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Cytogenetics and Chromosomal Abnormalities in Multiple Myeloma-A Review

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Abstract

Multiple myeloma constitutes about 1% of all malignancies. It has complex cytogenetic and heterogeneous clinical presentations. Recent advances in molecular diagnostic methods have shed light into the chromosomal and molecular changes underlying the pathogenesis of plasma cell dyscrasias, such as Monoclonal Gammopathy of Unknown Significance (MGUS), Smoldering Myeloma (SMM), Multiple Myeloma (MM), and plasma cell leukemia. It is now well established that majority of hypodiploid and hypotetraploid karyotypes, otherwise known as non-hypodiploid karyotypes, harbor chromosomal changes that are considered high risk and have an aggressive disease course. The hyperdiploid category consists of trisomies of uneven chromosomes, and majority of the patients have a good prognosis, although a minority of patients have aggressive disease with up-regulation of proliferative genes. Risk stratification with gene expressions profiling and aCGH studies have helped classify patients into high risk, intermediate risk and good risk categories which are helpful in guiding therapy. While t(4;14) and del(17p) are considered to be the most deleterious cytogenetic abnormalities, del(13) by FISH analysis is considered an intermediate risk and t(11;14) is considered as a good risk marker. The worst outcomes are observed in the high risk category, and even the most intensive treatments cannot fully overcome the negative impact of genetic findings. Although some novel agents are showing promise in changing the outcomes of t(4;14), del(17p) remains a challenging disease. While many targeted therapies are under development, more work needs to be done in establishing and integrating routine testing of these cytogenetic markers into clinical practice to individualize treatment although within specific genetic subgroups there remains a high degree of variability in outcome determined by other factors, mainly the extent of the disease.

Keywords: Multiple myeloma; Cytogenetics; Molecular genetics

Introduction

The understanding of the biology of multiple myeloma has evolved rapidly with the introduction of molecular genetics and major advances have been made in the last few decades, which have changed myeloma from an incurable into a curable disease in at least a substantial fraction of patients [1,2]. Technological advancements have identified chromosomal and molecular abnormalities that underlie the pathogenesis of myeloma and are forming the basis for future targeted therapies. Multiple myeloma is a heterogeneous disease with some patients relapsing early after treatment, while about 50% of patients achieving a complete remission; enjoy remissions for more than 10 years [1,2].

Here we present a review of (1) known chromosomal and molecular abnormalities occurring in multiple myeloma and their effect on prognosis and treatment. (2) The utility of conventional cytogenetics, interphase *FISH*, Gene Expression Profiling (*GEP*) and array-Comparative Genomic Hybridization (*aCGH)* studies, and (3) a brief outline of the newer therapies and targeted agents. We will also review the known literature on chromosomal abnormalities with respect to age, race and extra-medullary disease in multiple myeloma.

Aneuploidy and Chromosomal Abnormalities

Using sensitive techniques, chromosomal abnormalities are considered universal in multiple myeloma [3]. High density array-Comparative Genomic Hybridization (aCGH) and Single Nucleotide Polymorphism (SNP) based mapping pathways have identified the presence of abnormalities in 100% of myeloma patients [4,5]. This is in contrast to the previously known literature that showed presence of abnormalities in approximately one third of the patients with conventional metaphase cytogenetics.

Over the years much has been learnt about the chromosomal aberrations and the heterogeneity that involves the cancer cells including those in multiple myeloma [6]. Multiple myeloma reveals multiple numerical and structural chromosomal abnormalities. It is different from most other hematologic malignancies, which are typically less complex, and myeloma resembles more the complexity of solid tumors [7]. Plasma Cells (PC) that have transformed to myeloma exhibit complex immunophenotypic as well as cytogenetic changes. Unlike leukemia, the karyotypic complexity in multiple myeloma results in delay in identification of abnormalities involved in the pathogenesis of the disease even with cytogenetics [3,6]. The prognostic significance of the difference in ploidy (hyper and hypo) was first proposed by Smajda et al. [8]. In a large series published by Mateo et al. [9] consisting 915 patients, nearly 43% of the patients evaluated showed hyperdiploid DNA content (H-MM) [9]. Tetraploidy or near- tetraploid status was seen in only 2%, Hypodiploid cell (NH-MM) content was seen in 46% of the patients and bi-clonality was seen in 26% of the population [9]. The study also demonstrated differences in antigen expression of the plasma cells which may explain not only the difference in clinical features but also in the genetic variability. The study was able to distinguish antigenic profile of myeloma clones by correlating them to the ploidy status. Of note, cells with increased expression of CD 20+, CD 28+ and loss of reactivity to CD 56- and CD 117 were predominant in the NH-MM clones. It has been hypothesized that the interplay between differences in expression of antigens and immunophenotypic leads to genetic and clinical variability [9].

