

# The molecular pathogenesis of multiple myeloma

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## Abstract

Multiple Myeloma (MM) is characterized by uncontrolled proliferation and accumulation of clonal plasma cells within the bone marrow. However, the cell of origin is a B-lymphocyte acquiring aberrant genomic events in the germinal center of a lymph node as off-target events during somatic-hypermutation and class-switch recombination driven by activation-induced-deaminase. Whether pre-germinal center events are also required for transformation, and which additional events are required for disease progression is still matter of debate. As early treatment in asymptomatic phases is gaining traction in the clinic, a better understanding of the molecular pathogenesis of myeloma progression would allow stratification of patients based on their risk of progression, thus rationalizing efficacy and cost of clinical interventions. In this review, we will discuss the development of MM, from the cell of origin through asymptomatic stages such as monoclonal gammopathy of undetermined significance and smoldering MM, to the development of symptomatic disease. We will explain the genetic heterogeneity of MM, one of the major drivers of disease recurrence. In this context, moreover, we will propose how this knowledge may influence future diagnostic and therapeutic interventions.

## Introduction

Random mutagenesis is a frequent and likely ubiquitous phenomenon in replicating tissues, stemming from slight intrinsic infidelity of DNA replication and repair processes, and enzymatic modification of DNA bases.<sup>1</sup> Additionally, exogenous processes may increase this mutation rate. Rarely, these events will result in creation of a variant conferring a proliferative or survival advantage to the cell. In such case, a

small pre-clinical proliferation will be observed.<sup>2</sup> Further acquisition of additional variants may then dictate evolution from these small clonal proliferations, never recognized in clinical practice, to a clinically evident cancer following natural selection acting on the resulting phenotypic diversity.<sup>3</sup> The complex multicellular microenvironment, the competition for metabolites, oxygen, growth factors, and the necessity for immune escape<sup>4</sup> will also dictate which clone is the fittest for growth. Genomic plasticity, conferred by the loss of DNA-repair mechanisms and/or acquisition of a hypermutator phenotype, will certainly facilitate the ability to adapt of the tumor cells.

Plasma cell dyscrasias are frequent hematological malignancies, and are usually regarded to as a more complex disease from a genomic point of view as compared to leukemias and lymphomas.<sup>5</sup> The most frequent conditions, forming a continuous spectrum that can often be observed over time in the same patient, are clinically categorized as monoclonal gammopathy of unknown significance (MGUS), smoldering multiple myeloma (SMM) and active MM.

The diagnosis of MGUS requires the presence of a serum monoclonal protein of  $<3$  g/dL and  $<10\%$  clonal bone marrow (BM) plasma cells, in the absence of myeloma defining events or amyloidosis. MGUS may progress to the more advanced asymptomatic stage of SMM, defined by a serum monoclonal protein of  $\geq 3$  g/dL or 24h urinary monoclonal protein  $\geq 500$  mg, and/or  $10\text{--}60\%$  clonal BM plasma cells in the absence of myeloma defining events or amyloidosis. Active MM in turn is diagnosed in presence of clonal BM plasma cells  $>10\%$  and/or a biopsy proven bony or extra-medullary plasmacytoma, and one or more myeloma-defining events: end-organ damage (hypercalcemia, renal failure, anemia, lytic bone lesions),  $\geq 60\%$  bone marrow clonal plasma cells, serum free light-chain (FLC) ratio  $\geq 100$  (for kappa) or  $<0.01$  (for lambda), and  $>1$  focal lesion on MRI.<sup>6</sup>

These categories reflect differences in management to prevent the development of end-organ damage, or its prompt recognition and treatment.<sup>6</sup> From a genomic point of view, the question then is whether this clinical evolution is paralleled by a similar biological evolution of the neoplastic clone, from initiating lesions to those associated with progression and development of an aggressive disease, and whether this can be exploited clinically.

In this review, we will describe recent advances on the molecular pathogenesis of MM. Cell-intrinsic factors involved in the initiation processes of monoclonal gam-

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mopathies will be described in the context of the normal B-cell development. We will then focus on the different patterns of evolution from asymptomatic to aggressive stages of disease and the impact MM heterogeneity has in this process.

