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REVIEW ARTICLE

# Increasing genomic discovery in newly diagnosed multiple myeloma: defining disease biology and its correlation to risk

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## Abstract

Our understanding of MM genomics has expanded rapidly in the past 5–10 years as a consequence of cytogenetic analyses obtained in routine clinical practice as well as the ability to perform whole-exome/genome sequencing and gene expression profiling on large patient data sets. This knowledge has offered new insights into disease biology and is increasingly defining high-risk genomic patterns. In this manuscript, we present a thorough review of our current knowledge of MM genomics. The epidemiology and biology of chromosomal abnormalities including both copy number abnormalities and chromosomal translocation are described in full with a focus on those most clinically impactful such as 1q amplification and del(17p) as well as certain chromosome 14 translocations. A review of our ever-expanding knowledge of genetic mutations derived from recent whole-genome/exome data sets is then reviewed including those that drive disease pathogenesis from precursor states as well as those that may impact clinical outcomes. We then transition and attempt to elucidate how both chromosomal abnormalities and gene mutations are evolving our understanding of disease risk. We conclude by offering our perspectives moving forward as to how we might apply whole-genome/exome-level data in addition to routine cytogenetic analyses to improve patient outcomes as well as further knowledge gaps that must be addressed.

**Keywords** MM · Treatment · Mutations · Cytogenetics

## Abbreviations

| | |
|---|---|
| amp | Amplification, ≥4 copies |
| ASCT | Autologous hematopoietic cell transplant |
| B2M | $Beta_2$ microglobulin |
| CA | Chromosomal abnormalities |
| CCF | Cancer clone fraction |
| CNAs | Copy number alterations |
| CNV | Copy number variations |
| EMN | European myeloma network |
| FDA | Food and Drug Administration |
| HD | Hyperdiploid |
| HR | Hazard ratio |
| IFM | Institute Francophone du Mye´lome |
| IGH | Ig heavy chain locus |
| IMWG | International Myeloma Working Group |
| ISS | International Staging System |
| LDH | Lactate dehydrogenase |
| IgK | Light chain kappa |
| IgL | Light chain lambda |
| MGP | Myeloma genome project |
| MGUS | Monoclonal gammopathy of undetermined significance |
| MM | Multiple myeloma |
| MMRF | Multiple Myeloma Research Foundation |
| MMSET | Multiple myeloma SET domain |
| MV | Multivariate |
| NDMM | Newly diagnosed multiple myeloma |
| NHD | Non-hyperdiploid |
| OS | Overall survival |
| PCL | Plasma cell leukemia |
| PFS | Progression-free survival |
| PI | Prognostic index |
| R-ISS | Revised international staging system |
| RRMM | Relapsed refractory multiple myeloma |
| SMM | Smoldering myeloma |
| XPO1 | Exportin 1 |

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## Introduction

Multiple myeloma (MM) is an acquired malignant plasma cell disorder that typically develops late in life with a median age at diagnosis of 69 years. Although it is a rare disorder accounting for just 1.8% of all new cancers in the USA and a lifetime risk of just 0.76%, it is the second commonest hematological malignancy [1]. With the advent of new therapeutics and the increasing utilization of high-dose melphalan and autologous stem cell transplantation (ASCT) over the last 20 years [2], 5- and 10-year overall survivals (OS) have improved across all age, race, and ethnic groups [3]. These benefits are more tempered in those with high-risk disease with revised international staging system (R-ISS) stage III [4] patients achieving only a 24% 5-year progression-free survival (PFS) and 40% 5-year overall survival (OS). MM remains incurable with only 10–15% of MM patients achieving or exceeding expected survival as compared with the matched general population.

Our understanding of MM genomics has expanded rapidly in the past 5–10 years. This is a consequence of cytogenetic analyses obtained in routine clinical practice guided by the International Myeloma Working Group (IMWG) [5] and R-ISS [4] staging systems as well as the ability to perform whole-exome/genome sequencing and gene expression profiling on large patient data sets. Consequently, the driving genomic events that lead to the development of MM from its precursor states as well as the cytogenetic and mutational changes that drive disease progression and relapse are being elucidated. Given the clonal heterogeneity between and within MM patients, this knowledge has not directly translated into targeted therapeutics but has greatly increased our understanding of drug resistance mechanisms and the biology of risk.

