptoms*#

Symptomatic multiple myeloma
- Presence of M-protein in serum and/or urine
- Bone marrow (clonal) plasma cells* or plasmacytoma
- Related organ or tissue impairment (end organ damage, including bone lesions)*#

Solitary plasmacytoma of bone
- No M-protein in serum and/or urine*
- Single area of bone destruction due to clonal plasma cells
- Bone marrow not consistent with multiple myeloma
- Normal skeletal survey (and MRI of spine and pelvis if done)
- No related organ or tissue impairment (no end organ damage other than solitary bone lesion)*#

Non-secretory myeloma
- No M-protein in serum and/or urine with immunofixation
- Bone marrow clonal plasmacytosis ≥ 10% or plasmacytoma
- Related organ or tissue impairment (end organ damage, including bone lesions)*#

*A small M-component may sometimes be present.

Myeloma-related organ or tissue impairment (end organ damage) (ROTI): Calcium levels increased: serum calcium > 0.25 mmol/L above the upper limit of normal or > 2.75 mmol/L; Renal insufficiency: creatinine >173 mmol/L; Anemia: hemoglobin 2 g/dL below the lower limit of normal or hemoglobin <10 g/dL; Bone lesions: lytic lesions or osteoporosis with compression fractures (MRI or CT may clarify); Other: symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections (> 2 episodes in 12 months)

Serum free light chains

Development of antibody able to detect epitopes that are hidden when light chain is bound to the heavy chain has now allowed quantitation of free light chain not bound to heavy chain in serum. The serum free light chain (SFLC) assay involves measurement of both κ and λ free light chain in serum and the κ/λ FLC ratio. In a number of benign conditions, such as immune dysregulation and renal dysfunction, SFLC levels may be abnormal; however, only in plasma cell monoclonal disorders is the SFLC ratio abnormal. This test has provided a new measure of myeloma disease burden especially in cases where light chain is produced. SFLC measurement is especially indicated in monitoring patients with AL amyloidosis, oligosecretory or non-secretory myeloma, myeloma with renal failure where 24-hour Bence-Jones protein (BJP) measurements are not reliable, and light chain disease–only MM. In fact over two-thirds of patients previously considered non-secretary have detectable protein by SFLC measurement. The frequency of abnormal SFLC ratios observed in various plasma cell disorders is shown in **Table 3**. However, SFLC measurement complements and does not replace 24-hour urine BJP measurement. SFLC measurement has become an important tool for following response to therapy as well as disease progression. Emerging data also suggest a role for SFLC measurement as a prognostic marker in MGUS, smoldering myeloma and MM. In MGUS an abnormal κ/λ free light chain ratio predicts a higher likelihood of progression to myeloma.[3-7]

Bone marrow examination

The presence of clonal plasma cells in the bone marrow is the second important test in the diagnosis of myeloma. The proportion of infiltrating plasma cells in the marrow (**Table 2**) help define MGUS from MM and smoldering MM. The pattern of bone marrow involvement, diffuse versus nodular, is not critical in diagnosing the disease; however, morphologic features, such as plasmablastic cell type, may suggest aggressive disease.[8] In addition to the number of plasma cells, it is important to confirm the clonal nature of the plasma cells. The most common measure is to detect pres-

Table 3. Rates of abnormal free light chain ratio (rFLC) in plasma cell disorders.

Disease	n	Pts with abnormal rFLC, %
Symptomatic MM[5]	456	96
Non secretory MM[35]	28	68
Light chain MM[36]	224	100
Smoldering MM[37]	273	90
MGUS[3]	1148	33
Amyloidosis[38]	262	98

ence of single type of cytoplasmic light chain by flow cytometric analysis or immunohistochemical staining of plasma cells.[9] An extensive immunophenotypic evaluation of the plasma cell compartment in bone marrow has been used to identify myeloma cells from normal plasma cells. Plasma cells are considered normal if they meet the following criteria on immunophenotyping: $CD38^{+++}$, $CD56^{-}$, $CD45^{+}$, $CD20^{-}$, $CD28^{-}$, $CD33^{-}$, and $CD117^{-}$.[10] This analysis allows an objective marker to distinguish reactive from clonal plasmacytosis and to detect small numbers of clonal plasma cells in minimal disease setting, post therapy. It is important to keep in mind, while evaluating clonal cells using immunophenotyping, that myeloma cells display both intra- and inter-patient variability in surface phenotype. In a recent study the combination of CD38/CD56/CD19/CD45 surface markers were able to detect residual myeloma plasma cells from normal plasma cells in more than 90% of cases and for the remaining patients CD28, CD117, CD33, or CD20 were utilized based on MM cell phenotype at the diagnosis.[11]

