Role of genetics in myeloma risk stratification

R. Fonseca

Mayo Clinic Cancer Center, Collaborative Research Building, Scottsdale, AZ, USA

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A B S T R A C T

Risk stratification, mostly done via genetic testing is of paramount importance in the prognostication of myeloma for counseling treatment planning and prognostication. This paper discusses the implications of these novel genetic factors and current therapy for the disease.

Introduction

It is now widely accepted that multiple myeloma (MM) is a heterogeneous disease with the major subtypes being defined by the underlying genetic aberrations of the clonal plasma cells (PC).[1,2] These genetic aberrations have been used as the foundation to classify the disease, establish prognostic categories, and to a limited degree, serve as predictive indicators.[1–3] Many aspects of the disease are directly influenced by the presence of specific genetic changes. These include clinical features, "natural history of the disease," aggressiveness markers and other.

Obtaining this genetic information is now considered routine clinical care for MM, and should be required for all ongoing and future clinical trials.[4,5] In general, five to eight subgroups of MM have been identified using the various methodologies, although many of these categories largely overlap.[6–8] Subdivision into many small subgroups carries the risk of creating fragmented analyses that lack statistical power to be reliable[9] In response to this challenge, a dichotomy classification has been proposed that segregates patients into high and standard risk categories (+/- and intermediate risk category) (www.msmart.org).[9,10] While no specific therapy exists that is primarily directed at high-risk MM, three important corollaries have emerged: i) clinical trials aimed at high-risk MM are being developed; ii) identification of risk categories has allowed standard-risk patients to be treated with more chronic disease controlling strategies; iii) and identification of high risk features is critical for proper counseling of patients with these variants of the disease (avoidance of "MM is a chronic disease" hue to the patient counseling discussion).

Background and techniques

The field of MM cytogenetics has invigorated since the first observations were made in the mid 1980s and 1990s regarding a potential role for cytogenetic changes to discriminate patients with more aggressive disease.[11–13] The field quickly evolved to the application of molecular cytogenetics (i.e., FISH)[3,14,15] and more recently high throughput genomic platforms (i.e., gene expression profiling (GEP)[16–18] and array based comparative genomic hybridization (aCGH).[19,20] Deployment of more modern genomic tools has been hampered by practical limitations regarding reimbursement and regulatory considerations. However, a recent commercialization of GEP provides a test case in which modern genomics is introduced into routine clinical care for the disease.[21]

Until such advances are widespread, FISH remains the standard for disease prognostication.[2,3,14,15] It is imperative, however, that FISH be done in such a manner that the scoring is restricted to PC only.[22] Many commercial laboratories continue to perform FISH in unsorted, or otherwise unselected, nuclei making results interpretation unreliable. Samples submitted to cytogenetic laboratories often represent the last pull of those collected for clinical purposes. It is, therefore, not uncommon to observe a much lower fraction of PC in those aspirates, mostly as a consequence of hemodilution. Clinicians must be wary and look for the specific nomenclature provided in the cytogenetic response. A standard "*nuc ish*" (for nuclear interphase FISH) should be considered evidence of unreliable testing. PC must be either sorted (for instance using anti-CD138 magnetic beads)[3] or the FISH analysis must be coupled with immunofluorescence detection of the clonal PC.[22] PC identification can readily be done using cytoplasmic light chain staining (e.g., cIg-FISH).[22]

Diagnosis and disease classification

All cancers can be classified from various perspectives that describe features and attributes of the clonal cells (and sometimes the host as well).[23,24]. In MM, the disease

diagnosis does not require genetic studies *per se*, as the morphology is obvious and consistent enough that no specific genetic marker is required for diagnosis. A rare exception to this occurs in cases where MM must be differentiated from other late B-cell malignancies, such as Waldenström macroglobulinemia (WM).[25,26] For instance, the rare version of MM that produces IgM monoclonal proteins (IgM MM) can be differentiated from WM, since the former has a high prevalence of t(11;14)(q13;q32)[26] and the latter is more likely to have 6q deletions and never has t(11;14)(q13;q32).[27]

Despite recent treatment advances, namely derivates of thalidomide and proteasome inhibitors[28,29] being touted as overcoming some of the negative prognostic implications for patients with high risk genetic features (e.g., t(4;14)(p16;q32)), these patients still tend to have more aggressive clinical courses with overall worsened prognosis from the time of diagnosis.[15,30,31]

