

Chapter 2

Prognostic Implication of Genetic Changes (Cytogenetics, and FISH, Gains and Losses of DNA by SNP Array and aCGH) in Risk Stratification in Myeloma

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Abstract Multiple myeloma (MM) is characterized by a huge heterogeneity in survival. Most of these differences can be captured by the variability of genetic events occurring within the malignant plasma cells. At the chromosomal level, the two most important changes are the del(17p) and the translocation t(4;14), both associated with a poor outcome. Recent data using modern genomics, such as gene expression profiling, or SNParray, revealed another level of complexity, which can be utilized for a better prognostic assessment. However, these techniques are still research tools. Whether there will be routine techniques in the future is an open question.

As for other hematopoietic malignancies, and especially acute leukemias, chromosomal abnormalities have been shown to represent very strong predictors of evolution in multiple myeloma. However, and in contrast with acute leukemias, the use of genetics to predict patient evolution, and thus to adapt therapy to this risk, is only in its infancy. This gap between myeloma and leukemias is especially related to technical pitfalls (see next section). With the development of novel technologies and the systematization of genetic analyses in the diagnostic evaluation of the patients, there is no doubt that genetics will take a major place in the management of patients with myeloma. The goal of this chapter is to summarize our current knowledge of chromosomal abnormalities in myeloma as well as to show their role in the oncogenesis, their impact on the natural history of the disease, and their potential utility in patient management.

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2.1 Definitions and Technical Aspects

Classically, the landscape of the chromosomal abnormalities observed in a specific disease is obtained through the analysis of hundreds of patients using classical cytogenetics. In myeloma, this approach has been less successful than in leukemias because of the difficulty to generate metaphases within the tumor clone. Recent studies using high-density CGH (comparative genomic hybridization) or SNP (single-nucleotide polymorphism) arrays have shown that virtually 100% of the patients with myeloma display chromosomal abnormalities [1, 2]. In contrast, cytogenetic analyses reported in the literature have shown that an abnormal karyotype is identified in <30% of the patients [3–8]. This discrepancy is not related to the resolution differences between the two techniques, since many of the genetic changes identified by CGH involved large chromosomal regions. The two major explanations are related to the low proliferation of malignant plasma cells and to the usual low percentage of plasma cells within the biological specimens sent to the cytogenetic laboratories. Thus, normal karyotypes in myeloma should not be interpreted as the absence of chromosomal changes within the tumor clone, but as the result of the division of normal myeloid bone marrow cells, representing the constitutional DNA.

This failure to obtain informative karyotypes has led researchers to use other techniques, not dependent upon the generation of clonal metaphases, especially fluorescence in situ hybridization (FISH) on interphase cells [9–12]. This technique enables to assess the presence or not of specific chromosomal changes in every patient with myeloma, whatever his/her in vitro proliferation potential. However, because of the common low plasma cell percentage within the bone marrow specimens sent to the lab (median=6% in the IFM experience), the technique cannot be performed straightforward, as usual in other hematopoietic malignancies. The plasma cells have to be selected, either by a previous cell sorting or by the concomitant labeling of the cytoplasmic Ig light chains, thereby enabling an unambiguous identification of the plasma cells. Both strategies are equivalent: cell sorting enabling to perform further analyses, like gene expression profiling or copy number analyses on pure plasma cell populations.

2.2 Specific Chromosomal Changes

2.2.1 *Aneuploidy*

Despite the scarcity of large cytogenetic studies, the analysis of the literature reveals that abnormal karyotypes can be separated in two groups, almost identical in numbers: patients with a hyperdiploid karyotype (i.e., with more than 46 chromosomes), and those with a hypo- or pseudodiploid one (i.e., with less than 46 chromosomes, or 46 chromosomes with structural aberrations). Although this classification (hyperdiploidy vs. non-hyperdiploidy) is somewhat artificial, hyperdiploidy appears as a relatively homogeneous group. Most of the patients in this category present with a

