Review

What Is Genomic High-Risk Myeloma?

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Abstract: Although treatment of multiple myeloma has changed dramatically over time, there is still a subpopulation of patients who do not respond to treatments and are labeled as high risk. A combination of serum and genomic markers can be used to identify and stratify these patients according to associations with outcome. The most common method of identifying the genomic markers of high-risk multiple myeloma is using fluorescence in situ hybridization using probes to identify IgH translocations or copy number changes including the t(4;14), t(14;16), t(14;20), gain 1q, and del(17p). However, as research studies utilize newer technologies, such as whole genome sequencing, more high-risk factors are being identified including mutations of TP53, DIS3, BRAF, and complex structural events. Integration of comprehensive genomic studies into clinical trials will aid in defining the genomic high-risk landscape of multiple myeloma, which in turn can be transferred to individual patient diagnostics and treatment management.

Keywords: genomics; high-risk; myeloma

1. Genomics of Multiple Myeloma
1.1. Primary Genetic Events

The genomics of multiple myeloma (MM) are complex but can be divided into subgroups based on the primary events. The cell of origin for MM is a post-germinal center plasma cell where aberrant class switch recombination of the immunoglobulin heavy chain (IgH) locus on chromosome 14 results in translocations occurring with partner chromosomes [1–4]. As a result of the translocation, the highly expressed IgH locus super-enhancers are placed next to an oncogene resulting in its overexpression. There are five main partner chromosomes resulting in a t(4;14), t(6;14), t(11;14), t(14;16), and t(14;20) which in turn overexpress the oncogenes FGFR3/NSD2, CCND3, CCND1, MAF, and MAFB, respectively.

The IgH translocations are early initiating events which are present in all myeloma cells within a patient and can be detected in ~40% of newly diagnosed patients. Therefore, approximately 60% of patient samples do not have a primary IgH translocation. Instead, ~50% of newly diagnosed patients have chromosome trisomies, the most frequent of which involve chromosomes 5, 9, 15, and 19, and are also considered to be initiating primary events. This group are collectively known as hyperdiploid MM. IgH translocations and hyperdiploidy primary events are both found in all disease stages of plasma cell malignancies including monoclonal gammopathy of undetermined significance (MGUS), smoldering MM (SMM), MM, and plasma cell leukemia (PCL).

1.2. Secondary Genetic Events

Built on top of these primary genomic abnormalities are secondary abnormalities which include copy number changes, mutations, additional translocations, and epigenetic modifications. As these are secondary events, they are not present in every MM cell, and they can be subclonal. However, due to selective pressure, the secondary events become clonal if they give an evolutionary advantage to the cell through, for example, increased
proliferation or treatment resistance. These secondary events accumulate in the plasma cell and eventually drive the cell through the asymptomatic MGUS and SMM stages to symptomatic MM.

Secondary copy number changes appear at the MGUS stage but increase in frequency as the disease progresses. In MM, most chromosomes are affected by copy number change to some degree. The most frequent copy number changes in newly diagnosed MM, excluding hyperdiploid trisomic chromosomes, are gain of 1q (36%) and deletions of 1p (30%), 6q (33%), 8p (25%), 12p (15%), 13q (59%), 14q (39%), 16q (35%), 17p (7%), 20 (12%), and 22 (18%) [5].

Significantly mutated genes, those mutated at a higher frequency than expected, are detected but there are few genes that are mutated in more than 5% of newly diagnosed MM patients. The most frequently mutated genes are KRAS (21%), NRAS (17%), DIS3 (10%), TENT5C (9%), BRAF (8%), and TP53 (59%). However, there is a long tail of genes that are mutated below 5% including ATM (4%), EGR1 (4%), H1.4 (4%), IRF4 (3%), and XBP1 (1%). The mutation rate in MM is ~60 nonsynonymous mutations per sample, or 1.2 mutations/Mb. However, this mutation rate is not consistent across all MM subtypes, and the t(14;16) and t(14;20) have significantly more mutations and are defined by an APOBEC mutational signature that is rarely seen in other subgroups [7].

Secondary translocations are also acquired as the disease progresses. The most common of these involves MYC, which is involved in Ig and non-Ig translocations to place it next to active super-enhancers resulting in its overexpression. Other common secondary translocations involve TXNDC5, FOXO3, and TENT5C which all interact with MYC translocations [8], and also MAP3K14 (NIK), the central regulator of the NF-κB pathway [9].