## Initiating events

Asymptomatic clonal expansion of hematopoietic cells are nowadays well recognized at the expense of plasma cells,<sup>7</sup> having been found decades ago through the identification of a monoclonal antibody in the serum through protein electrophoresis. On the contrary, the presence of an asymptomatic clonal B-lymphocytosis has been identified more recently thanks to flow cytometry.<sup>8</sup> Stem cells can also show similar instances of asymptomatic clonal expansions, identified through next-generation sequencing (NGS).<sup>9,10</sup> All these evidences confirm the multi-step nature of cancer evolution. In fact, it is thought that all MMs are preceded by an MGUS stage, even if not clinically evident.<sup>11</sup>

Contrary to lymphoproliferative diseases, plasma cell dyscrasias are not classified based on the cell of origin, since the lat-

ter – a post-germinal center B-lymphocyte is morphologically different from the neoplastic cell encountered in the bone marrow at the time of diagnosis – a plasma cell.

Events leading to transformation of a naïve B cells upon antigen encounter within the germinal center (GC) of lymph nodes are thought to arise from errors during class-switch-recombination (CSR) and somatic hypermutation (SHM) of the B-cell receptor (BCR). These are two processes aimed at increasing antigen affinity to a peculiar antigen and conferring specific effector functions, catalyzed by the activation-induced-deaminase (AID) enzyme. Through the creation of double strand breaks and mutations, AID activity is at risk of off-target mutations and rearrangements.<sup>12</sup> It is unknown if the transformed cell would need some priming in the form of pre-existing mutations or genomic lesions permissive to the survival upon this AID off-target activity, but this is subject of intense research. Another question is whether this transformation is favored by a germline predisposition. Indeed, the risk of developing a plasma cell dyscrasia is increased two-fold in relatives of MM patients,<sup>13</sup> and germline transmission of several risk alleles has been described.<sup>14,15</sup>

The transformed B-cell will then home to the BM and differentiate into a plasma cell, giving rise to the clonal expansion clinically recognized as MGUS. Importantly, crucial to this process is the interaction with the microenvironment.<sup>16</sup>

MGUS therefore displays common genetic features with MM: it carries either recurrent translocations of oncogenes with switch regions of the IGH locus or an hyperdiploid (HD) karyotype. The latter consists in multiple trisomies of odd chromosomes 13 and 17.<sup>17</sup> IGH translocations are caused by aberrant CSR promoted by AID, as proved by the fact that the rearrangement hotspot is close to the canonical CSR breakpoints. Furthermore, translocation partner genes show mutations with a signature of AID-induced mutations, consistent with a germinal center origin of the event.<sup>18</sup> Subsequent to the translocation, Ig enhancers promote the overexpression of the recurrent partner genes, consisting in the known oncogenes *CCND1*, *WHSC1*, *MAF*, *MAFB*, *CCND3* in t(11;14), t(4;14), t(14;16), t(14;20) and t(6;14) respectively. Being initiating events, IGH translocations are almost always clonal, mutually exclusive with each other and with the HD karyotype.<sup>12,19</sup> On the contrary, mechanisms leading to the generation of an HD karyotype are less clear. In hyperdiploid acute lymphoblastic leukemia, the HD karyotype

is thought to derive from a single abnormal cell cycle duplication.<sup>19</sup> However, analysis of the activity of mutational processes with a constant mutation rate on trisomic chromosomes showed that the number of pre-gain and post-gain mutations is often different from chromosome to chromosome. This implies that different trisomies can be acquired in different time windows.<sup>20</sup> Furthermore, only mutations in HD chromosomes acquired before the gain show an off-target AID signature, while mutations acquired after the gain don't show any sign of AID activity.<sup>21</sup> This demonstrates a germinal center origin of the trisomy. Last, mechanisms linking trisomy to neoplastic transformation are unclear, but may be linked to the expression of oncogenes within the duplicated chromosomes.<sup>22</sup>

The same analysis of the activity of mutational signatures with a constant activity over time has provided bases to enquire when the transformation happens in the life of the patient. Using serial samples from the same patients, the activity of these mutational processes could be extrapolated back in time, concluding that the transforming event could take already in the second or third decade of life.<sup>21</sup> Subsequently, decades of clonal proliferation and acquisition of additional events would ensue before the clone becomes clinically evident in the form of MGUS.

## Genomic features of MGUS

In MGUS, differently from MM, clonal BM plasma cells are low to absent, the monoclonal protein in the serum is low, and there are no signs of end-organ damage, active MM or amyloidosis.<sup>23</sup>

IGH translocations and HD are transforming events, however they are not sufficient for MM development. In fact, MGUS may display these abnormalities and remain clinically stable. This is the case for the majority of cases, since MGUS progresses at an average rate of 1% of cases each year, and this rate does not increase even after decades.<sup>24</sup> This argues against a model of continuous acquisition of additional lesions to drive progression, and on the contrary suggests that clonal sweeps may be driven by stochastic events. The question is therefore what additional genomic events are required for progression. From a point of view of prevalence, the t(11;14) is more frequent in MGUS, while all other translocations are more prevalent in MM. On the contrary, del(13) and other copy-number alterations (CNAs) are more prevalent in MM. This suggests a differential propensity

towards transformation conferred by karyotypic events that can be assessed by FISH or SNP-arrays.<sup>25–27</sup> Furthermore, the fact that many CNAs can be found at the sub-clonal level confirms their acquisition after the PC clone has been established.