high number of chromosomes (median = 54), involving nonrandom gains. Actually, trisomies involve especially odd chromosomes, mostly chromosomes 3, 5, 7, 9, 11, 15, 19, and 21. In contrast, non-hyperdiploid karyotypes are much more heterogeneous, even though some chromosome losses and some structural aberrations appear also nonrandom. The most recurrent ones are monosomy 13, 8p deletions, 1q gains, and 14q32 translocations. Nevertheless, these abnormalities are not specific of the non-hyperdiploid category and can be observed in hyperdiploid karyotypes. Of note, most (if not all) of the human myeloma cell lines derived from patient specimens belong to the non-hyperdiploid category. This bias has to be considered when cell lines are used as models of the human disease.

Few studies have analyzed the prognostic value of this classification [8, 13–15]. Hyperdiploidy seems to be associated with a better prognosis. However, these analyses are hampered by several methodological biases, including the retrospective nature of the analyses, the heterogeneity in the patient population, and the disparity of the treatment strategies. More recently, a few studies have used interphase FISH approaches to define ploidy [16, 17]. These studies did confirm the better prognosis of hyperdiploidy, but it has to be demonstrated that this prognostic value is not dependent of other confounding parameters. In the IFM experience, hyperdiploidy was not an independent prognostic factor but was associated with a lower incidence of del(13), t(4;14), and del(17p) (see below, [18]).

2.2.2 *Chromosome 13 Abnormalities*

Chromosome 13 is frequently abnormal in myeloma. Most of the abnormalities are monosomies and, less frequently, translocations or interstitial deletions are observed, usually involving the 13q14 region. In cytogenetic series, chromosome 13 abnormalities, or del(13), are observed in about half of the abnormal karyotypes. This frequency has been confirmed in interphase FISH studies [19–23]. The role of del(13) in the oncogenesis of myeloma is still a matter of debate. The abnormality is probably an early event (and even possibly a primary genetic event), since it is observed with similar frequencies in premalignant MGUS stages and in patients at relapse. The molecular consequences of these chromosomal losses are almost unknown, although a molecular signature has been identified using expression profiling [24, 25]. However, because most of del(13) are in fact monosomies, many genes are lost and may be deregulated. The quest for the Holy Grail is still opened!

The first recognition of the negative impact of del(13) on survival came from Little Rock in 1995, based on cytogenetic data [26]. Later on, several groups demonstrated that this poor-prognosis feature was retained when the del(13) was identified by interphase FISH. However, a debate still exists regarding the prognostic value of del(13) depending on the technique used. Few reports have compared the prognostic value of both techniques on the same patients. However, those reports are concordant to show that del(13) identified by interphase FISH only (with a concomitant normal karyotype) does predict for a shorter survival

than those patients lacking del(13) [27, 28]. In the recent IFM study, del(13) identified by FISH was not an independent prognostic factor, since its association with a poor prognosis is related to the concomitant t(4;14) or del(17p) (see below) [18]. Actually, del(13) per se may not confer a specific prognosis in myeloma and may be considered as a marker frequently associated with other more specific poor-prognosis factors.

2.2.3 *Translocations Involving the 14q32 Region*

Cytogenetic analyses did identify the chromosomal 14q32 region as a recurrent hotspot of translocations in myeloma, with a frequency of about 30%. In most cases, these translocations identified on the karyotype were t(11;14)(q13;q32) [29, 30]. The interest for this region grew in 1997 with the publication by Bergsagel et al. showing that at least 90% of the human myeloma cell lines did present an illegitimate molecular rearrangement of the *IGH* gene, located at 14q32 [31]. Interestingly, some of the cell lines displaying a rearrangement by Southern blot looked normal at karyotype, leading to hypothesize cryptic rearrangements. This hypothesis has been demonstrated by several authors, using different techniques. Actually, the analysis of primary tumors from patients showed that a 14q32 translocation was present in about 60% of the patients [12, 13, 32, 33]. Furthermore, it has been shown that these 14q32 translocations did involve several chromosomal partners, and that some of these translocations were karyotypically silent, explaining, at least in part, the frequent discrepancy between FISH and cytogenetics. However, the picture is different than that observed in some non-Hodgkin lymphomas, in which a unique, typical 14q32 translocation is the hallmark of a lymphoma subtype. In myeloma, at least 30 different chromosomal regions have been involved in translocations with the 14q32 region. Despite this heterogeneity, a few recurrent specific translocations have been described: the t(11;14)(q13;q32) in ~20% of the patients, the t(4;14)(p16;q32) in ~15% of the patients, and the t(14;16)(q32;q23) in ~5% of the patients. These more frequent translocations may actually define myeloma subtypes.