End-Organ Damage Evaluation

As described in **Table 2**, the diagnosis of symptomatic MM requiring therapeutic intervention depends on identification of associated end-organ damage. The four common features are detection of anemia, hypercalcemia, renal dysfunction and bone lesions. Detection of lytic bone lesion is by skeletal survey. MRI is more sensitive in detecting both bone lesions and bone marrow involvement by myeloma. Although MRI is not yet incorporated in the standard diagnostic algorithm, if a questionable lesion is observed on skeletal survey it may need to be confirmed by MRI. Detailed discussion of various imaging techniques in myeloma is provided in the subsequent chapter. Similarly, a renal biopsy is indicated only if the association of renal dysfunction with myeloma is in question. For example, if a patient with a long-standing history of diabetes and hypertension has MGUS with renal dysfunction, then a renal biopsy may help identify whether renal involvement is from monoclonal protein, in which case therapeutic intervention may be indicated. In a patient with MGUS, renal damage may be due to amyloid deposition or due to light chain deposition disease, while in a patient with asymptomatic myeloma renal damage may be more frequent due to myeloma cast nephropathy. Additional investigation to detect end-organ effects of myeloma includes symptomatic hyperviscosity confirmed by serum viscocity measurement, detection of amyloidosis by tissue biopsy, and history of recurrent bacterial infections (> 2 episodes in 12 months).

Response determination

Determination of response is a critical element of any therapeutic intervention in general practice as well as clinical studies. Standard criteria are established for such reporting.[12] The most recent update of these criteria incorporates SFLC measurement to allow inclusion of patients previously considered inevaluable and to allow determination of responsiveness in such oligosecretory patients (**Table 4**).[13] Accurate and predictable response determination has now greater importance with increasing evidence that attainment of complete response (CR) is associated with improved survival and that the novel agents and combinations are able to achieve CR in more than 30% of patients. The importance of CR is described further in the chapter on induction therapy. Efforts are underway to define the CR more stringently by including immunophenotypically negative bone marrow as well as resolution of MRI-detected lesions. There is also renewed interest in revisiting the role of achieving molecular CR, which previously was not considered to provide any added benefit; however, with novel agents it may allow prediction of long-term disease-free survival.

Prognostic Evaluation

Following the initial diagnostic work up, more detailed cellular and molecular studies are required to evaluate prognostic variables that determine the disease behavior, define therapeutic strategies, compare results of clinical trials, and predict overall long-term outcome. A number of prognostic factors have been identified in different patient populations and following various therapies. These include factors related to the tumor burden such as β2-microglobulin, > 3 lytic bone lesions, hemoglobin, presence of hypercalcemia and percentage of bone marrow plasmacytosis; factors related to tumor biology such as cytogenetic abnormalities, plasma cell–labeling index, Bartl grade, IgA myeloma, C-reactive protein, LDH and soluble IL-6 receptor; and factors related to host and micro-environmental influences such as bone marrow microvessel density, serum syndecan-1 levels, and MMP-9 and soluble CD16 levels. Finally patient-related factors such as age, serum albumin and performance status may influence overall outcome. It is also important to note that treatment-related factors, such as tandem transplant, use of novel agents and, importantly, achieving CR also influence outcome.

Staging system

A clinical staging system using standard laboratory measurement, developed by Durie and Salmon, was predictive of clinical outcome after standard-dose chemotherapy.[14] However, with the use of high-dose therapy and novel agents the Durie-Salmon system is less predictive of outcome. This may be explained by the fact that the Durie-Salmon system is predominantly focused on tumor burden and these newer therapies are better able to reduce tumor burden, making tumor biology–related factors more predictive of outcome.