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These commonly known aberrations in myeloma are arbitrarily classified under H-MM or NH-MM karyotypes as discussed earlier. Studies have shown that the distribution of the patients between the two groups is nearly uniform and has important prognostic information.

Hypodiploid karyotype

The abnormalities associated with this karyotype include chromosomal additions, deletions, translocations or whole chromosomal losses. In addition to hypodiploid status, pseudodiploid and near tetraploid chromosomal karyotypes are also included in this category [8,10,11]. IgH translocations are most frequently found abnormality in this group of patients, reportedly in up to 60% of the patients [12-14]. The other important category of aberrations is deletions, commonly involving chromosomes 13,14,16 and 22 [8,10,11,15,16]. This karyotype group has been associated with chromosomal abnormalities that not only portend poor survival but also poor response to therapy [8,17-20]. It should be noted that neoplasms other than myeloma, such as acute lymphocytic leukemia, are also associated with poor outcomes when hypodiploid [21-23].

Hyperdiploid karyotype

This group constitutes recurrent abnormalities of mostly the uneven chromosomes: 3,5,7,9,11,15,19 and 2 [5] and is generally associated with a good prognosis [8] unless gene expression profile indicates a proliferative gene pattern [24]. IgH translocations are not exclusively seen in the hypodiploid group; about 10% of the hyperdiploid patients carry such translocations [25].

Ig H translocations: The translocations involving the IgH locus on 14q32 are postulated to be present in nearly 60% of the cases of myeloma and are more prevalent in NH-MM than in H-MM [12-14]. The most common recurrent translocations involving the IgH locus are t(4;14), t(14; 16) and/or t(11;14); less common are t(6;14) and t(14;20), whereas 14q32 translocations without previously specified translocations are not associated with this karyotypes at a higher frequency [13]. IgH translocations are commonly involved with the chromosome loci of 11q13, 16p23 and 4p16 resulting in dysregulation of oncogenes cyclin-D, *c-maf* and Fibroblast Growth Factor 3 (FGFR3), respectively [26-28]. The t(6;14) involves the Cyclin D3 gene [29] and the t(14; 20) involves the up regulation of maf-b gene [30-33]. Boersma et al. [32] performed a study using a double-color immune-Fluorescence In Situ Hybridization (imuno-FISH) technique. They reported finding t(14q32) in nearly all clonal plasma cells indicating the ubiquity of this particular translocation in MM [32]. Also, the high frequency of these translocations already present at diagnosis supports previously published reports that these translocations are an early event in the pathogenesis of multiple myeloma [34].

Translocation (11;14)(q13;q32): These translocations involve the IgH gene on chromosome 14 and the CCND 1 (cyclin D) oncogene on 11q13 and are seen in nearly 15% of patients with multiple myeloma [12,35]. This results in over expression of the CCND/PRAD 1 oncogene which is seen in many lymphoid malignancies as well as in myeloma [36-38]. This translocation is found to be associated more with light chain only myeloma and AL amyloidosis [12,35,39,40] and it is present almost always in cases of IgM myeloma [41].

These findings indicate that the immunoglobulin subtype involved in myeloma and its association with the recurrent translocations may not be just a random process [35]. This translocation is often associated with lymphoplasmacytic morphology and the plasma cells are often found to be positive for CD20. t(11;14) is associated with a favorable outcome when compared to patients without this translocation in a study by Fonseca et al. [42] and others; however, the differences lacked statistical significance [42]. The study from UAMS reported two distinct subsets of patients with t(11;14) with different outcomes and only the subgroup with CD20 positive plasma cells had a better outcome [43]. This difference in observations of the impact of t(11;14) may be attributed to the underlying genetic heterogeneity as there were some groups of patients harboring this translocation with a more aggressive disease like plasma cell leukemia. The t(11;14) can also be useful in distinguishing Waldenström's disease from monoclonal IgM myeloma as the former is almost never associated with IgH translocations [41,44]. Patients with this translocation tend to remain sensitive to chemotherapy even after relapse. This continuing sensitivity disappears after patients acquire a del(17p) abnormality (Tricot, personal observation).