The **t(11;14)(q13;q32)** is identical to that observed in mantle cell lymphomas. The breakpoints involve the *IGH* gene at 14q32, and the *CCND1* gene at 11q13, encoding the cyclin D1 protein [34, 35]. One of the molecular consequences of the translocation is the upregulation of cyclin D1. So far, the oncogenic role of the translocation is unknown. Although the cyclin D1 has been involved in the activation of proliferation, the t(11;14) myelomas are characterized by a low proliferative index and a frequent morphology of small mature plasma cells [36, 37]. They are more likely to express the CD20 at the cell surface [38]. Clinically, this type of myeloma is not remarkable. Even though preliminary reports did show on a better survival [39], more recent and larger studies did not confirm this prognostic impact [18, 40].

The **t(4;14)(p16;q32)** is so far specific of myeloma and has never been described in other malignancies. However, this specificity has to be taken with caution since

the t(4;14) is “cryptic,” meaning that it is not detectable by the karyotype. The molecular cloning of the translocation revealed a peculiar situation so far unique in hematology [41–43]. The translocation leads to the deregulation of two genes located at 4p16. The *FGFR3* gene, which encodes a receptor for the fibroblast growth factors, is located on the telomeric side of the breakpoints. The translocation displaces the *FGFR3* gene to the 14q32 region, leading to the molecular activation of the gene transcription. Because FGFR3 has a tyrosine kinase activity, it is a good candidate for an oncogenic function. Several tyrosine kinase inhibitors are currently tested in order to inhibit this function. However, even though in vitro and animal models did favor this hypothesis [44, 45], several reports did show that about one-third of the patients with t(4;14) did not display FGFR3 overexpression [46, 47]. This observation supports the hypothesis of another molecular consequence of the translocation. Actually, the translocation disrupts another gene located at 4p16, a gene identified with the cloning of t(4;14) translocations. This gene has been named *MMSET* (for *Multiple Myeloma SET domain* gene), because of some degree of homology with other genes containing a *SET* domain, like *MLL*. The translocation disrupts the *MMSET* gene within the first introns, leading to the generation of a novel chimeric *IGH-MMSET* gene. So far, neither the physiological function of *MMSET* (it could be involved in the regulation of chromatin remodeling), nor the consequences of its deregulation by the translocation are known. However, this chimeric gene is constantly present in the t(4;14) cases, and *MMSET* may represent the primary target of the t(4;14).

Several studies did show that the t(4;14) is associated with a poor prognosis (Fig. 2.1). However, recent large-scale studies did suggest that all the patients with t(4;14) do not present a very short survival and that other factors may have an influence on outcome [18]. For instance, the IFM did show that patients with t(4;14) and a low β 2-microglobulin level may enjoy longer survivals than those presenting the translocation with a high β 2-microglobulin level [48]. Recent data suggest that the poor prognosis associated with the t(4;14) might be (at least partially) overcome by novel therapies, especially bortezomib-based combinations [49]. Interestingly, genetic studies using FISH did show that at least 85% of the patients with t(4;14) do also present del(13). The reasons for this strong association are so far unknown, but clinically it appears that patients displaying the two genetic abnormalities have a poorer prognosis than those lacking the del(13). Finally, it has to be highlighted that the frequency of the t(4;14) is higher in human myeloma cell lines than in the patients (25 vs. 15%). This discrepancy may reflect the intrinsic aggressiveness conferred by the translocation, possibly facilitating the generation of cell lines.