The combination of serum β2-microglobin, one of the most consistent predictors of survival in myeloma, with serum albumin has been proposed as a new International

Table 4. Uniform response criteria from the International Myeloma Working Group.[12,13]

Response categories	Response criteria
CR	No M protein in serum and urine; negative immunofixation and disappearance of any soft-tissue plasmacytomas and < 5% plasma cells in bone marrow.
VGPR	Serum and urine M-component detectable by immunofixation but not on electrophoresis or ≥ 90% or greater reduction in serum M-protein plus urine M-protein < 100 mg per 24 h.
PR	≥ 50% reduction of serum M protein and reduction in 24-h urine M-protein by ≥ 90% or to < 200 mg per 24 h. If the serum and urine M-protein are unmeasurable a ≥ 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria. If serum and urine M-protein are unmeasurable, and serum free light assay is also unmeasurable, ≥ 50% reduction in bone marrow plasma cells is required in place of M-protein, provided baseline percentage was ≥ 30%. In addition to the above criteria, if present at baseline, ≥ 50% reduction in the size of soft-tissue plasmacytomas is also required.
MR	≥ 25% but < 49% reduction of serum M protein and reduction in 24-h urine M-protein by 50%-89%, which still exceeds 200 mg per 24 h. In addition to the above criteria, if present at baseline, 25%-49% reduction in the size of soft-tissue plasmacytomas is also required. No increase in size or number of lytic bone lesions (development of compression fracture does not exclude response).
SD	Not meeting criteria for CR, VGPR, PR or progressive disease.
PD	Increase of 25% from lowest response value in serum M protein (absolute increase must be ≥ 0.5 100 mL) and/or urine M-component (absolute increase must be ≥ 200 mg/24 h) and/or bone marrow plasma cell percentage (absolute % must be 10%) and/or only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg per 100 mL). OR Definite development of new bone lesions or soft-tissue plasmacytomas or definite increase in the size of existing bone lesions or soft-tissue plasmacytomas. OR Development of hypercalcemia (corrected serum calcium >11.5 mg per 100 mL) that can be attributed solely to the plasma cell proliferative disorder.

Abbreviations: CR, complete response; VGPR, very good partial response; PR, partial response; MR, SD, PD, FLC, free light chain

Staging System (ISS) (**Table 5**). This system predicts outcome following both high-dose therapy and treatment based on novel agents in both younger and older patients. It has gained acceptance because it uses two simple laboratory measurements. However, lack of inclusion of tumor biology related–factors such as cytogenetics or molecular markers may limit its eventual utility.

Cytogenetics and FISH

MM is associated with significant chromosomal abnormalities that evolve over the course of the disease. Although virtually all patients with myeloma have genomic rearrangement, as the myeloma cells have low proliferative activity, only one-third of the patients have detectable abnormality at the time of diagnosis; on repeated analysis, the detection improves to one-half of patients. The normal karyotypic pattern observed in the remaining half most likely originates from dividing normal hematopoietic cells. Numerical abnormality is observed with hyperdiploid karyotypes and infrequent translocations, while non-hyperdiploid karyotype is associated with high prevalence of translocations predominantly involving the 14q32 Ig gene locus.[15,16] Almost half of the patients have hyperdiploidy and they present with high chromosome number (median = 54) involving non-random gains of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21. Patients with hyperdiploidy have better overall survival, while patients who present with hypodiploidy have more resistant disease and shorter survival.[17]

Table 5. International Staging System.[39]

Stage	Criteria	Median Survival, mo
I	Serum β2-microglobulin < 3.5 mg/L Serum albumin ≥ 3.5 g/dL	62
II	Not stage I or III	44
III	Serum β2-microglobulin ≥ 5.5 mg/L	29

Certain recurrent changes have been identified and correlated with overall outcome. By conventional cytogenetics, abnormalities such as t(4;14), t(14;16), part or whole chromosome 13q deletion and loss of 17p13 carry a poor prognosis in patients undergoing high-dose therapy; hyperdiploidy and t(11;14) translocations are associated with better outcome.[18,19] The significance of chromosome 13q deletion remains enigmatic as it is also observed in patients with MGUS with unclear relationship to its transfor-

mation to myeloma. Additional recurrent cytogenetic markers associated with poor prognosis, such as gain of 1q arm and loss of 1p, are being identified. Importantly, recent studies have shown that bortezomib is able to overcome the adverse outcomes associated with t(4;14) and chromosome 13 deletion.[20] These studies highlight a point that the prognostic factors, both clinical as well as molecular, may be more closely related with the type of therapeutic intervention rather than disease itself and that novel targeted therapy may be able to overcome the adverse effects of these molecular changes.