Translocation (4;14): The t(4;14) (p16;q32) was first reported by Chesi et al. [28] and constitutes nearly 20% of the IgH translocations in multiple myeloma. It results in dysregulation of the oncogene FGFR3 [28]. These findings were supported by similar findings by other studies [45,46]. Clinically, the t(4;14) was associated with a higher frequency of IgA myeloma [12,35] which is known to be associated a worse outcome. The association of t(4;14) and IgA was also confirmed by Moreau et al. in their study [35]. It has been reported that this translocation results in activation of 2 genes, FGFR3 and Multiple Myeloma SET domain (MMSET domain) which are located at the locus 4p16.3 [28]. However, FGFR3 is only involved in two thirds of patients with this translocation while MMSET is always involved and it remains unclear how this results in the development of multiple myeloma [26]. They also reported that this translocation occurs earlier in the development of the myeloma and that the FGFR 3 gene activation leads to the initiation of mitogen-kinase pathway through *ras* [47]. It has also been shown that the FGFR 3 activation confers IL-6 independence to the plasma cell [48]. IL-6 independence is also seen with activating N- *ras*, K-*ras* mutations [7,49,50] which are seen in about 40% of the myeloma patients and they are believed to play a role in tumor progression [7]. It could be concluded that the FGFR3 and *ras* mutations function in an analogous manner but are mutually exclusive as was found in their study on mouse by Chesi et al. [26]. The activation of FGFR3 can be blocked by specific small molecule inhibitors or monoclonal antibodies that are under development [51].

The association of t(4;14) with poor survival and worse outcome was confirmed in many studies [13,52-55]. It is associated with short remission duration and high rate of relapse even after stem cell transplantation. In the ECOG trial with 199 patients, Fonseca et al. [56] studied the relationship between ploidy status, the IgH translocations and the deletion 13 status by using FISH analysis [56]. They made the following observations on the association of deletion 13 with the three most common IgH translocations. While 94 % of patients with t(4;14) had del(13) abnormalities, 67% of patients with t(14;16) and 49% of patients with t(11;14) harbored the deletion 13. On the other hand, among patients who had del(13), 29% of patients had t(4;14), 19% had t(11;14) and 6% had t(14;16) translocation. Of note, patients without an identified IgH translocation, 45% of patients had del(13) changes. In conclusion, del(13) was reported to be more commonly associated with non-hyperdiploid karyotypes. In addition, the del(13) is strongly associated with t(4;14) abnormality not only in MM but also in SMM as well as MGUS [56]. This study also found that del(13) plays an important role in the pathogenesis of both MGUS and MM and is not just a promoting factor responsible for progression of MGUS to MM. They also concluded that the very high association of del(13)

abnormalities in t(4;14) patient and not vice versa suggests that the translocation event occurs later than the deletion of 13 [56].

The long-term follow-up analysis of the IFM 99 trial published by Avet-Loiseau [55] in 2012, involving 520 patients found an association between t(4;14) and chromosome 1q amplification, but this was not statistically significant. There was however no association between t(4;14) and del(17p) [55]. This study concluded that t(4;14) and del(17p) had the worst impact on the overall survival while β-2 micro globulin >5.5 mg/L and age >66 were also found to have poor survival outcomes.

Translocation (14;16)(q 32; q23): The t(14;16) is an IgH rearrangement involving chromosome 16. This translocation results in up regulation of the oncogene *c-maf* [26] and it has been reported to be occurring in nearly between 3-7% of myeloma patients [13,57]. Various groups have published different observations about the prognostic value of this translocation. Initially, the Mayo group reported that the t(14;16) carried an adverse prognostic value with shortened survival and an aggressive disease course [13]. The Arkansas group has also observed similar outcomes in patients with t(14;16) [58]. More recently, a larger series consisting of 1003 patients was published [57] in which the authors observed no difference in clinical outcomes in patients carrying t(14;16) when compared to patients that did not have the translocation. The difference in the outcomes in the two series was due to many confounding variables such as in Mayo group the number of patients with t(14;16) was small(n=15) and there was higher incidence of del(17p13) (33%) versus (9%) seen in the group reported by Avet-Loiseau et al. [57]. Also, the intensity of treatment was different as the Mayo group treated patients with conventional chemotherapy variations [59] whereas 60% of the patients studied by Avet-Loiseau et al. [57] received double intense chemotherapy which could have contributed to better outcomes [57]. However, even this study suffers from small numbers due to the very low incidence of this particular translocation. Further prospective studies with larger groups are needed to resolve the debate.

Translocation (14;20): The translocation of two loci on chromosome 20 with IgH t(14;20) (q 32;q 11) was described by Kuipers et al. [60] in myeloma cell lines [60] and was later described in detail with using FISH analysis by another author [33]. This translocation results in an ectopic expression of the MAFB oncogene [32,33]. In a large case series involving 2207 patients with Multiple Myeloma, MGUS and SMM, the incidence of t(14;20) was found to be <1%, 1.5% and 5% in MM, SMM and MGUS, respectively [61]. This study concluded that the t(14;16) was associated with a more stable disease when observed in MGUS but with a shorter survival in MM patients. It must be noted that similar to translocations t(4;14) and t(14;16), a majority of patients with t(14;20) in this study also had a high frequency of 13q deletions (70%) and constituted a majority of the NH-MM group (30/36) [61]. There was a trend towards a strong affiliation with 1q gain although the number of patients is small.