Below, we first present a thorough review of our current knowledge of MM genomics including chromosomal abnormalities and genetic mutations. We subsequently explore how they drive MM disease pathogenesis and have translated into better risk stratification in newly diagnosed multiple myeloma (NDMM) patients. We conclude with our perspective moving forward as to how we might apply whole-genome/exome-level data in addition to routine cytogenetic analyses to improve patient outcomes as well as define and prioritize further knowledge gaps moving forward.

## Chromosomal abnormalities in multiple myeloma pathogenesis: copy number abnormalities and translocations

MM is known to evolve from the precursor diseases monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM). Genetic events detected at the MGUS stage are likely to be primary events involved in tumor development whereas events present at the MM stage and absent in MGUS are likely secondary events leading to tumor progression. The genetic abnormalities in MM are complex and heterogenic but molecular subgroups have been clearly defined. Primary events in MM can be generally grouped into two categories: cases with primary immunoglobulin translocations; and those that are hyperdiploid (HD) with trisomies of the odd-number chromosomes also referred to as cases with copy number abnormalities (CNAs known as aneuploidies, abnormal in whole chromosome or arm level) [6].

Of note, the methodology of detecting cytogenetic abnormalities has evolved in MM over the past several decades. During early risk stratification and genomic assessment of MM patients, specific abnormalities on metaphase cytogenetics were found to be associated with inferior survival. However, this assay relies on the presence of actively dividing cells, and as terminally differentiated B cells, plasma cells have limited proliferative capacity [7, 8]. For example, the t(4;14) translocation is karyotypically silent with conventional cytogenetics but detected by FISH probes for breakpoints on chromosome 4 in the *FGFR3* gene. Consequently, only one-third of MM patients have metaphase cytogenetic abnormalities at diagnosis. Interphase FISH (iFISH) is a more sensitive modality for identifying specific cytogenetic abnormalities of pathological significance. Subsequent to the International Staging System (ISS) development [9], chromosomal abnormalities (CA) detected by iFISH have become a standard of care in risk stratifying MM patients [10, 11]. MM may have chromosomal aberrations carried by only a subset of tumor cells, and the cytogenetic heterogeneity of individual cases reflects the coexistence of cytogenetically defined aberrant plasma cell clones [12].

### Copy number abnormalities

Karyotyping and iFISH studies have demonstrated that almost all MM are aneuploid. Based on the types of CNAs, MM is divided into two main groups: HD and non-hyperdiploid (NHD) which compose 60% and 40% of MM respectively. HD (with a number of chromosomes between 48 and 74) is characterized by trisomies of odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19, and/or 21 [13–15]; while NHD is characterized by hypodiploid (up to 44/45 chromosomes), pseudodiploid (44/45 to 46/47), near-tetraploid (more than 74), hyperhaploid (loss of nearly a haploid set of chromosomes with 24–34 chromosomes), and tetraploid [14]. The HD state may be a consequence of simultaneous gain of all additional chromosomes in a single abnormal mitosis. Only a limited percentage of HD

tumors (<10%) have a concurrent primary translocation affecting IGH [16]. Single-cell sequencing has shown that HD can precede primary translocations affecting the Ig heavy chain (IgH) locus in some patients [17].

In addition to aneuploidy changes, copy number gains or losses of chromosomes at an entire arm level are frequent including del13q (45–59%), +1q (35–40%), del14q (39%), del6q (33%), del1p (30%), and del17p (8%) [18]. Below, the biology and pathogenesis of several CNAs of note are discussed.

**1q amplification (+1q)** The gain/amplification of *CKS1B* gene at chromosome region 1q21 (1q+) is seen in approximately one-third of NDMM patients making it one of the commonest copy number abnormality [19]. *CKS1B* is a member of the cyclin kinase subunit 1 protein family and is essential for cell growth and division [20]. It is widely expressed in various tissues but universally in the bone marrow where it associates with p27kip1-Cdk/cyclin complex and acts as a cofactor for Skp2-dependent ubiquitination of p27 [19–21]. When amplified, it activates the Cdk/cyclin complex and leads to greater degradation of p27, and ultimately cell cycle upregulation by promoting the G1/S transition this leading to MM cell survival and growth.