The **t(14;16)(q32;q23)** is also specific for myeloma. The cloning of the translocation did show that the 16q23 breakpoints occur in the vicinity of the *MAF* gene, leading to its translocation on the derivative chromosome 14, and finally to its overexpression [50]. Further investigations did show that *MAF* is a transcription factor that positively regulates other genes like *CCND2* or *ITGB7* [51]. The translocation is rare in myeloma (~3% of the patients), whereas it is frequently observed in myeloma cell lines (~25%). Here again, the discrepancy in frequencies between patients and cell lines might be related to the aggressiveness conferred by the t(14;16). Actually, very

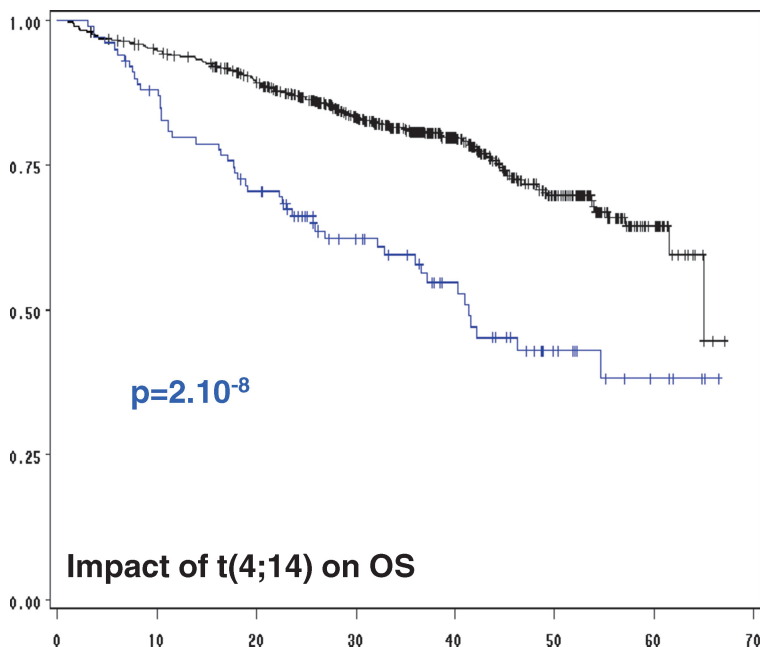


Fig. 2.1 Overall survival for patients presenting a t(4;14) or not. *Black* represents patients without and *blue* with t(4;14). This research was originally published in *Blood*. Ref 18 © the American Society of Hematology

few reports have analyzed the prognostic impact of this translocation. The rare publications on this topic did report a shorter survival for patients presenting the translocation, which is almost always associated with del(13). This translocation has to be related to another 14q32 translocation, the **t(14;20)(q32;q11)** observed in only a few percentage of patients [52]. This latter translocation deregulates *MAFB*, a gene belonging to the *MAF* family, with molecular consequences so far unknown.

Finally, almost 20% of the patients display a 14q32 translocation with other multiple chromosomal partners. The role of these nonrecurrent translocations is totally unknown. In contrast to the recurrent 14q32 translocations described above, these ones are mostly observed in patients with hyperdiploid karyotypes. They may reflect a genetic instability, especially focused on the 14q32 region and the *IGH* gene, which is physiologically rearranged at several stages of the B-cell differentiation.