2. High-Risk Genomic Markers

2.1. Translocations

Fluorescence in situ hybridization (FISH) has been extensively used to detect IgH translocations in the clinical setting [10–12]. This technique is beginning to be replaced by sequencing technologies such as whole genome sequencing or targeted sequencing, but while this is broadly used in research it has been only slowly implemented in clinical practice. It is widely accepted that the t(4;14), t(14;16), and t(14;20) are associated with a poor prognosis and are present in newly diagnosed MM patients at frequencies of 12%, 3%, and 2%, respectively [13–15]. These constitute the CCND2 translocation groups, where downstream expression of CCND2 is increased [16]. Conversely, the t(11;14) and t(6;14), present in 15% and 1% of newly diagnosed MM patients, are considered to be standard risk, do not express CCND2, but instead highly express CCND1 or CCND3, respectively [2,17].

MYC translocations are more difficult to comprehensively detect with FISH, due to their interaction with multiple chromosomes, the positioning of the breakpoints both telomeric and centromeric of MYC itself, and deletions or gains of chromosomal material in proximity to MYC [8,18,19]. This has led to a general underestimation of the prevalence of MYC translocations, where FISH generally only captures the t(8;14) IgH-MYC rearrangement. Previous sequencing data [8] indicated that MYC translocations were present in 24.6% of newly diagnosed MM patients, but only 4.5% were to the IgH locus. The remainder of MYC translocations were to the Ig light chain loci (6.7%) or to non-Ig loci (13.5%). In addition, there were copy number changes surrounding the MYC locus including focal gains and deletions, tandem duplications, and inversions that may affect regulatory sequences and, therefore, MYC expression, bringing the total of MYC abnormalities to 36% of MM patients.

Given the complexity of detecting MYC abnormalities, it is difficult to determine the true impact on prognosis, as most clinical trials do not capture all the rearrangement types. Of those trials with sequencing data, the UK Myeloma XI trial showed a significant multivariate association with progression-free survival [20], but there was no association with outcome in the more clinically diverse observational MMRF CoMMpass study [21].
However, an association with outcome was seen with immunoglobulin lambda (IgL)-MYC translocations in the same dataset, which can be extended to all IgL translocations [22].

2.2. Complex Structural Variants

In addition to the primary IgH translocations and secondary MYC rearrangements, new classes of structural variants and their impact on prognosis are being identified using whole genome sequencing. There are three main classes of complex structural variants: chromoplexy (concatenation of multiple translocations resulting in loss of copy number), templated insertions (concatenation of multiple translocations resulting in focal gain of copy number), and chromothripsis (chromosome shattering and random assembly with oscillating copy number) [23], and they have been identified at frequencies of 10%, 19%, and 24%, respectively, in the CoMMpass dataset [24]. These complex events are linked to DNA instability and alterations in TP53. In addition, chromothripsis is an independent event affecting poor outcome in the CoMMpass dataset. Further investigation of these complex events in other datasets will be key to understanding their interplay with outcome, DNA instability, and TP53 abnormalities.

2.3. TP53/Del(17p)

Since the identification of del(17p) as a prognostic marker using FISH [25], it has become clear that abnormalities of TP53 are incredibly important in identifying high-risk patients. Through the FISH era, many studies have continued to show that del(17p) was perhaps the strongest marker of high-risk disease and was present in between 8–11% of newly diagnosed MM patients [15,26,27]. Using FISH, it is possible to determine accurately the percentage of cells with the abnormality, and most institutions have used >10% of cells with the abnormality as the cut-off for the presence of the deletion in a sample. As such, it has been possible to further stratify patients based on the percentage of cells with del(17p) and, in general, the more cells with the deletion, the worse the association with poor prognosis and early relapse [28,29]. This makes biological sense given that the more clonal this abnormality is, the more high-risk cells are present to provide therapy resistance, resulting in a shorter time to relapse. Two studies have provided an optimal cut-off of del(17p) to determine high risk, using 55% and 60% of cells [28,29], but patients with lower percentages of abnormal cells are still considered to be high risk.