NGS has the potential to allow a much deeper analysis of the genome of MGUS, highlighting initiating events beyond recurrent translocations and CNAs. However, single-cell RNAseq studies have clearly highlighted how at this stage there is a large number of contaminating, non-clonal PC which may hamper bulk cell analysis.<sup>28</sup> However, initial targeted DNA-sequencing studies highlighted recurrent mutations in the myeloma genes *NRAS*, *BRAF*, *KRAS*, *DIS3*, *EGR1* and *LTB*. Mutations were less frequent than in active MM and within each case allelic frequencies were suggestive of a late acquisition of the mutation.<sup>27,29</sup> Importantly, no mutations have been detected in tumor suppressor genes such as TP53, or in genes involved in DNA repair mechanisms as *ATM* or *ATR* usually enriched in more advanced phases of the disease. Indeed, MGUS does not display an early and specific single-nucleotide mutational activity that may explain expansion of the tumor clone. This is very different, for example, from what observed in NPM1-mutated acute myeloid leukemia,<sup>30–32</sup> and is more in line with a slowly evolving disease driven by structural events as seems to be the case for most mature lymphoid neoplasms.

Very recently, through the combination of multi-parametric flow-sorting strategy and low-input whole genome library preparation, the genome of highly purified clonal MGUS PCs has been sequenced.<sup>33</sup> With the addition of SMM and MM cases, a comprehensive analysis of progressive vs non-progressive asymptomatic cases and their comparison to active MM cases has been possible. Results suggested a quite striking difference between MGUS and SMM cases that remained stable in the long term. Stable asymptomatic conditions displayed very little activity of mutational processes besides the AID-activity responsible for disease initiation,<sup>34</sup> along with reduced numbers of CNAs. Interesting, while the number of trisomies in HD cases were not significantly different between the various conditions, stable asymptomatic cases showed fewer instances of chr(1q) gain or amplifications, del(6q), gain(8q24) involving the *MYC* locus, del(16q) as compared to progressive cases. Finally, structural variants and particularly complex events like chromothripsis and templated insertions<sup>20</sup> were strikingly enriched in progressive cases, suggesting that in the future a molecular signature may

prognosticate indolent asymptomatic cases much better than the current clinical and laboratory parameters.

## Genomic features of SMM

SMM carries a higher disease burden than MGUS, as by definition clonal plasma cells in the BM must be >10% and <60%. The rate of progression of SMM is 10% per year in the first 5 years, then declines to 3% for the next 5 and to 1% after ten year from diagnosis.<sup>35,36</sup> SMM is therefore quite heterogeneous from a clinical point of view, suggesting its definition includes patients ranging from an actual active MM that does not yet satisfy criteria for diagnosis to others with a biologically indolent form similar to MGUS just with more BM PCs.

MGUS patients do not routinely undergo BM examinations during follow-up, therefore it is unusual to catch an evolution from MGUS to SMM even if this is what it is supposed to happen in all progressing cases. This makes it hard to ascertain events associated with initial progression of this asymptomatic conditions, and most of what we know about SMM comes from cases diagnosed *ab initio* as such. Furthermore, since SMM itself can be stable for years, our knowledge of its evolution is biased towards more aggressive and more rapidly evolving cases.

From a genomic point of view, SMM appears to carry similar genetic abnormalities to active MM, just at a lower frequency.<sup>29,37,38</sup> An interesting observation

came from the whole-genome analysis of paired samples from ultra-high risk SMM progressing to MM. At baseline, the genomic structure of SMM was similar to MM in terms of driver events. This included translocations, CNAs and gene mutations but particularly complex structural events. Known secondary CNAs such as del(13q), del(6q), del(8p), del(16q) and amp(1q) were also frequent. Differently from MGUS, the structure of high-risk SMM was therefore very similar to that of MM.<sup>39</sup> Comparing the genome of paired samples, clonal evolution followed one of two main modalities. Authors described a “static progression model”, where the subclonal structure grows unchanged from SMM to MM, and a “spontaneous evolution” model, where the subclonal structure of SMM changes because of the acquisition of one or more subclones and/or loss of others at the time of progression to MM. On average, patients would progress in less than one year in the static model, and at a much slower pace in the spontaneous evolution model. Furthermore, analysis of mutational processes active in each subclone was also particularly revealing. AID activity was preponderant in the ancestral clone of each case, again confirming a germinal center origin of the disease. Subclones evolved later in the disease course and responsible for progression showed instead enriched activity of the APOBEC family of DNA deaminases, an aberrant mutational process active across a variety of cancers<sup>40</sup>, shedding some light onto aberrant genomic processes responsible for the acquisition of

additional genomic lesions.