Chromosome 13

Observations about the aberrations involving chromosome 13 and its association with poor prognosis in multiple myeloma was initially reported by Tricot et al. [16]. The same findings associating decreased survival and deletions of chromosome 13 were also reported by Fonseca et al. [56]. Tricot et al. [15] also first reported the association of poor prognosis in myeloma with partial or complete deletions of Chromosome 13 and 11q but not other karyotype abnormalities [15]. They also published that the adverse prognostic impact seen with the presence of deletion 13 by FISH is entirely contributed by the concomitant presence of abnormal metaphase cytogenetics. Deletion of chromosome 13 by metaphase cytogenetics, present in 17% of patients, has consistently been associated with a poor outcome irrespective of the ploidy status [20,62]. In contrast, deletion of chromosome 13 by FISH (present in 50% of patients) has a very weak correlation with outcome [63]. Chromosome 13 abnormalities are observed in nearly 50% of all cases of multiple myeloma by FISH analysis, out of which 85% are monosomies [64-67]. Also, data are emerging that chromosome 13 deletion is a prerequisite for clonal expansion of tumors as 90% of cases with t(4;14) (p16;q32) are associated with chromosome 13 deletions [64-67].

13 q deletions are considered to be associated with poor survival by itself in some reports [15,68-70]. Other studies reported that the prognostic value is indirectly related to its association with poor cytogenetics like t(4;14) (p16;q32) [3,13,68,71]. More recently, the authors of the long term analysis of the IFM 99 trials have also confirmed these previously known findings that del(13) was strongly associated with t(4;14) as well as del(17p) in 85% and 86% of patients respectively in a study involving 52 patients [72]. It can be concluded at this time that chromosome 13 is a surrogate for poor prognosis in association with hypodiploid karyotype but probably not an adverse prognostic factor independently [42,73]. It should be noted that deletion 13 in chronic lymphocytic leukemia is associated with a good prognosis when it is the sole cytogenetic abnormality [74], although the same gene appears to be involved in both malignancies.

Deletion of 17p

Deletion of the 17p13 locus of the chromosome that codes for tumor suppressor gene p53 is considered as a very important prognostic factor in multiple myeloma [3,13,75]. This is considered to be a progression marker and has been reported as a late event that transforms a more indolent myeloma into an aggressive disease [3]. It is present in only a small percentage (8-10%) of patients at diagnosis [73], but the frequency increases during disease progression. Myeloma cell lines, which are derived from terminal myeloma patients, show del(17p) abnormalities in 70%. The deletion of 17 is considered a poor prognostic factor and imparts very high mortality, shortened survival in addition to a higher prevalence of CNS disease [54,76] and other extramedullary manifestations [77]. These features are unaffected regardless of the intensity of treatment [3,54,75,78]. Also, deletion of 17p13 is associated with short time to relapse following high dose chemotherapy [54] and a negative survival even following an allogeneic stem cell transplant [79]. The recent IFM 99 follow up study also established, like previous other studies, that deletion 17p13 along with t(4;14), age and elevated β-2 microglobulin levels carry the worst prognosis and outcomes in myeloma patients [72].

Chromosome 1 abnormalities

Most common aberrations of chromosome 1 are interstitial deletions of 1p or amplifications of 1q [80]. It has been reported that Chromosome 1 abnormalities involved structural aberrations that involve both arms resulting in reciprocal translocations [81]. The majorities of chromosomal 1 abnormality are in the form of jumping translocations and involve decondensation and rearrangements of pericentromeric chromatin region [81]. Three different mechanisms of translocations of 1q gains have been proposed [82].

Chromosome 1 abnormalities have been found to be a major prognostic indicator by Shaughnessy et al. [80] in their study validating the gene expression profile signatures for high risk disease [80]. Over expression or gain of the 1q21 (CKSIB gene) which leads to

proliferation and survival of myeloma cells [83] and other regions of the Chromosome 1 along with loss of several regions on 1p are associated with shortened survival and poor prognosis in studies performed by the Arkansas group [81]. However, it has not been validated by other studies from the Mayo Clinic group [84]. The IFM 99 follow-up study recently did report the prognostic importance of chromosome 1q gains towards overall survival but not to progression free survival indicating its importance in relapsed disease. Also, their study highlights the strong association between t(4;14) and chromosome 1q and the importance of including its routine testing in myeloma patients for prognostication [72]. Chang et al. [85] have showed that loss of 1p31-32 is associated with short survival and is an independent prognostic factor [85]. However, the relationship of this abnormality with other known risk factors such a t(4;14), t(14;16), 17p13 deletion and 1q21 gain remains to be established in larger clinical trials using FISH probes.