With the introduction of new techniques, such as next generation sequencing, it has become possible to identify both deletions and mutations of TP53 simultaneously. The incidence of biallelic TP53 alterations (combinations of deletion and/or mutation) at diagnosis is 2–3% [21,30]. Now that information on both alleles of TP53 is available, two studies have shown that the poor prognostic impact of del(17p) by FISH was, in fact, driven by biallelic alterations in TP53, and that patients with one intact TP53 allele fared as well as patients without any abnormality [21,28]. However, this observation has not been universally accepted, with one study showing that deletion of TP53 alone also conferred a poor outcome, though not to the extent of biallelic alterations [31]. The difference in results could be explained as the latter study only analyzed patients where del(17p) was present in >55% of cells, whereas the former studies incorporated all patients with TP53 abnormalities. This difference in the pathogenicity of TP53 has been experimentally tested using cell lines with engineered TP53 deletions and/or mutations to generate mono- or bi-allelic mutants, and in a co-culture system the biallelic mutants outgrow wild type and mono-allelic TP53 cells to become the dominant clone [32]. Unfortunately, most centers do not yet test for TP53 mutations and so del(17p) is still used as a proxy. Additional abnormalities of TP53 at the RNA and protein level likely contribute further to outcome, as has been seen through expression of p53 isoforms generated through alternative splicing [33].

2.4. Gain and Amplification of 1q

Extra copies of 1q are common in MM, having been identified in ~30–40% of newly diagnosed MM patients. The most common method of detection in diagnostic labs is with
FISH using a probe against the CKS1B locus on 1q21.3. Gain (three copies) of the whole of 1q is the most common abnormality of 1q, but focal gain or amplification (four or more copies) of 1q21 has also been seen [34,35], especially in relapsed samples [36]. Although CKS1B is commonly assayed for 1q abnormalities, there is no consensus on the driver gene of importance in this gene dense region of the genome, and many others have been suggested including BCL9, MCL1, ANP32E, IL6R, and ADARI [5,37–39].

Gain or amplification of 1q is associated with poor prognosis, and it has been shown that there is a stepwise association between the number of gained copies and poor outcome, where patients with gain of 1q perform worse than those without gain, and those with amplifications perform worse that those with gain [21]. However, in a meta-analysis of large clinical trials, there was competing evidence that there was no difference in outcome between patients with gain or amplification of 1q [40], but patients with amplification were more likely to be associated with other high-risk markers such as t(4;14), t(14;16), and stage III of the International Staging System (ISS) and Revised ISS (R-ISS).

2.5. Mutations

Aside from TP53, there is little information on the prognostic importance of mutations in MM. Often other mutations in the DNA repair pathway, including ATM (3.9%) and ATR (1.6%), are associated with poor outcome. In the UK Myeloma XI trial, the combined signal from ATM and ATR mutations was independently associated with poor progression-free and overall survival [20]. In the CoMMpass study, only TP53 mutations/deletions had an impact on prognosis [21]. However, a major limitation of these studies was the duration of follow-up, which was 25 months and 20 months, respectively. Another study with a much longer follow-up of 98 months [41], identified that mutations in DIS3 and BRAF, in addition to TP53, were associated with poor outcome. The association of BRAF and DIS3 mutations with prognosis is yet to be verified in an additional dataset.

DIS3, encoding part of the RNA exosome complex, is mutated in 10% of newly diagnosed MM patients [42] and is associated with poor outcome [41]. DIS3 is situated on chromosome 13, and as such is also frequently deleted. Similar to TP53, the poor outcome associated with DIS3 was due to biallelic alterations (mostly deletion and mutation of the remaining allele) which occurred in 5% of patients. It is not clear how a mutation of DIS3 affects outcome, but it has been associated with genomic instability in B cells [43].

BRAF, part of the MAPK pathway, has been shown to be mutated in 8% of newly diagnosed MM patients and mostly consists of the V600E variant (46%) but also has mutational hotspots at G466, G469, and D594 [41]. Based on studies in melanoma [44], these mutations can either be kinase activating (V600E, G469A/R/V) or kinase dead (G466E/R/V, D594E/G/N). Although both types of mutation have a poor prognostic impact, the kinase dead mutations had a significantly bigger impact on outcome. Kinase dead BRAF mutations have also been highly associated with concurrent mutations in NRAS or KRAS and have been linked to chemoresistance in other cancers [45].

2.6. Gene Expression

Gene expression profiling (GEP) using microarrays was a prolific methodology in the early 2000s that enabled whole transcriptome analysis of MM. These arrays were used on clinical trial samples to identify high-risk expression signatures including GEP70 and SKY92 [46,47]. These expression signatures both identify a high-risk set of patients comprising 15–20% of all newly diagnosed MM patients. The technology used for these signatures is becoming obsolete, and therefore, they are not as widely used as DNA-based risk classifiers. However, their use in several clinical trials has shown applicability in identifying high-risk patients who may benefit from novel therapies [48,49]. It is likely that these array-based expression signatures will be transitioned over to RNA-sequencing platforms, extending their applicability in a new technological era.