2.2.4 Deletions 17p

More recently, a loss of the short arm of the chromosome 17, i.e., del(17p), has been described in about 10% of the patients with myeloma [53, 54]. These deletions are not specific to myeloma and have been reported in numerous tumor types, like

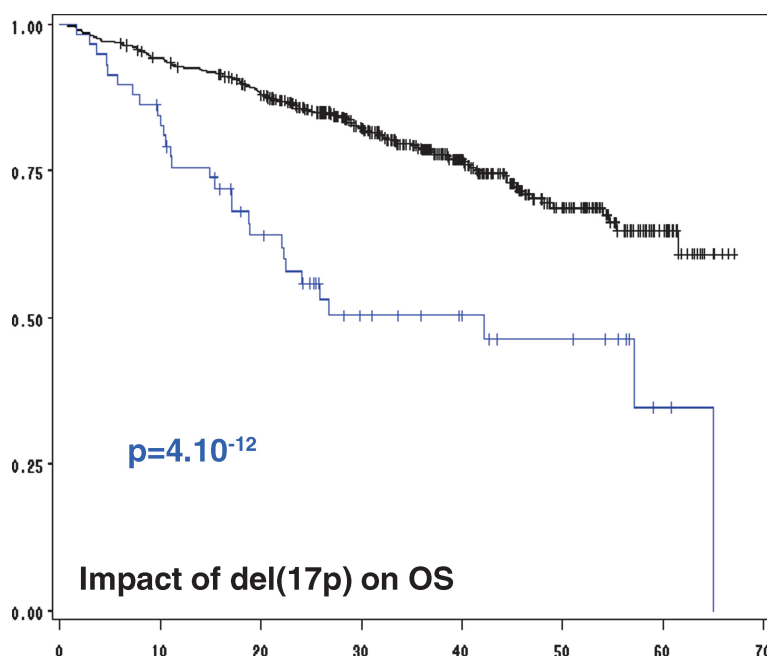


Fig. 2.2 Overall survival for patients presenting a del(17p) or not. *Black* represents patients without and *blue* with del(17p). This research was originally published in *Blood*. Ref 18 © the American Society of Hematology

chronic lymphocytic leukemia, acute myeloid leukemia, or many solid tumors. These losses generally involved the major part of the short arm of chromosome 17, thus leading to the loss of many genes, this region being particularly rich in coding sequences. However, most authors did focus on the *TP53* gene, located at 17p13, since this gene is mutated in about half of the tumor types. Nevertheless, this hypothesis would suppose a mutation of the other allele. This hypothesis is attractive since patients presenting a del(17p) usually display a poor prognosis, whatever the type of treatment (conventional or intensive). Since P53 is involved in the mechanisms of cell death induced by most chemotherapeutic agents, its invalidation may participate to the chemoresistance presented by patients with del(17p). However, this hypothesis has to be demonstrated. Mutations of the *TP53* gene are a rare event in myeloma, especially at diagnosis [55–57]. The IFM recently reported that *TP53* mutations are exclusively observed in patients with del(17p), with a frequency of around 30–40% [58]. Whatever the mechanism, these deletions are associated with a poor outcome, observed in all the studies reported so far (Fig. 2.2). In contrast to translocations involving the 14q32 region, they are thought to be secondary events and can be acquired during evolution.

2.2.5 Abnormalities of the 1q Region

More recently, the Arkansas group did report on the prognostic value of 1q gains [59]. In cytogenetic studies, extracopies of the long arm of chromosome 1 have been described in about one-third of the patients [60]. Actually, this abnormality is not restricted to myeloma and has been reported in many tumor types, both in hematological neoplasms and in solid tumors. In the Arkansas study, this abnormality came up as the strongest prognostic factor. They did show that patients with either a gain of the 1q21 chromosomal region or with overexpression of the *CKS1B* gene (located at 1q21) presented a poor outcome in the “Total Therapy” program. Since this pioneering report, several groups did confirm the poor outcome of patients with 1q gains. However, the Mayo Clinic [61] and the IFM (unpublished results) did show in independent cohorts of patients that this parameter was not retained in multivariate analyses and that its prognostic value disappeared when combined with other classical biological and genetic prognostic factors. Thus, further studies are required in order to understand the real prognostic impact of 1q gains.