Testing

Standard investigation of chromosomal abnormalities of suspected new diagnosis of Multiple Myeloma should include metaphase cytogenetics, interphase FISH testing and/or cIg (cytoplasmic immunoglobin) FISH analysis.

Metaphase chromosomes/conventional cytogenetics

Metaphase chromosome analysis has been the standard method of cytogenetic analysis in multiple myeloma. This is based on Giemsa-banding of chromosomes. Metaphase cytogenetic analysis is successfully obtained in only 30% of the cases owing to the low proliferative capacity or low mitotic index of the myeloma cells [86]. Also, translocations such as t(4;14) that carry very important prognostic information are seldom detected due to the cryptic nature in normal cytogenetic analysis [87]. To overcome these pitfalls and to increase the likelihood of detection of cytogenetic abnormalities, multiple harvests of the specimen were attempted as was performed by the Arkansas group where they used 24 hr, 48 hr and 72 hour cultures.

It has been believed that metaphase analysis is of sorts a test of the biology of the disease [88] as it carries vital information on the "stromal dependence" of the myeloma cell. In the early stages of disease the myeloma cell is considered to be a slow diving and to be dependent on the bone marrow stroma for multiplication. This may give a falsely low detection rate when the marrow aspirate is sent for testing as the myeloma cells may not survive outside the marrow microenvironment. However, with advancement of the disease, myeloma cells can proliferate independent of the stroma. Detention of chromosomal changes by metaphase cytogenetics is considered as the best surrogate marker to determine stroma independence and thereby proliferative capacity of the disease [63]. This may also be the reason why extra medullary myeloma carries a grave prognosis. Hence, metaphase testing is considered to provide very vital information on the biology of the disease and it is very important to perform this study despite the advent of FISH and cIg FISH.

	PR	LB	MS	HY	CD-1	CD-2	MF
TT_2							
Hyperdiploid	55	67	43	93	20	20	25
Nonhyperdiploid	45	33	57	7	80	80	75
TT_3							
Hyperdiploid	53	25	33	86	0	50	17
Nonhyperdiploid	47	75	67	14	100	50	83
Values indicate the percentage of the total number of cytogenetics abnormality cases within each subgroup having the variable indicated. P<.001 for both TT_2 and TT_3 groups. Table 1 adapted with permission from Zhan et al. [63]							

Table 1: Percentage of hyperdiploidy and non hyperdiploid karyotypes in training (TR) and test (TE) sets.

Interphase chromosomes-FISH (Fluorescence In Situ Hybridization)

Introduction of testing interphase nuclei for chromosomal analysis with FISH has improved the detection rate of cytogenetic abnormalities in multiple myeloma. This study does not require actively proliferating cells in contrast to the conventional cytogenetics [89,90]. However, the low percentage of plasma cells in the marrow specimens still poses a challenge as it may hamper the detection rate even with FISH analysis [87]. The IMF group have recommended performing FISH analysis on enriched plasma cell (CD138 selection) or plasma cells detected by cytoplasmic immunoglobin light chain staining (cIg FISH). There are only a few studies comparing the yield of FISH in enriched studies to those done on whole bone marrow cells [91,92]. Stevens-Kroef et al. [87] published that the detection rate of clinically relevant cytogenetic abnormalities is superior with enriched plasma cells compared to whole bone marrow cells [87]. The consensus statements of the myeloma working group in 2011 recommended that interphase FISH studies of chromosomes probes for 17p13, t(4;14) and t(14;16) as a standard work up at the time of diagnosis [93]. In our opinion, it is also required to test for hyperdiploidy (probes for chromosomes 5,7,9,11,15) and for t(11;14) and t(6;14). These entities are associated with a good prognosis. We also test for the t(14;20) and amplification of 1q21, which are associated with a poor prognosis.

Dong et al. [94] published their study comparing conventional FISH to cIg FISH and reported that the cIg FISH is superior in detecting plasma cell neoplasm when compared to the former [94]. However, it also suffers from low detection rates in samples with low plasma cell burden.

The biggest challenge remains the early detection of cytogenetic information in patients with relapsed disease and in the post-treatment phase when there is a paucity of residual disease and plasma cell burden [95]. Identification of high risk disease early in the course of relapse will help in guiding treatment strategies and early intervention [78,96-98].

Gene expression profiling

The University of Arkansas has identified a set of 70 gene signature that is capable of identifying high risk multiple myeloma, present in approximately 17% of patients, with high-throughput sequencing and genomic tools [80]. This has been further narrowed to 17 genes that are highly capable of predicting prognostic information by the same group. These models predict and distinguish high risk disease with a great ability. The IFM group has also identified a 15 gene model that could predict poor prognosis [99]. Although other markers such as proliferation index, centrosome index and cancer testis antigens [100-102] are developed using GEP, these need to be further validated.