**1p deletion** *CKS1B* amplification often co-occurs with *CDKN2C* gene deletion at chromosome 1p32.3 (1p-) locus [19]. Approximately 10% of NDMM will have a 1p deletion [22]. *CDKN2C* is a tumor suppressor gene that leads to deregulation of the G1/S transition resulting in proliferation of plasma cells in patients with MM [22].

**Del(17p)/TP53** Loss of the short arm of chromosome 17 [17p13 [del(17p)] is seen in 5–10% of ND-MM. The methodology for identifying deletions varies but the techniques employed include metaphase cytogenetics, iFISH, Seq-FISH, and whole-genome/exome sequencing. Importantly, cytogenetic analysis of chromosome 17p deletions which spans the *TP53* gene is typically performed by FISH probes against 17p and does not probe *TP53* in isolation. Although the clinical relevance of del17p is well established in MM, the exact mechanism by which del17p promotes aggressive disease biology remains unclear [23]. The length of the deleted region can vary from a few megabases (MBs) to deletion of the entire short arm of chromosome 17. The *TP53* gene is located in the minimally deleted region (0.25 MB) suggesting that it is a critical gene in the 17p13 region [24]. *TP53* mutation has been identified as a driver mutation in MM [25]. However, a deletion event usually involves several genes, and co-deletion of *TP53* along with *Eif5a* and *Alox15b* has resulted in more aggressive disease [26] and it remains unclear how genes other than *TP53* contribute to tumorigenesis.

From the myeloma genome project (MGP), Walker et al. [ 25] demonstrated that *TP53* deletion is the most common abnormality at 8%, followed by mutation (~6%) and bi-allelic inactivation (~4%). Deletions in *TP53* induce clonal immortalization and survival of tumor cells as well as drug resistance which is thought to drive poor prognosis [27, 28]. Missense mutations of *TP53* might associate with even worse outcomes in some cases. As in other tumor types, *TP53* mutations in MM are spread across the entire gene, with many mutations occurring within the DNA-binding domain. Missense mutations in *TP53* produce mutant *TP53* proteins that not only result in loss of normal *TP53* function (LOF), but also gain of oncogenic functions (GOF) [29, 30].

Early studies from analyses of small numbers of patients suggested an association between deletion on one allele and mutation on the second allele of chromosome 17p, putatively resulting in complete inactivation of P53 function [31]. In a larger NDMM dataset ($n = 779$) where both *TP53* mutation and FISH data were available ($n = 72$), a significant correlation between mutation and deletion was observed [32]. The MGP data also showed a significant association between the presence of del(17p) cancer clone fraction (CCF) >0.55 and mutation on the second allele of TP53, where 27 of 28 patients with a TP53 mutation had CCF >0.55 [25]. Finally, the relationship between mono and bi-allelic del(17p) and TP53 mutational status remains to be clarified. Additional research is needed to improve our understanding of drivers of high-risk biology in MM patients with del17p and or *TP53* deletions.

## Chromosome translocations

NHD MM includes tumors that harbor chromosomal translocations and often involve the IgH locus, light chain kappa (IgK) locus at chromosome 2, light chain lambda (IgL) locus at chromosome 22, and MYC locus in the chromosome 8q24 region. The largest majority (>90%) of chromosomal translocations in MM affect chromosome 14, specifically the IGH locus at *14q32.33*, which is one of the most heavily transcribed genes in plasma cells [33]. The landscape of translocations with particular attention to those involving the immunoglobulin (Ig) heavy chains (IGH) is described below.

Translocations into the immunoglobulin heavy chain locus located at 14q23

The translocation of oncogenes to the IgH locus at 14q32 is thought to be the seminal event in the development of MM [33, 34]. Translocations into the immunoglobulin heavy chain locus located at 14q23 occur in early stages of disease development suggesting this may be a primer driver event.