2.3 Practical Use of Cytogenetic Data in Routine Practice

The first question is as follows: Should we perform chromosomal analysis for every patient and (in case of a positive answer) how? Analyzing the prognostic impact of some chromosomal changes, it is clear that cytogenetics (conventional or molecular) displays a prognostic value in myeloma, similar to other hematological neoplasms. Thus, in agreement with general hematological practice, cytogenetic analysis at diagnosis should be considered as a “good clinical practice,” at least to define the prognosis of the disease in each typical patient. Furthermore, the improvement of our knowledge of myeloma biology will definitely have a major impact in the improvement of patient management, either by selecting optimal treatments for each patient or by helping in the development of novel drugs.

The way to perform chromosomal analysis is more debatable. Both conventional cytogenetics and molecular cytogenetics present their own advantages. Conventional cytogenetics allows a global envision of the chromosomal abnormalities throughout the entire genome. Furthermore, the prognostic value of a typical chromosomal abnormality will probably be stronger if detected at karyotype rather than with FISH, essentially because an abnormal karyotype is, by definition, linked to proliferation (at least in vitro). Taking into account the prognostic impact of proliferation in myeloma, karyotypic abnormalities display a strong prognostic value. However, this is not a sufficient reason to recommend to perform conventional cytogenetics. This theoretical advantage has to be faced with the frequent absence of any detectable chromosomal change on the karyotype and with the heaviness of cytogenetic assessment. Actually, karyotyping is highly time consuming, and regarding its low informativity in myeloma, it has to be seriously evaluated before it is proposed, especially in a multicenter setting. Thus, more and more cooperative groups did

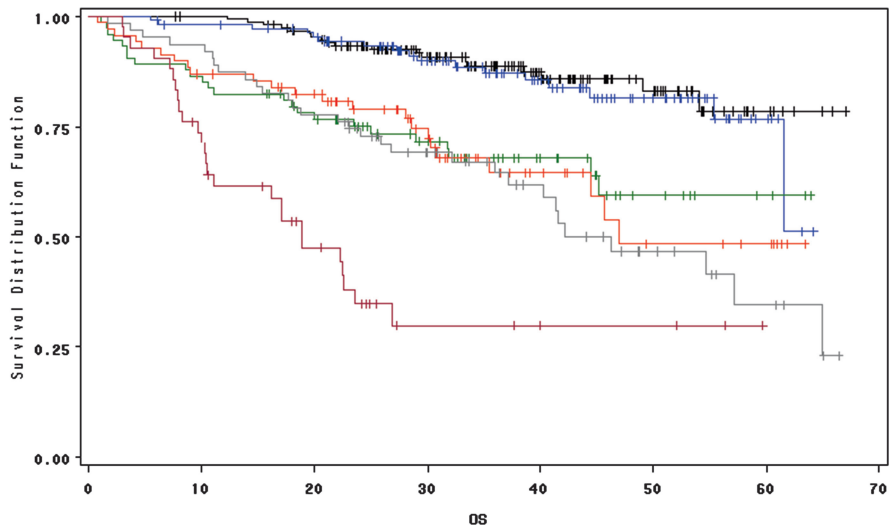


Fig. 2.3 Influence of $t(4;14)$, $del(17p)$, and $\beta 2$ -microglobulin level on overall survival. The *black* curve is for patients lacking $del(13)$, $t(4;14)$, and $del(17p)$, and presenting a low $\beta 2$ -microglobulin level (≤ 4 mg/L). The *green* curve represents the similar patients, but with a high $\beta 2$ -microglobulin level (> 4 mg/L). The *blue* curve depicts patients lacking $t(4;14)$ and $del(17p)$ with a low $\beta 2$ -microglobulin level, but presenting a $del(13)$. The *red* curve represents patients lacking both $t(4;14)$ and $del(17p)$ with a high $\beta 2$ -microglobulin level and with a $del(13)$. The *gray* curve shows patients with either a $t(4;14)$ or a $del(17p)$ in more than 60% of their plasma cells, and a low $\beta 2$ -microglobulin level. Finally, the *pink* curve shows the overall survival of patients with either a $t(4;14)$ or a $del(17p)$ in more than 60% of their plasma cells, and a high $\beta 2$ -microglobulin level. This research was originally published in *Blood*. Ref 18 © the American Society of Hematology