Biological classification

A biological classification of the disease is primarily driven by the orderly acquisition of genetic changes that drive clonal proliferation.[2,7,8,32] Because the classification identifies discrete subgroups of the disease, these classes may be associated with dissimilar outcome, but not necessarily. This biological classification is primarily driven by genetic features that can be observed since the premalignant phases of the disease are associated with specific genetic progression events (from benign stages to malignant).[33,34] In MM, this is best exemplified by chromosome 14 translocations as one group and trisomies leading to hyperdiploidy as another.[35–37] Accordingly, at the very top hierarchical level, MM can be classified into hyperdiploid and non-hyperdiploid.[36] This classification is one that stands the test of time and is usually revealed by testing performed via various technologies.[2]

Genetic prognostic classification

While many systems exist that can estimate prognosis for MM patients, recent efforts are all focused on the identification of genetic markers that can discriminate clinical outcomes.[3,15,38] These genetic prognostic markers may be the same that also form biology subclasses (e.g., t(4;14)(p16;q32)), but can also include genetic progression events, such as chromosome 17 deletions and abnormalities of chromosome 1 (deletions of 1p and 1q amplifications). Genetic prognostic classifiers are accordingly exclusively focused on those features of the clonal cells that dictate outcome. In contrast, clinical classifications identify host features that may portend a higher likelihood of death only because of host specific features and not truly describing a more aggressive nature of the clonal process.[23,24]

Genetics have been used by several large studies to predict the outcome of MM patients treated with conventional[14,39] and high dose chemotherapy followed by stem cell transplant (SCT).[3,15,16] The majority of these studies were done when treatments were primarily based on alkylator therapy. Now multiple studies are underway to understand better the prognostic implications of classic genetic classifiers in cohorts of patients treated with proteasome inhibitor and imuunomodulatory drugs.

While there is still a paucity of data regarding the value of prognostic factors for novel therapeutics, it appears that bortezomib can neutralize some of the negative effects of some of the high-risk markers,[40–42] and emerging data exists regarding thalidomide and lenalidomide.[43–45] Despite the initial excitement all of the studies addressing the role of proteasome, inhibitors in patients with high-risk disease have been relatively underpowered to address the question conclusively, and most have been done in the setting of relapsed and refractory MM, with only few addressing the question in the upfront setting.[30,41,43] It is also important to note that the value of prognostic factors needs to be validated according to the specific stage of the disease; prognostic factors validated in the upfront treatment of the disease may not have similar effects in previously treated patients.

Predictive capacity

In some instances, genomic markers may be used to predict responsiveness to specific therapy (e.g., herceptin in her2neu positive breast cancer). As of yet, there are no validated predictive markers for MM. This information, if available in an expeditious, economical, and practical manner could be of major significance in choosing the various therapeutic options for the disease. Since most patients currently still receive all agents at one point or another of their disease, it is likely this information would only determine sequencing of treatment. The only exception to this would be if we had such predictive power to know with great certainty that a proposed therapy would be of no value in patient care, and then attempts at it would be futile. One unvalidated example of a predictive marker is determination of constitutive upregulation of the NF-kB pathway via mutations.[46] Another possibility is that a pathway or a cell surface marker is specific enough so that therapy would only be dictated in cases with such phenol/genotype.

Specific genetic categories

Deletions of 17p

Among all MM, genetic factors deletions of chromosome 17 remain the single most important prognostic factor.[3,14–16,47–50] These abnormalities were identified originally in patients treated with conventional forms of chemotherapy and SCT but have persisted as prognostic in patients treated with lenalidomide and bortezomib.[43–45,51] A recent study of a large group of patients receiving induction treatment with bortezomib and dexamethasone followed by SCT showed that novel therapy has had a minimal impact among patients with 17p13 deletions.[45] One large study continues to show the importance of -17 as a marker of negative outcomes.[30] While the exact gene involved in the negative prognosis associated with these deletions has not been fully elucidated, all evidence points to *p53* deficiency as a culprit in the more aggressive clinical course.[47]

t(4;14)(p16;q32)

This translocation was originally described as associated with an inferior outcome in a series of patients treated with alkylators, both at standard doses and with