There are well-established translocation partners with varying frequencies:

- Common; seen in more than 10% of patients: t(4;14) and t(11;14) translocations [34]
- Less common; ≤5% of patients: t(14;16), t(6;14), t(8;14), and t(14;20) translocations [34].

Deregulation of a D group cyclin is consistent across translocation subgroups. This can occur either directly as seen in t(11;14) (cyclin D1) and t(6;14) (cyclin D3); or indirectly as seen in t(4;14) or in the MAF translocation (t(14;20) and t(14;16)) [33–38]. Translocations into 14q32 influence prognosis due to an upregulation of oncogenes. Depending on the partner, this varies but can include both the MAF family members (such as MafA, MafB, and c-Maf) as well as D-type cyclins (such as cyclin D1, D2, and D3) [33–36].

t(4;14) was initially identified based on breakpoints on chromosome 4 in the *FGFR3* gene and subsequently involved the *MMSET* gene (*MMSET*: multiple myeloma SET domain; also known as Wolf-Hirschhorn syndrome candidate 1 (WHSC1) or nuclear receptor-binding SET domain 2 (NSD2)). The t(4:14) translocation was the first example of an IgH translocation that simultaneously dysregulated two genes with oncogenic potential: *FGFR3* on der(14) and *MMSET* on der(4) [36]. Importantly, *FGFR3* shows only weak transforming activity and is eventually lost in 30% of patients suggesting that it is not the main oncogenic factor [37], whereas *MMSET* is known to have histone methyl transferase activity and is deregulated early on in the genesis of developing MM [38].

In 15–20% MM cases whose IgH spilt can be detected by FISH, no specific partner chromosomes can be identified. Despite its remarkable prevalence with t(14;unknown) being as common as t(4;14) or t(11;14), its impact on risk and prognosis as well as biology is not well described albeit it is thought to be neutral [39]. Finally, the partners in ~15% of IgH translocations are defined most recently by using next-generation sequence techniques. Partner genes in this group are mainly *MYC*, and less frequently *WWOX*, *B2M*, *ERF*, *RND3*, *JUN PAX5*, *DPF3*, and *MTMR1* [40, 41].

**IgK and IgL translocations** IgK and IgL translocations are detected in 4.5% and 10% of ND-MM cases [42, 43]. Approximately 41% of IgL translocations and most IgK translocations involve MYC locus. Other recurrent partners include *MAP3K14*, *CD40*, *MAFB*, *TXNDC5*, *CCND1*, *CCND2*, and *CCND3* but with lower frequencies (1 to 7%).

**Myc translocations** Myc translocations and aberrations are common and frequently lead to an upregulation of the oncogene *MYC* which like in other B-cell malignancies has been shown to be unfavorable. In the CoMMpass study, a comprehensive natural history myeloma genomic project spearheaded by the Multiple Myeloma Research Foundation (MMRF) that has currently enrolled over 1150 patients from 76 sites in North America and Europe, MYC translocations occurred in 182 (23%) of 795 patients evaluated on trial and were juxtaposed to a large number of regions [43]. Among MYC structural alterations, translocations involving the immunoglobulin lambda (IgL) locus are present in 9.8% of patients and of which IgL-MYC translocations accounted for 41% of IgL translocations overall. t(8;14) is uncommon in myeloma with a prevalence of only 1–2%. Of note, there are significant associations between Myc and other abnormalities highlighting oncogenic dependencies in MM. For example, Myc rearrangements can lead to deregulation of *FAM46C* which has been associated with hyperdiploid MM [25]. 8q24 breakpoints have been found to partner with the immunoglobulin enhancers IGH, IGK, and IGL, important B-cell maturation loci including *XBP1*, *FAM46C*, *CCND1*, *KRAS*, and other superenhancers such as *NSMCE2*, *TXNDC5*, *FOXO3*, *IGJ*, and *PRDM1* [40].