include in their strategy the use of interphase FISH for the assessment of chromosomal changes observed in patients enrolled in those trials. Even though this technique is also technically demanding (plasma cell labeling or sorting), it is much lighter to set up, especially for multicenter trials. Samples can be sent to a reference lab, enabling a huge improvement in cost-effectiveness, and also high reproducibility. So FISH karyotyping is probably the method of choice for the analysis of chromosomal changes in myeloma. Combining Influence of $t(4;14)$, $del(17p)$, along with beta 2-microglobulin level may provide a superior model to predict overall survival (Fig. 2.3).

2.4 Genomic Abnormalities in MM

Genome-wide CGH and SNP DNA arrays have demonstrated their utility to identify acquired chromosome abnormalities in myeloma cells [1, 2, 62–69]. Despite dramatic differences of resolution between metaphase-based CGH and array CGH (aCGH) or high-density SNP arrays (SNP array), the skyline recurrence plot for copy number abnormalities (CNAs) is similar in different studies [2]. High-resolution molecular karyotyping using whole-genome DNA provided molecular evidence

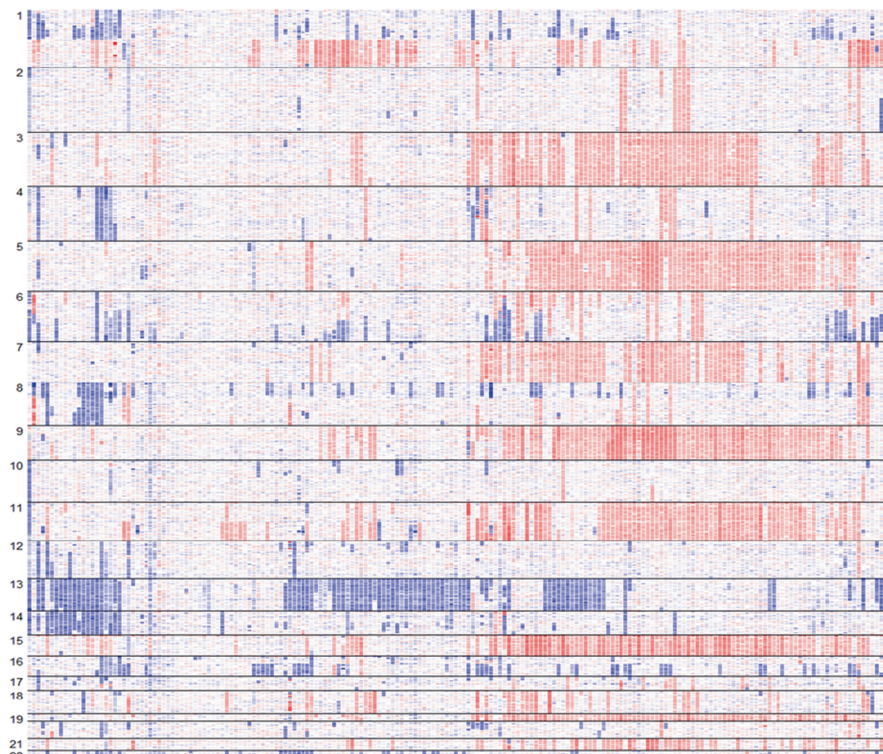


Fig. 2.4 SNP array plot of 192 patients analyzed with the Affymetrix 500 K chip. Legend: each column represents a single patient. SNPs are ordered from chromosome 1p to chromosome 22q. Blue represents losses, whereas red represents gains. This research was originally published in *Blood*. Ref 18 © the American Society of Hematology

that MM is a heterogeneous genetic disease with an average of at least seven numerical and/or structural chromosomal abnormalities (Fig. 2.4) [1, 2].