More recent studies on much larger sample cohorts have further expanded these findings, and translated them into information that could be used in clinical practice. In particular, *MYC* translocations<sup>41</sup> or *MYC* abnormalities, mutations in *MAPK* genes or DNA repair genes and the t(4;14) all independently predicted progression to MM.<sup>42</sup> This evidence makes it tempting to assume that genomics can really help prognostication of SMM by identifying at diagnosis cases that will behave like MGUS, and will rarely progress in years owing to the acquisition of additional genomic events, and cases that are de facto MM and already show all features of an aggressive neoplasms. This also highlights the inadequacy of current prognostic scores, mostly based on the tumor burden of SMM<sup>43</sup>.

## Genomic features of MM

Genomic studies in MM have much changed the perception of the disease in the last 10 years. Dozens of mutated genes, mutational processes, CNAs and complex structural events have been added to the genomic landscape of what initially seemed to be a disease with few karyotypic events<sup>44</sup> and gene mutations. Initial enthusiasm for the discovery of actionable mutations such as *BRAF* V600E<sup>45</sup> has nevertheless been curbed by the evidence that MM at diagnosis is a highly heterogeneous disease,<sup>18,46,47</sup> so that targeted treatment can trigger rapid subclonal outgrowth outcompeting the main

**Table 1. Main genomic features of MGUS (1a), SMM (1b) and MM (1c).**

Stage of disease	Genomic features
Stable asymptomatic cases	Fewer instances of chr(1q) gain or amplifications, del(6q), gain(8q24) involving the MYC locus, del(16q) as compared to progressive cases
Progressive cases	Structural variants and particularly complex events like chromothripsis and templated insertions are strikingly enriched
Table 1a MGUS	
Genomic feature	Clinical significance
MYC abnormalities/translocations, MAPK or DNA repair genes mutations t (4; 14)	Independently predict SMM progression to MM
Table 1b SMM	
Genomic feature	Clinical significance
Mutations in CRBN t (11;14)	Predict IMiDs and PI resistance Predict targeted treatment (venetoclax) responsiveness
High-risk lesions:	Simultaneously resistance to PIs and IMiDs and worse prognosis
- bi-allelic events in tumor suppressors,	
- amp(1q),	
- acquisition of an APOBEC signature	
Table 1c MM	

clone at diagnosis.<sup>48</sup> At the same time, this wealth of information has failed to improve a biological classification of the disease. This is still based on the main cytogenetic events, confirming these are the initiating events that shape the subsequent trajectory of evolution, providing some constraint on the type of alterations required for progression<sup>20</sup>. HD and IGH-translocated cases still provide the mainstay of classification. Within HD cases, about a third associate with CNAs – mostly del(1p), amp(1q), del(13q), del(14q), del(16q) – and the other two thirds with mutations, with a preponderance of mutations of the RAS family. T(4;14) cases cluster in two categories, frequently associate with del(13q), and in the first they also associate with CNAs, in the second they show fewer CNAs but mutations in *DIS3* and *FGFR3*. t(11;14) cases also fall in two different categories, one with *CCND1* and *IRF4* mutation, and one with *TP53* bi-allelic inactivation. A seventh category is not characterized by any particular structural event, but by a hypermutated genotype.<sup>20</sup> This evidence reinforces the notion that gene mutations are late events, whose impact is not strong enough to define a genetic category of the disease. Furthermore, most mutated genes are not even expressed in MM.<sup>49,50</sup> On the contrary, and similar to solid cancers, structural events – many of which are complex and non-recurrent, yet impacting recurrent driver genes – are the events that drive and define the disease.<sup>51</sup>

However, genomic analysis has revealed several important prognostic correlates, most of which are not captured by the R-ISS<sup>52</sup>. A well-known example is what is somehow improperly referred to as “double-hit” multiple myeloma, *i.e.* cases with ISS stage 3 and chr(1q)amp, or cases with *TP53* bi-allelic inactivation.<sup>53</sup> The definition is somewhat improper since it has been observed how several combinations of genomic events have prognostic relevance, some showing a clear interaction, others simply highlighting an additive effect.<sup>54,55</sup> Other events associated with worse prognosis include a high activity of the APOBEC mutational process,<sup>56</sup> IGL-MYC translocations,<sup>57</sup> *TP53* mutations.<sup>58</sup> While this list is not at all inclusive, the main examples are cited to stress the point that the use of genomic analysis for MM prognostication is still in its infancy, and a comprehensive analysis will be required over large datasets to understand the independent prognostic role of these and other variables to inform clinical decisions.