Zhan et al. [24] have identified and validated 7 sub-groups in newly diagnosed multiple myeloma based on common gene expression signatures [24] (Table 1). The groups were classified according to the over- or under-expressions of certain genes, related to specific translocations, hyperdiploidy, low bone disease and proliferation. They were able to identify and distinguish high risk and low risk categories based on the gene signatures [24] (Figure 1).

In a recent study, GEP comparisons between samples at baseline, after induction chemotherapy and at relapsed MM were performed. A total of 56 genes were significantly up-regulated expression both

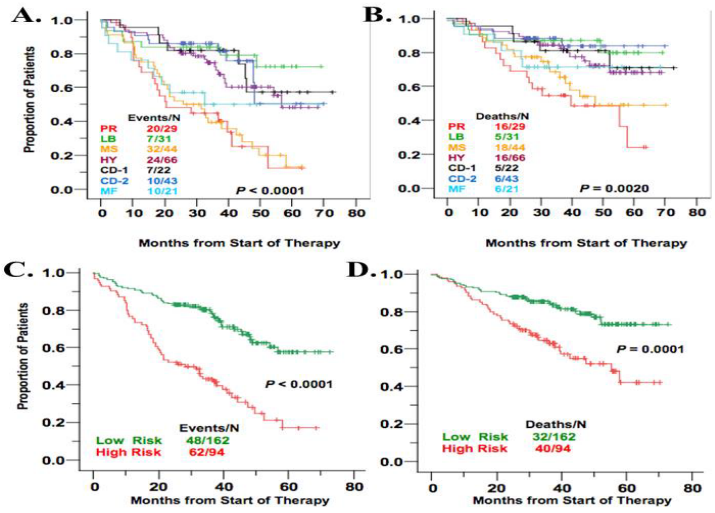

Figure 1: Adapted with permission from Zhan et al. [63].

after chemotherapy and at relapse [103]. The major functional group including ten genes (*TOP2A*, *CDC20*, *TRIP13*, *NEK2*, *AURKA*, *RRM2*, *CCNB1*, *KLF4Al*, *CEP55*, and *PBK*), belongs to the well-established Chromosomal Instability (CIN) signature [104]. Clearly, an increase of CIN signature or a stable high level of CIN signature was observed in samples obtained pre-first, pre-second, and post-second Autologous Stem Cell Transplants (ASCT) by serial GEP analyses, supporting our hypothesis that myeloma cells with high CIN signature are resistant to chemotherapy and ASCT. Furthermore, a high drug-resistant score is associated with significantly shorter duration of response, of event-free survival, and of overall survival (OS). *NEK2* was the gene most strongly associated with inferior survival in unadjusted log rank tests. We subsequently performed Comparative Genomic Hybridization (CGH)-array and FISH using cell lines transfected with NEK2 versus wild-type (WT) cell lines, and cell lines transfected with EV versus WT cell lines. Increased Nek2 expression induces DNA gains and losses resulting in chromosomal instability [103].

array-Composite Genomic Hybridization (aCGH)

aCGH is a very useful and powerful tool in the investigation of the cytogenetic basis of disease especially in multiple myeloma as it overcomes the shortcomings of the metaphase cytogenetics and FISH studies. FISH studies suffer from inability to discover new chromosomal anomalies as they are capable of identifying only known, recurrent patterns. aCGH testing does not require metaphase cells and it can detect genome wide copy changes [85]. Unsupervised aCGH studies can discover new markers of disease prognosis that can be further put to use by FISH studies [89]. This technique has identified recurrent abnormalities including loss of 1p, gain of 1q, loss of 12p and gain of chromosome 5 as predictors of outcomes [89].

Using a combination of aCGH and microarray data we identified recurrent copy-number aberrations And Minimal Common Regions (MCRs) of gain/amplification and loss/deletion in genomic DNA from purified plasma cells obtained from patients with newly diagnosed MM [80]. Unsupervised clustering of 65 cases based on recurrent gains and losses of genomic DNA identified four classes of disease. We demonstrated that gains of chromosomes 1q and 7, deletion of chromosome 13, and the absence of chromosome 11 in one hyperdiploid subtype. We also observed that high-level amplification of 1q21 and deletions of 1p and chromosome 13 in another non-hyperdiploid subtype [80].

In a study on 127 patients, Chang et al. [85] identified recurrent aCGH aberrations that are associated with short survival and have validated their findings with FISH [85]. They were able to identify and validate the loss of 1p31-32 and 20 p 12.3-12.1 as being associated with shorter survival as discussed earlier. Although the study suffers from lack of high resolution and small sample size, it demonstrates the utility of aCGH in identifying chromosomal aberration in unbiased genomic platforms and provides prognostic information and the promise of identification of new genomic information, DNA gains and losses and other epigenetic information that may provide prognostic information in the future [85].