MM patients are characterized by highly frequent (>30%) gain of the odd numbered chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, loss of chromosomes 13 and X (in females cases), and gain of 1q and frequent (>10%) deletion of sub-chromosomal material including 1p, 6q, 8p, 12p, 14q, 16p, 16q, and 20p and gain of 6p. Based on these frequent CNAs, MM are clustered into several groups, with one predominant group including the vast majority of hyperdiploid MM (≥ 47 chromosomes) that almost exclusively harbored chromosome gains (whole or segmental) [2]. Among hyperdiploid MM, a clear survival advantage was demonstrated for either hyperdiploid patients with trisomy 11 [1] or 5q31 gain [2].

Besides their high resolution, genome-wide SNP arrays combine intensity and genotype data to simultaneously evaluate copy number and loss of heterozygosity in cancer cells. The use of adapted analysis tools such as CHAS (<http://www.affymetrix.com>), CNAG and AsCNAR (<http://www.genome.umin.jp>), dChip (<http://www.dChip.org>), and Partek GS (<http://www.partek.com>) allows to establish both allele frequency and determine allele-specific copy number, facilitating exploration

of acquired uniparental disomy (UPD) and minor populations in cancer. Few studies have analyzed distribution, size, and frequency of UPD in MM [2, 67, 69]. Two mechanisms of acquired UPD can occur in MM: whole-chromosome UPD that arise from a chromosomal segregation error in mitosis and segmental UPD that occur through mitotic recombination events. Whole-chromosome UPD is a rare event, apparently not random, affecting predominantly even chromosomes. Segmental UPD is more frequent and affect preferentially chromosomes 16q and Xq. UPD has no prognostic impact in MM.

2.4.1 Minimal Common Regions of Interest with Prognostic Value

Analysis of genome-wide aCGH or SNP arrays in large series of MM allows identification of recurrent minimal common genetic lesions [1, 2, 69]. These genomic analyses confirmed prognostic significance of 1q21 (*CKS1B*), 8q24 (*MYC*), and 17p13 (*TP53*) and revealed novel loci at 1p, 5q, 8q, 12p, 14q, 16q, 20p, 20q, and 22q, which impact on survival. However, prognostic significance of these lesions has to be confirmed in larger genomic studies using standardized genomic analysis tools.

2.4.2 Genes of Interest Residing in MGCL

Integration of recurrent amplifications and deletions with their correlated gene expression changes identified a number of candidate genes associated with poor survival. Among them, genes with “oncogene-like” pattern defined as genes residing in amplified genomic loci showing significant overexpression in amplified genomic locus included *ADAR*, *CKS1B*, *IL6R*, *ILF2*, *MCL1*, *SHC1*, *UBAP2L*, and *UBE2Q1* at 1q21 locus; *MYC*, *FBXO32* at 8q24; and *YWHAB* at 20q13. Genes with “tumor suppressor gene-like” pattern defined as downregulated genes located in deleted genomic regions included *DFFA* at 1p36; *CD27/EV11* at 12p13; *CYLD* at 16q12; and *TP53* at 17p13.

2.4.3 Multivariate Analysis of Lesions Independent of Risk Stratification

Based on the frequent genomic lesions (>10%) with the most significant prognostic impact identified in newly diagnosed MM enrolled in IFM trials, we built a survival model. Multivariate analysis retained two adverse genetic markers: amp(1q23.3) and del(12p13.31) and one favorable marker: amp(5q31.3) [2]. Further genomic studies by other cooperative groups are required to evaluate the prognostic impact of the model in patients treated with different regimens.

2.5 Conclusion

To conclude, genetic changes represent probably the most important prognostic factor in MM, as previously shown in other hematological malignancies. Several techniques can be used to identify these factors, including FISH, SNP, or CGH array, but also gene expression profiling (described in another chapter). The goal now will be to define what is (are) the best technique(s) to use to detect these prognostic factors.

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