Aside from prognostic markers, genomics has also opened the search for predictive markers, *i.e.* events that correlate

with response to a specific treatment or lack thereof. However, no such biomarkers have been found to correlate with response to proteasome inhibitors (PIs) or immunomodulatory drugs (IMiDs), the two most used drug classes in induction. Indeed, mutations in *CRBN* in IMiD-resistant cases<sup>59</sup> and in proteasome subunit genes in PI-resistant cases<sup>60</sup> account for a tiny fraction of cases and are not found – or are found at the sub-clonal level – in the majority of cases that do not respond to such treatments.<sup>50,61</sup> Indeed, analysis of cases that are simultaneously resistant to PIs and IMiDs suggested instead that chemoresistance in MM is achieved through the acquisition of high-risk lesions, such as bi-allelic events in tumor suppressors, amp(1q), and acquisition of an APOBEC signature.<sup>50</sup> The described sub-clonal heterogeneity is responsible for this dynamic evolution of the tumor through lines of treatment and is especially visible in cases of extra-medullary evolution.<sup>18,62</sup>

### Potential clinical applications of genomic technologies in plasma cell dyscrasias

The recent progress prompted by genomics discoveries in MM raised the question as to whether these merit incorporation into routine clinical practice. The paradigm has been set by myeloid malignancies and particularly AML, where genomics has dramatically impacted classification<sup>63</sup> and prognostication,<sup>64</sup> prompting the development of clinical-grade NGS sequencing panels.<sup>65–67</sup> In MM, the nature of the genome of the disease requires that translocation in IGH regions and copy-number abnormalities are captured along with gene mutations, posing additional hurdles to the design of the panel. While several NGS attempts have been successful at matching or outperforming the accuracy of FISH for the detection of such structural events,<sup>68–70</sup> the perception still is that NGS is a much complicated technique and the extra-information added to common FISH panels is not going to change the way we make clinical decision soon.

This perception may soon be challenged. Starting from SMM, novel prognostic markers incorporating cytogenetic events have been validated,<sup>71</sup> accepting the notion that disease biology should be more relevant than disease burden for SMM prognostication.<sup>72</sup> In newly diagnosed MM, treatment paradigms still follow a “one-size fits all” approach, but the example of venetoclax, a new drug class in relapsed-refractory MM that seems to offer a survival

advantage in cases with t(11;14) but a disadvantage in other subgroups<sup>73</sup> will open the field for personalized treatment in MM based on patient-specific gene lesions. Furthermore, several trials are exploring a risk-adapted approach, if not a “basket” design where personalized treatment is offered based on each patient’s gene mutations.<sup>74</sup> The most immediate application of NGS to the clinic will nevertheless be that of reliable measurement of minimal-residual disease through the sequencing of the patient-specific B-cell receptor rearrangement. The prognostic value of this technique seems to be extremely high,<sup>75</sup> and this is likely explained by the fact that this technique may overcome the heterogeneity of other phenotypic and genotypic markers of the tumor clone.

Limits of NGS in MM could be represented by the difficulty of obtaining enough DNA from bone marrow aspirates, and by the spatial heterogeneity of the disease.<sup>62</sup> In this respect, analysis of circulating tumor cells or cell-free tumor DNA could represent a suitable alternative for longitudinal disease monitoring. While initial approaches in MGUS and SMM have been in part disappointing,<sup>76,77</sup> MM at diagnosis seem to offer more circulating cells and cfDNA thus allowing a more informative analysis.<sup>78,79</sup> Limiting amounts of circulating DNA seem also to limit the analysis of peripheral blood to track disease response to treatment so far<sup>80</sup>. However, there is little doubt that knowledge banks built on thousands of cases of MM, including genomic and clinical details are highlighting prognostic groups that can’t be captured by FISH alone.<sup>55,53</sup> More such efforts are underway and will likely soon reach a consensus on a reduced set of genomic lesions that may explain most of the risk of MM at diagnosis and may amenable to routine clinical-grade detection. Furthermore, the advent of new drug classes will improve the treatment landscape of MM, but potential benefits may be offset by increased costs and toxicity. This mandates that novel biomarkers are found to rationalize treatment, implying that genomic analysis will become routine clinical practice at diagnosis and at each relapse.<sup>81</sup>

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