Age and Chromosomal Abnormalities in Multiple Myeloma

Multiple myeloma has been regarded as a disease of old age with the median age at presentation being 69 years [105] and the incidence has been expected to increase with advancement of age [106]. Less than 2% of patient diagnosed with MM are under 40 years of age and about 40% are above 70 years of age [107-109]. Age along with race has been regarded as an important prognostic indicator in myeloma as young people are considered to have better survival compared to the older population [105,107]. A study by Butler et al. compared groups of patients aged less than 60 years to patients older than 60 years. Chromosomal abnormalities investigated by FISH studies showed multiple cytogenetic abnormalities in the group older than 60 years. Also, IgH gene translocations were found more commonly in this group and were attributed to a higher frequency of t(4;14) and the results were statistically significant [110]. This is the only study showing significant differences and has been contradicted by other studies, where no statistically significant differences were observed [111]. Additionally, the study by Sagaster et al. [111] also did not show any difference in patterns of chromosomal aberrations expressions between age groups of <45, 45-7 and >70 years [111]. A study by Ross et al. [112] also did not show any relationship between del (13q14) and age, but the t(14;16) was an independent prognostic variable along with age [112]. Butler et al. further reported superior survival rates in younger patients, but only those with standard risk cytogenetics. However, the survival rates in

individuals with higher risk markers were age-independent [110]. This study did not include probes for chromosome 1q and 1p, which were shown to be predictors of poor prognosis [4,80] in gene expression profiling.

These findings are then contradictory to previous studies that showed a lower incidence of t(4;14) and del(17p) in older patients [78]. It was demonstrated that age was not an independent factor on the overall survival or event free survival and is not considered as a prognostic variable in patients receiving auto transplants [113]. It is unclear at this time to say to what degree age is an independent prognostic factor for outcome, but if it is, it is unlikely related to differences in genetics, but rather to differences in treatment and the ability of older patients to tolerate more intensive treatment approaches [114].

Race and Ethnicity

Race is believed to play an important prognostic role in the biology of cancers among other factors. This could be attributed to multiple features such as variation in critical tumor genes, epigenetic changes, dietary habits, inflammatory responses, tumor micro-environment and importantly, societal factors [115].

There is a two to three fold higher rate of diagnosis of MGUS and Multiple Myeloma in African Americans compared to the European population in a large study done in veterans by Handerson et al. [115]. Although the exact cause for this dissimilarity was not found, a biologic cause was thought be the underlying phenomenon. Characteristics of myeloma among African Americans and eastern Europeans were compared in a study done by Landgren et al. [116]. In this study, FISH studies revealed that IgH translocations were found in lower frequency in the African American population than in European counterparts; the difference was statistically significant. There was no difference in the type of translocation with respect to t(4;14), t(11; 14) or t(14;16). Also, array based Comparative Genomic Hybridization (aCGH) showed no difference in the frequency of hypodiploid or hyperdiploidy in the two populations. It should be noted that this study showed a low frequency of mutations in the African-American cohort. Clinical data comparing the two cohorts was missing in this study [117]. It remains unknown if other mutations such as k-*ras*, n-*ras*, 17p53 and NFkB signaling pathways are expressed in varying frequencies in different ethnic groups [117]. Further investigation is still needed to prove cytogenetic differences translating in to clinical outcomes among African-American and European cohorts.

Extra Medullary Involvement of Myeloma

Extra Medullary Disease (EMD) occurs in a small set of patients with multiple myeloma where the clonal plasma cells spread to organs, not in direct contact with bone marrow. There has been a difference in opinion about the definition of EMD as some authors have defined it as a contiguous spread of the plasma cells from the bone marrow to the adjacent soft tissue [118]. Other series have defined it as occurrence of plasma cell clones at distant organs via hematological dissemination [119,120]. We favor the latter definition, since the former is not necessarily related with an inferior prognosis. Due to the disparities in definition, the incidence of extramedullary manifestation widely varies and has been reported to be between 7% and 18%. The incidence has been reported to go up to 20% with the progression of disease or relapse. There has not been a great amount of data on the true incidence rates and the pathogenesis and the underlying chromosomal abnormalities in EMD. The most common site of EMD is the skin at initial diagnosis whereas liver is the most common site at relapse [121].

It has been postulated that the extramedullary spread of multiple myeloma cells is a result of decreased adherence to bone marrow stroma [122]. This might be a result of decreased expression or loss of various adherence molecules and receptors such as VLA- 4 (CD49d), CD44 and CD56, down regulation of P-selectins, chemokine receptors etc [122].

It has been observed by some authors who reported that exposure to targeted therapies may predispose the emergence of extramedullary disease [123]. Although there are not many controlled trials validating this observation, Baker et al. [117] have reported in their study that there was no correlation between a prior treatment with bortezomib, thalidomide of lenalidomide and an increased likelihood of development of extramedullary disease [118].