As technologies advance and more global genomic assays are performed on patient samples at a high rate, there is an inevitable influx of potential new high-risk biomarkers.
A recent crowd sourcing challenge identified that expression of PHF19 was associated with high-risk disease [50]. PHF19 encoded a polycomb-like protein that associated with the PRC2 complex and chromatin [51]. In a similar fashion to NSD2, which was overexpressed by the t(4;14), PHF19 resulted in a modified chromatin landscape. A combination of NSD2 and PHF19 expression with age and ISS resulted in a simpler risk stratification algorithm that was as effective as GEP70 [50]. PHF19 expression also increases in the transition from SMM to MM, indicating that it could also be a marker for disease progression [52].

2.7. Relapsed Myeloma

In general, the high-risk secondary genomic abnormalities seen in newly diagnosed MM increase in frequency at relapse. Not only is the incidence of gain/amp 1q or deletion/mutation of TP53 at a higher frequency in relapsed patients, but the clonal composition within one patient of these markers also increases, meaning that abnormalities that were subclonal at diagnosis are often clonal at relapse [53–55]. For TP53 and other tumor suppressor genes such as CDKN2C and RB1, the incidence of biallelic loss also increases in frequency at relapse. This indicates a selection bias for high-risk markers and treatment resistance.

In addition to the markers at diagnosis, other genomic abnormalities can rise to dominance at relapse. For example, mutations in PRDM1 have been associated with decreased outcome at relapse [56]. There are also treatment-related abnormalities that are selected due to drug resistance mechanisms. For instance, IMiD treated patients relapsed with a variety of abnormalities in the cullin E3-ubiquitin ligase complex including cerebon (CRBN). A variety of CRBN abnormalities have been described in IMiD-resistant patient samples including mutations, deletions, and RNA splice variants and these are associated with inferior outcomes [57,58]. As an extension to the cullin E3-ubiquitin ligase complex, the COP9 signalosome is also important for IMiD sensitivity and the genes COPS1, COP2, COPS4, and COPS5 have been identified in genome-wide screens of myeloma cells as determining drug sensitivity [59–61].

With the advent of targeted immunotherapies in MM, there are a new raft of resistance mechanisms being identified. As most immunotherapies target cell surface molecules, there is a selection process for cells with reduced or no surface expression of the antigens. This type of intrinsic immunotherapy evasion can include deletion of the gene at the DNA level, reduced expression of the gene, post-translational modifications, secretion of the antigen, or other mechanisms to reduce the efficacy of the therapy. A key example of this is B cell maturation antigen (BCMA, encoded by TNFRSF17) which is targeted by chimeric antigen receptor (CAR)-T cells, bispecific T cell engagers (BiTEs), and antibody-drug conjugates (ADCs). It has been shown that patients relapsing from these treatments can have homozygous deletions of TNFRSF17 resulting in loss of expression of BCMA and lack of efficacy of the drug [62,63].

3. High-Risk Smoldering Multiple Myeloma

Smoldering multiple myeloma (SMM) patients are increasingly being stratified according to risk of progressing to MM. Currently, risk stratification is based on clinical parameters including bone marrow plasma cell percentage, serum free light chain ratio, and the presence of focal lesions [64]. Those patients associated with a high-risk of progression may benefit from treatment before clinical symptoms appear, whereas low risk patients may benefit from the watch and wait approach and not suffer any adverse side effects from un-necessary treatment.

Increasing the specificity of these stratification methods is likely to include incorporating genomic markers. Two studies examined time to progression in SMM patients with FISH markers [65,66] and both studies identified that those patients with a t(4;14) or del(17p) were associated with a shorter time to progression. More recently DNA sequencing has been used to explore the genomics of SMM more fully by comprehensively analyzing copy number and mutations, in addition to translocations. From these studies [67–69], the key abnormalities associated with a shorter time to progression to MM were Ras mutations.
(KRAS and NRAS), DNA repair pathway mutations (TP53, ATM, and ATR), mutations in DIS3, and MYC abnormalities. In addition, there were significant differences in the frequencies of some abnormalities between SMM and MM patient samples including fewer high-risk translocations, especially t(4;14) and MYC translocations; a lower frequency of high-risk copy number changes including del(1p), gain 1q, and del(17p); fewer mutations in NRAS and TENT5C; and fewer biallelic events in tumor suppressor genes such as CDKN2C and TP53.