## Genetic mutations in MM pathogenesis

The development of MM from precursor diseases such as MGUS and SMM is driven by secondary events which provide a fitness advantage to a particular subclone and are required for tumor progression. For example, most copy number variations (CNV), translocations involving *MYC*, and somatic mutations affecting *MAPK*, *NF-κB*, and DNA repair pathways are observed during MM and less frequently in pre-malignant stages. Next-generation sequence techniques have demonstrated recurrent gene mutations detected in almost all MM cases. There is significant molecular heterogeneity and a well-defined hierarchy of frequencies but most mutations are found in less than 10% of patients. Genes that are involved in MM mutations include *KRAS* (23–36%), *NRAS* (20–21%), *BRAF* (6–8%), *FAM46C* (11–12%), *DIS3* (11–16%), *TP53* (8–16%), *TRAF3* (5%), *CYLD* (2%), *RB1* (3%), *PRDM1/BLIMP1* (5%), *ACTG1* (2%), *RYR2* (5%), *SVIL* (5%), *TTN* (8%), *MUC16* (8%), *IGKC* (2%), *FAT3* (4–7%), *SP140* (7–12%), *CCND1* (3–10%), *ROBO1* (2–5%), *EGR1* (4–6%), *P140* (5–7%), *IgHJ6*, and *SDNAH5* [25, 44–48]. Through utilization of whole-genome sequencing in NDMM patients, Bolli et al. [ 48] showed the pervasiveness of driver mutations. When considering recurrent translocations and aneuploidies, deletions of tumor suppressor genes, amplification of oncogenes, and mutations pertaining to "oncogenic" or "possible oncogenic" classes, at least one such driver event was present in >99% of patients and overall a median of 6 events were present in each patient.

**Affected signaling pathways by genetic abnormalities in MM** Among all the genetic abnormalities in MM pathogenesis, the majority are involved in two main pathways: RAS/MAPK--43% (*KRAS*, *NRAS*, *BRAF*, *FGFR3*, *RASA2*, *PTPN11*); and NF-κB--17% (*TRAF2*, *TRAF3*, *CYLD*, *BIRC2*, *BIRC3*, *SP140*, *TWEAK*, *MAP3K14*, *COBLL1*, and *PRKD2*). Such mutations lead to abnormal activation of RAS/MAPK and NF-κB signaling, promoting the proliferation and survival of the mutant cells. NF-κB gene signature analysis suggests that NF-κB signaling is inversely associated with RAS-RAF mutations, suggesting 2 distinct subsets of diseases [25, 46, 49, 50]. The remaining affected genes are involved in the following pathways [51–54] (Fig. 1):

- Cell cycle: *LEMD2*, *CCND1/2/3*, *RB1*, *CDKN1B*, *c-MAF*, and *MAF-B*
- p53-DNA repair: *TP53*, *ATM*, *ATR*, *BAX*, *ZNFHX4*, *SAMHD1*
- Plasma differentiation: *IRF4*, *PRDM1/BLIMP1*, *MYC-MAX*, *ZNF208*, *HOXB3*
- RNA process: *DIS3*, *FAM46C*, *FCF1*, *RPL10*, *RP53A*, and *PABPC1*
- Protein translation: *FBXO4*, *RPN1*, *PTH2*, and *ST6GAL1*
- Epigenomic modification: *MMSET, PHF19, EZH2, HIST1H1E, KMT2B, KMT2C, CREBBP, ARID1A, ATRX, EP300, SETD2, TET2, KDM5C, ARID2, DNMT3A, KDM6A, NCOR1, IDH1, HistH1E, HistH14H*

During this disease development process, multiple genetic abnormalities are dynamically accumulated and can be detected in the same patient suggesting the collaboration of these abnormalities in disease development and progression. Primary genetic events including hyperdiploid and IgH translocations can be detected in all disease stages from MGUS to SMM to MM [6]. On the contrary, genetic mutations are thought to be secondary genetic events that are acquired during disease progression to smoldering myeloma and eventually to symptomatic MM [55]. These secondary genetic events cause activation of oncogenic signaling and inactivation of tumor suppression signaling. Del17p/*TP53* double-hit and *Myc* mutations (a rare event) are associated with drug resistance which are expanded in RR-MM cases [56, 57]. In addition, mutations that involve the proteasome, ER-stress, and CRBN as well as those that lead to an increased exportin 1 (XPO1) activation are also expanded in RR-MM cases and related to drug resistance (Fig. 2) [56, 57].