SCT.[14,16,52–54] This translocation is associated with unfavorable outcomes and more aggressive clinical features at the time of diagnosis.[14,16,52–54] In the study of Shaughnessy and colleagues, patients with this translocation appear to be more likely to have a high risk GEP signature.[16] While recent data initially showed that the negative prognostic implication of the translocation was eliminated with the use of bortezomib,[40,41] subsequent studies have failed to show this. In fact, the aforementioned large French series shows that while bortezomib has improved outcome in t(4;14)(p16;q32) patients as compared with older therapies, the genetic marker is still prognostic in a large group of patients treated with this drug.[45] Another large Spanish study has shown similar results and confirms that high-risk genetic features still portend an unfavorable outlook for patients.[30] It can now be summarized that while novel therapeutics have improved the outlook of most patients, these gains are distributed unequally, being minimal for -17 patients, moderate for t(4;14)(p16;q32), and greatest for standard risk disease.[30]

t(14;16)(q32;q23) and other MAF abnormalities

We originally identified abnormalities of C-MAF, associated with the t(14;16)(q32;q23) as a negative prognostic feature in MM among patients treated with conventional doses of alkylator therapy.[14] Subsequent studies showed similar effects when patients were treated with high dose chemotherapy. In a GEP study, those with t(14;16)(q32;q23) were more likely to exhibit features of disease aggressiveness.[16] Another study looking at MAF-b translocations also identified this factor as associated with an inferior outcome amongst MM patients.[55] Routine testing for MAF abnormalities has not been universally embraced given the less frequent nature of this aberration. A recent report by the IFM has questioned the prognostic significance of t(14;16)(q32;q23) given that a large set of patients tested showed a neutral effect on prognosis.[56] This remains puzzling, as all other previous reports have shown important effects on prognosis. In this French study, those with t(14;16)(q32;q23) had a high propensity to display many circulating cells, a hallmark of aggressive MM.[56] While we think it is quite possible this may be due to type II error, further studies are needed to clarify or dispel the importance of this translocation as a prognostic marker.

Chromosome 1

Multiple studies have shown that chromosome 1 abnormalities are associated with an inferior outcome in MM.[57–61] The interpretation of these studies is confounded since these aberrations, chromosome 1p deletions and 1q amplifications, are highly correlated, and discerning individual contribution is nearly impossible.[37] Chromosome 1 aberrations have not yet made their way into standard clinical practice and continue to be investigated.

Genomic testing beyond FISH

GEP

The power of GEP as a prognostic tool[17,18] has been best exemplified by the work of investigators at the University of Arkansas.[8,16] Using a large patient dataset, they have been able not only to provide a classification of the disease (albeit very similar to all others proposed),[8] but also more importantly, identify 15% of patients with a very poor prognosis.[16] The data have been validated in other scenarios but need further validation in the context of novel therapies, although it is likely to be useful in these setting as well.

Practical limitations exist for widespread applicability of this test, but they are being overcome with standardized methods for cell processing. The test must be done in purified PC that needs to be shipped to reference laboratories. The results are interpreted as "signatures" indicative of high-risk genetic features. A new commercial venture has begun offering the test as a reference laboratory.[21] GEP also needs to be validated further as the test of choice in patients treated with various combinations of modern agents. There is no doubt from the scientific perspective that these strategies represent the best method for prognostic estimation of MM. A balance between the power of the predictive ability of genomics and practical implications is needed to integrate this fully into clinical practice.

aCGH

A series of studies has looked at the role of aCGH as a prognostication tool for MM.[19,20] The advantages of this platform are that the results are more stable and therefore more reliable. The other advantage is that the test can be easily converted to other standard clinical testing, such as FISH. Nevertheless aCGH has not gained mainstream acceptance as a routine test for MM given some of the same practical limitations as GEP (such as the need for purified plasma cells, regulatory efforts, etc.). One study showed that patients could be readily classified into three major prognostic subgroups based on a combination of genomic loss/gain (amp5q and deletions of 12p) and 2-microglobulin.[19,20] Another smaller study also looked at the use of aCGH as a classifier for the disease and with possible prognostic implications.[19,20]

Sequencing

There is minimal information regarding sequencing strategies as it relates to clinical outcomes and prognosis in MM. Some studies have shown that classic oncogenes and tumor suppressor genes can be altered in MM, but these have not reached the point of clinical utility.[47,62,63] Recent efforts using next generation sequencing will soon be published but have not been yet implemented with a clinical slant (Chapman *et al*, In press, Nature 2011).

Practical aspects of genetic testing

As aforementioned, the accurate genetic classification of patients can have profound consequences on clinical decisions, including counseling and treatment selection.[10] Furthermore, identification of risk categories should be paramount in developing specific clinical trials that address a major unmet medical need; the management of high-risk MM.[2] Results obtained from clinical genetic testing should be actionable and provide information with utilitarian value to clinicians.