Information on chromosomal abnormalities underlying the extramedullary disease is emerging. In a series published recently, among patients with extramedullary disease the overall incidence of cytogenetic abnormalities associated with multiple myeloma was significantly higher than in solitary plasmacytomas [124]. The abnormalities detected in EMD were del(17p13), del(13q14), MYC-over expression and t(4;14), all markers associated with poor prognosis [124]. In another study a higher frequency of *k-ras* mutations in EMD were reported [117]. Also, it was reported that the frequency of t(4;14) and del(17p) was higher in the EMD group compared to multiple myeloma without EMD [125]. Showed twice the incidence of del(17p13) in EMD compared to BM specimen. However, it should be noted that the number of patients in the group with EMD was very small [119]. On the same note a recently published study observed that the EMD disease occurred mostly in "high risk" disease as detected by 70- gene and 80-gene risk models including MF sub group, representing over expression of MAF/ MAF-B genes responsible for t(14;16) and t(14; 20), respectively [119]. Also, in the same study, the proliferation subgroup was shown to have more EMD. Centrosome amplification was also associated with higher frequency of EMD seen in this study that was earlier reported by Mayo group by [85,119].

Some authors published that there is need to make a distinction between primary extra-medullary plasmacytomas and EMD as the response to localized radiation therapy on primary Extramedullary plasmacytoma is excellent in the absence of disseminated myeloma [126]. The same authors have also demonstrated that primary extra-medullary plasmacytoma and multiple myeloma share most of the chromosomal aberrations with the exception of t(11;14)(q13;q32), which was seen only in multiple myeloma [126]. A significantly decreased overall survival in patients with EMD has been reported by many authors [118-120,25,127]. It is not clear whether the response to conventional treatment in EMD is any different than that of non-EMD myeloma, but the duration of response is significantly shorter.

At this time, it is imperative to state that EMD at diagnosis or at relapse carries a poorer prognosis. EMD is best detected by routinely performing PET-CT scan studies. More studies are needed to identify and confirm the genetic features underlying the pathogenesis of EMD and to establish the association with causation and the relative aggressiveness of EMD compared to the bone marrow disease only [124].

Chromosomal Abnormalities in the Era of Newer Targeted Therapies and Other Biological Agents

The efficacy of novel agents such as bortezomib and lenalidomide as treatment for relapsed or refractory multiple myeloma has been demonstrated by some early phase II studies [96,128]. The Phase II

SUMMIT study did not show any significant difference in outcome, EFS and OS or response rates to bortezomib treatment patient with del(13q) status, detected by FISH. Although the phase III trial showed a trend towards an adverse affect on event free survival but not overall survival, these affects were not seen when the confounding parameters such as age, prior treatment, ISS staging, etc were controlled. No difference in outcome was seen with del(13) status when tested by FISH analysis [96]. Similarly, in a study by Sagaster et al. [129] the effects of del(13), 14q32 translocations had no influence on the response rate or overall survival when treated with bortezomib based regimen [129]. These results were also confirmed by Jiang et al. [130] who found that del(13q) and t(4:14) had no impact on the response rate and outcome in relapsed/refractory myeloma when treated with bortezomib [130]. The TT3 study reported that the bortezomib based regimens would overcome the poor prognostic impact of t(4;14) and 17p deletion but the IFM study could find the benefit in only t(4;14) group but not in 17 deletion as mentioned earlier [78]. There is a general agreement that bortezomib based regimens improve survival of patients with high-risk myeloma including t(4;14), as defined by FISH analysis [122].

Lenalidomide, a more potent derivative of thalidomide has shown promise in many studies. Various studies indicated the inability of Lenalidomide/dexamethasone to overcome the poor prognosis associated with the 17p deletion [130-132]. Also, the impact of this treatment combination on del(13) and t(4;14) remains controversial at this time [130].

Future of Treatment of Multiple Myeloma

With the emergence of technology and information-gathering capability, we are learning more about the chromosomal aberrations and molecular abnormalities and pathways that underlie the pathogenesis of multiple myeloma. Interphase FISH, gene expression profiling, CGH, SNP etc have uncovered molecular signatures which will hopefully help us to sub-classify patients and tailor therapies according to the risk classification. With the identification of multiple targets, new therapies are being investigated. More long term studies and follow up-analyses are needed to determine the efficacy and role of the newer immunomodulator pomalidomide, new generation proteasome inhibitors carfilzomib and oprazomib, etc, and monoclonal antibodies such as Daratumumab. Although it is unlikely that these targeted therapies will replace the current treatment algorithms, it is hoped that these therapies will be able to prolong remission durations in high-risk patients after responses have been obtained with already established treatments.

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