**"Double-hit" and myeloma pathogenesis** In HD-MM and NHD-MM, the increase or decrease in the numbers of whole chromosomes results in expansion or reduction of both oncogenes and tumor repressor genes which may partially neutralize the oncogenic effects. This might explain why such dramatic change fails to induce malignant transformation when it happens alone. Such level change of oncogenes or tumor suppressor genes seems insufficient to

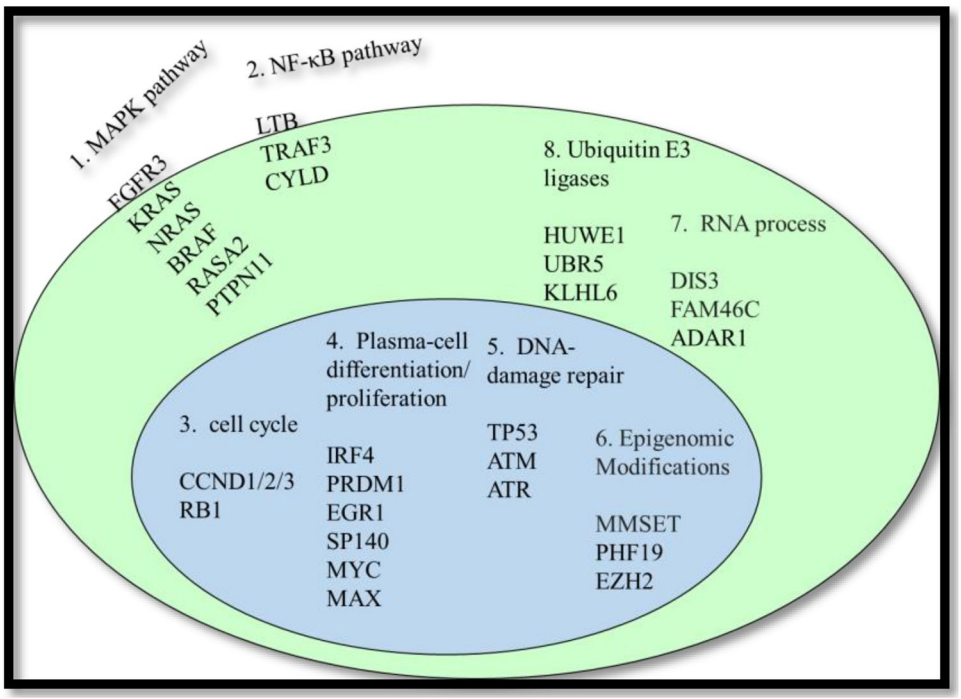


**Fig. 1** Genetic alterations involved pathways in MM. Genetic abnormalities in MM cause changes in at least 8 signaling pathways