Taken together, these studies indicate that the presence of known MM high-risk genomic abnormalities in SMM patients are important in driving the disease towards active myeloma. Current risk stratification of SMM patients is based on clinical and biochemical markers and minimal genomic information [70]. However, the incorporation of genomic information into MM risk stratification models has benefited patients, and therefore, the incorporation of more detailed genomics into SMM risk models will also improve risk stratification further and give valuable insights into the biology of the disease as it progresses.

4. Translating High-Risk Markers into Clinical Practice

One of the main problems with many risk stratification methods is implementing them in a clinical setting. FISH is used as a standard in many institutions, but even the panel of probes used can be inconsistent between institutions. In addition, the IMWG guidelines highly recommend that CD138 cell selection be performed on bone marrow aspirates to enrich for the malignant plasma cells before FISH is performed. This is incredibly important when detecting subclonal copy number changes in 1q and 17p which may be missed in unselected cell preparations.

Currently, the main risk stratification algorithms for newly diagnosed patients includes the International Staging System (ISS) [71], which uses serum albumin and β2-microglobulin levels. The ISS has been further refined by the addition of high-risk genomic abnormalities into the Revised ISS (R-ISS) [72] and the International Myeloma Working Group (IMWG) risk stratification methods [73]. The high-risk genomic abnormalities considered by these latter stratification methods are t(4;14), t(14;16), del(17p13) (TP53 locus), and gain/amplification 1q21+ (CKS1B locus) (Table 1), therefore, at the minimum these abnormalities should be analyzed to identify high-risk patients.

<table>
<thead>
<tr>
<th>Risk Stratification Method</th>
<th>Low</th>
<th>Standard</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISS [71]</td>
<td>I: serum β2m &lt; 3.5 mg/L, serum albumin ≥ 3.5 g/dL</td>
<td>II: Not stage I or III</td>
<td>III: serum β2m ≥ 5.5 mg/L</td>
</tr>
<tr>
<td>IMWG [73]</td>
<td>ISS I/II, age &lt; 55, and absence of all of t(4;14), del(17p13), and 1q21+</td>
<td>Not low- or high-risk</td>
<td>ISS II/III and t(4;14) or del(17p13)</td>
</tr>
<tr>
<td>R-ISS [72]</td>
<td>I: ISS I and absence of all of del(17p13), t(4;14), and t(14;16)</td>
<td>II: Not R-ISS I or III</td>
<td>III: ISS III with either high serum LDH or presence of any of del(17p13), t(4;14), t(14;16)</td>
</tr>
<tr>
<td>MGP Double-Hit [21]</td>
<td>Not high-risk</td>
<td>Biallelic TP53 or ISS III + amp1q</td>
<td></td>
</tr>
<tr>
<td>MASS [74]</td>
<td>No high-risk markers</td>
<td>One high-risk marker</td>
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</tr>
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In addition to these main risk stratification methods, it has been shown that the accumulation of high-risk markers also results in a progressively worse outcome. For example, patients enrolled in the UK Myeloma IX trial assessed for high-risk IgH translocations,
gain 1q, and del(17p) have a worse prognosis when analyzed for any one of these markers. However, when they are combined, it was found that patients with all three high-risk abnormalities have a worse outcome than those with two abnormalities, who in turn have a worse outcome than those with only one abnormality [15]. To this end, the Mayo clinic uses an additive algorithm to assess risk using the markers t(4;14), t(14;16), t(14;20), 1q gain or amplification, del(17p), ISS III, and elevated lactate dehydrogenase (LDH) [74]. Two or more of these markers is indicative of high risk.

None of these risk assessment methods take into consideration the incredibly important biallelic TP53 abnormalities that include mutation or deletion of both alleles of the gene. The recent analysis of the largest collection of sequencing data by the Myeloma Genome Project using a recursive partitioning model showed that the key variables defining risk were biallelic abnormalities in TP53 or amplification of 1q in combination with ISS III, and was termed double-hit [21]. With this definition, 6.1% of newly diagnosed patients were identified as high-risk myeloma.

As more information is gathered on patients’ genomic abnormalities, there will be a better understanding of what high-risk myeloma is comprised of and how to treat it [75]. Hopefully, future clinical trials will adopt comprehensive sequencing strategies to fully understand how the genomics of the tumor influences patient outcome and will not rely on information from a handful of FISH probes. In turn, this information can be passed down to smaller centers so that they can more quickly and cost-effectively use targeted sequencing approaches to identify the key prognostic information in non-trial patients, as well as identifying any targets of precision medicine [76].

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