Genetics prognostic tools should be reliable in predicting outcome of patients and also have desirable features to be developed as standard clinical laboratory tests (i.e., good reproducibility and accuracy). Ideally, they should be widely available and easy to interpret. Prognostic estimation with FISH-based strategies fulfills many of these requisites, albeit with GEP having a greater ability to discern outcome. If current genomic platforms can be employed in determining the outcome in a community setting for MM patients, they are likely to replace FISH as the diagnostic tool of choice (Table 1).

Recommended genetic clinical testing in myeloma

It is now accepted that all MM patients should have disease risk stratification done by employing one or more genetic tools.[2,10,15,30] In the past, this was mostly done using standard karyotype analysis, given the powerful implications of on outcome for patients with chromosome abnormalities. The limitations of this test made the diagnostic community move forward towards more modern techniques, such as FISH and now GEP. The GEP strategy promises to deliver the highest impact in selecting patients with high risk myeloma but still practical limitations have prevented widespread use.[16]

GEP recommendations

GEP is limited in that it is only available at reference laboratories,[21] needs to be done using purified PC, and is still fraught with complexities regarding reimbursement, coverage, and so on. It assumes a sufficient quantity of plasma cells is available for RNA extraction and of sufficient quality to perform the test. Unfortunately, not all patients have enough cells to be able to have GEP done, perhaps with smaller RNA requirements, the fraction of patients with successful results will increase. The purification process must be validated *post-hoc* for assurances that the product is composed predominantly of clonal PC. The cells and products should be maintained under optimal conditions to avoid degradation. The test is very appealing as it provides the most desirable genetic information in one simple step and has been shown to have the highest predictive value for any genetic classifier of MM. It has the capabilities of detecting those with the more aggressive disease, and can serve as comfort for those without this high-risk signature.[16] There is no doubt that GEP strategies will and should become standard of care in the future.

FISH testing recommendations

The recommended clinical testing will undoubtedly change over time if one uses FISH as a primary diagnostic tool, as new probes and markers will be discovered and validated (and some eliminated).[2] Some of the testing could be done only once as the information provided will not change, and some can be repeated since there may be changes over time.

FISH testing must be done always using a concurrent marker that identifies the PC or only with nuclei of previously sorted PC. Otherwise, the results should be considered unreliable. FISH is limited in that it addresses genomics only in a "close ended" question format and falls short of proving a comprehensive perspective on the genomic complexity of the disease. Its categorical allocation of patients into subsets, therefore, is intrinsically limited and overall, can only provide general guid-

Table 1. Comparison between FISH and GEP.

	FISH	GEP
Ability to discern highest risk categories	++	+++/++++
Clinical test	Yes	Now being deployed
Information obtained	Limited	Global genomics
Expense	Similar	Similar*
Can do overnight testing?	Yes	Yes
Need for reference laboratories	Yes	Yes
Number of cells needed for analysis	100	>100,000
Requires cell enrichment/purification?	No for clg	Yes
Suitable for cases of minimal plasmacytosis?	Yes	No
Number of cases with informative results	Most (>90%)	Low (<50%)
Covered by insurance	Yes	Sometimes

** Cost may be less for GEP depending on the number of probes used for FISH.*
*** Needs metadata generation and interpretation.*

Table 2. Risk stratified testing in myeloma.

Test	Markers	Frequency
High risk translocations	T(4;14) and t(14;16)	Needs to be tested only once to establish category
17p13 deletions	FISH -17p13	Can be tested at multiple times as it is acquired with progression
GEP	High risk GEP UAMS High risk IFM signature	Centrosome index Can be done at baseline and may be repeated. Data also emerging on predictive ability

ance. It is quite likely that a future that involves GEP would make FISH potentially dispensable (the only exception now being determination of 17p deletions not adequately covered by GEP).

Frequency of FISH testing recommended

An attempt has been made to summarize current recommendations and thoughts regarding the frequency of testing needed for myeloma (Table 2). While undoubtedly this will change over time, this can be used as a general guide on what to test and when.

Conclusion

Until the time that genomic tools (i.e., GEP, mRNA sequencing, genome sequencing) become standard clinical tools, at minimum, FISH must be done to establish prognostic criteria for MM patients. FISH identifies 25% of patients with high-risk disease and is fundamentally not that dissimilar to GEP, although clearly of less power. For now, the application of FISH techniques are likely to remain as the main way of determining the prognostic outcome of MM based on genomics.

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