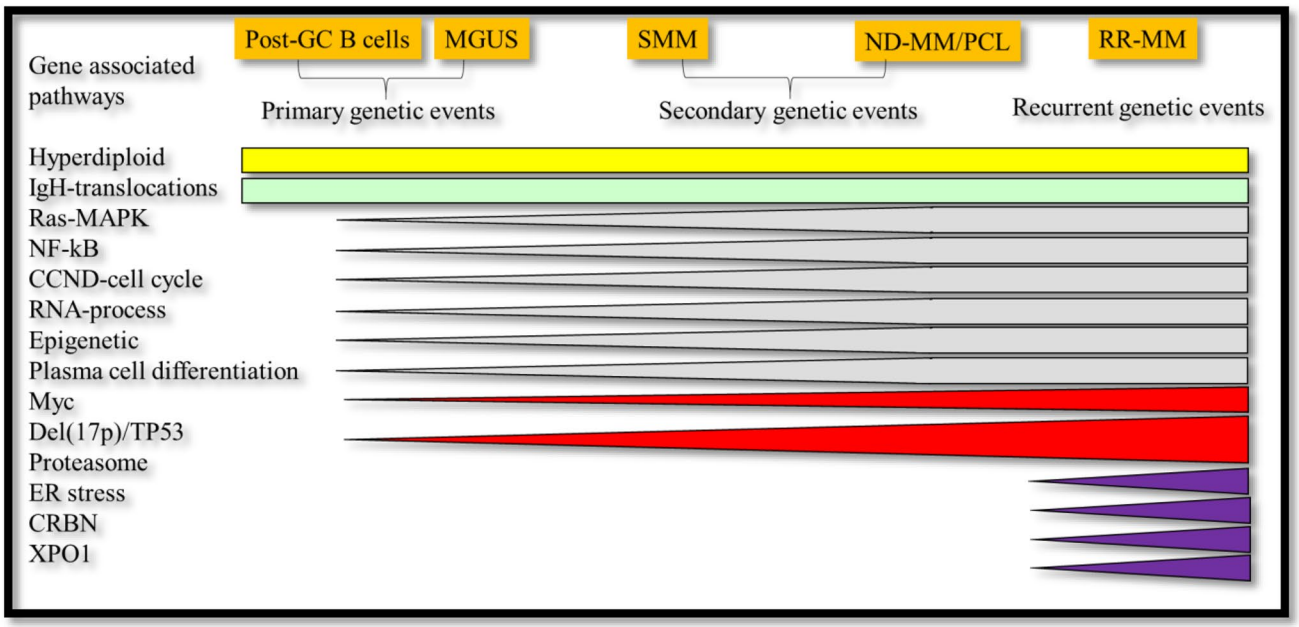


**Fig. 2** Genetic alterations involved pathways in MM development, progress, and relapse. GC: germinal center; MGUS: monoclonal gammopathy of undetermined significance; NDMM: newly diagnosed multiple myeloma; PCL: plasma cell leukemia; RRMM: relapsed refractory multiple myeloma; SMM: smoldering myeloma

induce malignant transformation. Clinical studies suggest that "double-hit" in the same gene or functional pathway are commonly observed in MM suggesting a critical role of threshold activity of the functional pathway in disease development. For example:

1. Several tumor repressor genes (*RB1, DIS3, DLEU2/miR-15a/16-1*) have been identified in the deletion region of del13q. *DIS3* inactive mutations commonly coincide with del13q which results in bi-allelic inactivation of *DIS3* [58].
2. Putative oncogenes in +1q include *CKS1B* (a negative regulator of *CDKN1B*), *MCL1*, *BCL9*, and *ADAR1* [59–61]. The common coincidence of +1q and del13q results in double hits on the CDKN1B-RB1 pathway.
3. The t(11;14) leads to cyclin D1 overexpression. An increase in *CCND1* mutations is detected in MM with t(11;14) [34–38].
4. The t(14;16) and t(14;20) lead to overexpression of *c-MAF* and *MAF-B* respectively. *MAF* is only significantly mutated in t(14;16) MM, while *MAFB* mutations are only detected in t(14;20) MM [62, 63].
5. The t(4;14) results in *MMSET* overexpression in 100% of cases and *FGFR3* overexpression in 70% of cases depending on the breakpoint site. *FGFR3* mutations are detected in 17% of patients harboring t(4;14), and probably result from somatic hypermutations on der14 [11, 64, 65].
6. MM with del1p often co-occurs with mutations of tumor repressors *CDKN2C* or *FAM46C* leading to double hits on the *CDKN2C* or *FAM46C* gene [66].
7. *MYC* region implication or duplication is commonly detected in MYC translocations, leading to double hit on the *MYC* gene [40, 41].
8. *TP53* mutation or deletion often detected in patients with del17p, especially RR-MM cases, results in bi-allele deletion (or mutation) in the *TP53* gene [19, 31].

MM pathogenesis is a result of multiple genetic abnormalities which collaboratively stimulate malignant transformation and uncontrolled expansion of malignant cells by inducing differentiation blockage and enhanced survival and uncontrolled proliferation. This explains the preferential coexistence of certain genetic abnormalities in the same patient. Several well-described and notable oncogenic co-occurrences and or dependencies of note include:

1. Patients harboring t(4;14) co-segregate with mutations in *FGFR3*, *DIS3*, and *PRKD2*, or with del12p, del13q, and gain of 1q9 [36, 67].
2. Patients with t(14;16) preferentially have co-occurring mutations in *MAF*, *BRAF*, *DIS3*, or *ATM* genes [25, 67].
3. Mutations in *CCND1*, *KRAS*, *IRF4*, *LTB*, and *HUWE1* genes are commonly detected in patients with t(11;14) [36, 48, 49, 67].