By using fluorescent in situ hybridization (FISH), specific genetic changes in interphase cells can be detected, overcoming the problem of lack of dividing cells required to obtain conventional cytogenetics. Probes targeting 17p (p53), t(11:14) (IgH, cyclin D1), t(4;14) (IgH, FGFR3) and 13q14 (Rb-1) are commonly used to identify and prognosticate. Recently, amplification of the 1q and/or deletion of 1p arm have been described as predicting poor outcome.[17] A number of large studies have identified the presence of del 17p, t(4;14) and t(14;16) as well as del 13q34 detected by FISH as predicting shorter overall survival, while t(11:14) is associated with improved survival (**Table 6**).[15,21-27] However, even in patients with these genetic abnormalities, outcome may be variable due to the role of other genetic and microenvironmental factors.[17] Both cytogenetics and FISH should be performed at the time of diagnosis to determine therapy as well as long-term outcome. The role for repeat cytogenetics and FISH at the time of relapse is unclear; however, a growing body of evidence suggests that myeloma cells evolve with progression and can acquire genetic features that may predict subsequent poor outcome. It is also possible that the genomic changes were not detected at the initial evaluation, and a repeat measurement may be able to pick up such changes.

Oncogenomic Profiling

High-throughput gene expression profiling has defined specific pathways important in the multistep transformation of normal plasma cells to MGUS and MM. These studies have allowed for molecular classification of myeloma, identified novel therapeutic targets, and provided the scientific rationale for combining these agents to overcome drug resistance and improve outcome.[28] The availability of large-scale expression profiling data in uniformly treated patient populations has provided the basis for an RNA-based prognostic classification system.[16] Moreover, a high-resolution array–based comparative genomic hybridization (aCGH) analyzing recurrent copy number alterations with integrated expression profiling data has allowed for further identification of DNA-based prognostic classification systems. These initial attempts at molecular classification and prognostication (**Table 7**) will need further validation and incorporation into more commonly available methods for wider application.

Cyclin D (D1, D2, or D3) expression is considered to be dysregulated in almost all MM and may be associated with pathogenetic mechanisms as well as prognosis. Bergsagel et al have used gene expression profiling to identify recurrent translocations, specific trisomies, and expression of cyclin D genes to divide myeloma into 8 groups (11q13, 6p21, 4p16, maf, D1, D1+D2, D2, and none). These groups have differences in the prevalence of bone disease, extramedullary tumors and progression. Although biologically this system shed some light on possible pathogenetic mechanisms, it is not a practical system for wider clinical application.[16]

Table 6. Prognostic significance of FISH-detected genetic changes in myeloma.

Study	No. patients	Therapy	Genomic abnormality/ FISH probe	Frequency, %	Median overall survival, mo
Zojer et al[21]	104	Conventional chemotherapy	13q34/Rb-1, D13S319	46	24 vs > 60 (P = .008)
Shaughnessy et al[22]	231	High-dose therapy x 2	13q34	51	Significantly shorter in del 13
Fonseca et al[15]	325	Conventional chemotherapy	13q34/Rb-1, D13S319	54	34.9 vs 51 (P = .021)
Chiecchio et al[23]	729	Conventional chemotherapy	t(4;14) t(11;14) Δ13 17p13	12 15 48 9	19 vs 44 (P = .002) NR vs 36 (P = .29) 29 vs 47 (P < .001) 19 vs 43 (P < .001)
Facon et al[24]	110	High-dose therapy	Δ13/D13S319	38	27 vs 65 (P < .0001)
Chang et al[25]	105	High-dose therapy	Del 17p13/p53	10	15 vs 48 (P < .0008)
Drach et al[26]	72	Conventional chemotherapy	Del 17p13/p53	33 newly diagnosed 55 relapsed	13.9 vs 39.7 (P < .0001)
Keats et al[27]	208	Conventional chemotherapy	t(4;14)	15	21 vs 43 (P = .006)

Abbreviations: NR, not reached.

Table 7. Oncogenomics-based myeloma classification system.

Study	Type of genomic study	No. patients	Outcome measure	Applications/limitations
Bergsagel et al[16]	Cytogenetics/FISH and expression profile	—	8 translocation/cyclin D (TC) groups	Combinations of methods being used. Difficult to apply. Clinical relevance unclear.
Shaughnessy et al[29]	Expression profile – 17-gene model*	532	High- and low-risk groups	Applicable to general population but requires validation.
Decaux et al[30]	Expression profile – 15-gene model†	182	High and low risk groups	Applicable to general population but requires validation.
Carrasco et al[31]	aCGH profile	67	Four groups based on amplifications and deletions	Difficult to apply in general population yet

* KIF14, SLC19A, CKS1B, YWHAZ, MPHOSPH1, TMPO, NADK, LARS2, TBRG4, AIM2, ASPM, AHCYL1, CTBS, MCLC, LTBP1, 242488_at, 1557277_a_at
† CNDP2, STMN1, AFG3L2, STK38, PARP1, CPSF6, LOC151162, TOX2, FRY, FLJ21438, MGST1, ALDH2, CTSF, ATF4, FAM49A

Shaughnessy et al investigated the gene expression profile of myeloma cells in 532 newly diagnosed patients with myeloma treated on 2 protocols incorporating tandem autologous transplantation to molecularly define high-risk disease. Using log-rank tests of expression quartiles, 70 genes, linked to shorter durations of complete remission, event-free survival, and overall survival were identified. The ratio of mean expression levels of upregulated to downregulated genes defined a high-risk score that was an independent predictor of outcome endpoints in a multivariate analysis ($P < .001$) that included the ISS and high-risk translocations. A subset of patients with high-risk scores had a 3-year continuous CR rate of only 20%, as opposed to a 5-year continuous CR rate of 60% in the absence of a high-risk score. Interestingly, multivariate discriminant analysis identified a 17-gene subset that performed as well as the 70-gene model.[29]

Decaux et al from the Intergroupe Francophone du Myélome studied gene expression profiles of myeloma cells obtained at diagnosis in 182 patients and identified the best 15 genes for calculating a risk score associated with the length of survival.[30] This analysis divided patients into a high-risk group characterized by the overexpression of genes involved in cell cycle progression and its surveillance, and a low-risk group with hyperdiploid signature and heterogeneous gene expression. The results were confirmed in a test set as well as in independent cohorts comprising 853 patients with MM. Overall survival at 3 years in a low-risk or high-risk group was 91% and 47%, respectively. These results were independent of traditional prognostic factors. It is interesting to note that although both these studies included patients undergoing high-dose therapy, the 15- and 17-gene models do not share a single common gene. This highlights the complexity of biological behavior of the tumor and the fact that the ultimate use of such expression data will require significant additional work. It also highlights the molecular redundancy in the tumor cells that drive its clinical behavior.

Carrasco et al have studied aCGH and identified areas of chromosomal amplifications and deletions in both MM cell lines and primary patient samples. Integration of these data with expression profiling has been used to develop a DNA-based classification system that has allowed for further identification of new therapeutic targets.[31] For example, this study showed that among hyperdiploid MM, ch11 gain confers a more favorable outcome, whereas ch1q gain and ch13 loss drive poor outcome. Overall, this study provides a framework that may help identify disease subgroups to improve clinical management and to discover targeted drugs for specific patients.

Development of Predictive Model

In an effort to develop a prospective pharmacogenomics platform, Mulligan et al have developed predictive classifiers of response and survival with bortezomib using data generated from multicenter international clinical trials of bortezomib in MM. Myeloma cells were enriched using a negative-selection procedure and used for gene expression profiling. Response and survival classifiers were significantly associated with outcome and confirmed on independent data.[32] Predictive models and biologic correlates of response showed specificity for bortezomib compared to dexamethasone. This study opens the possibility of patient-specific selection of agents that are likely to be effective and avoid unnecessary toxicity.[33] Prospective clinical trials are now necessary to validate such signatures prior to their general application.

Ongoing genomic and proteomic studies in MM have improved our understanding of myeloma pathobiology and allowed for molecular classification. Moreover, the high-risk features identified above are highly dependent on the therapeutic intervention used. For example, the newer biologically based therapies such as lenalidomide and bortezomib are able to overcome drug resistance, and some traditional adverse prognostic factors are no longer predictive of survival. Additional molecular studies with genomic and proteomic

analysis, including single nucleotide polymorphism, may identify a uniformly applicable prognostic system.

Disclosures

Conflict-of-interest disclosure: The author is on the Novartis, Millennium, and Celgene speakers bureaus. Off-label drug use: Use of Revlimid and Velcade in newly diagnosed patients.

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