4. *CDKN1B*, *FUBP1*, *NFKB2*, *PRDM1*, *PTPN11*, *RASA2*, *RFTN1*, and *SP140* are significantly mutated only in HD-MM samples [68, 69].
5. Del13q is commonly associated with NHD-MM [70–72].
6. Monosomy 17 or del17p is identified in patients with hyperhaploid [68, 69].

Several recently published large clinical databases have elucidated true genomic clustering resulting from oncogenic dependencies. In an enlightening analysis of both clinical and genomic data from the MGP, Walker and colleagues [25] evaluated whole-exome and in many cases whole-genome sequencing of 1273 NDMM patients. They identified 9 total clusters, two of which were hyperdiploid (cluster 1: gains in chromosomes 3, 5, 9, 15, 19, and 21; cluster 2: gains in the same chromosomes plus chromosome 11 and mutation of *FAM46C*) while 7 were non-hyperdiploid (cluster 3: del1p; cluster 4 by del12p and 13q; cluster 5: del13q and 14q and mutations in *MAX*, *TRAF3*, and *NFKBIA*; cluster 6: del16q; cluster 7: t(14;16), del11q, 1q gain, and mutation of *DIS3*; cluster 8: t(11;14); cluster 9: t(11;14) with gain 11q). Other groups have shown similar clustering and co-segregating genomic patterns [46, 49].

## The clinical significance of genetic abnormalities in multiple myeloma

As with other hematological malignancies, the accumulation of genomic aberrations is not only a hallmark of MM but also is associated with patient outcomes. Whole-genome/exome sequencing as well as the review of large patient data sets in the modern era has improved our knowledge of disease biology and pathogenesis from mutations to chromosomal. Although direct correlations with mutations have been linked to risk and patient outcomes, the implementation of this granulation genomic data into routine clinical practice remains an enormous barrier to improving risk stratification in NDMM. Thus, defining uniform characteristics of risk in NDMM remains elusive despite several validated risk stratification systemic in routine clinical use. The accurate assessment of risk at diagnosis is critical for many reasons including but not limited to:

1. The longest remission period being achieved by initial therapy and thus the duration of the first remission is one of the most important factors impacting patient prognosis and long-term outcomes in general
2. Accurate definition of risk for clinical trial design and enrollment
3. Establishing which clinical data should be obtained routinely in practice given certain tools such as gene expression profiling are challenging to obtain while others such as serum lactate dehydrogenase (LDH) can easily be incorporated into routine clinical care.

**Clinical staging systems** The international staging system (or ISS) is an early risk stratification for NDMM patients [9]. It incorporated a combination of serum beta$_2$ microglobulin (B2M) and albumin into a simple powerful three-stage classification predictive of OS. Subsequent to the ISS development, chromosomal abnormalities detected by iFISH have become a standard of care in risk stratifying MM patients and thus integrated genetic abnormalities into routine clinical practice. In 2014, the IMWG published and updated risk stratification focusing on differentiating high-risk patients, those with an expected OS of less than 2 years despite the use of novel agents, from low-risk patients, those with expected survival greater than 10 years. The staging system combines the ISS with certain high-risk iFISH changes including t(4;14), 17p13, and 1q21 [5]. The revised international staging system (R-ISS) was also developed which combines iFISH changes, serum (LDH), and ISS features and is the most widely recognized risk stratification tool for NDMM patients today [4]. Although it incorporates important genetic markers including t(4;14), t(14;16), and del17p, it does not include some important genomic data including 1q gain/amplification an increasingly important prognostic marker [19, 25] nor mutational data from TP53 as the data were not available.

**High-risk genetic lesions including copy number abnormalities, chromosomal translocations, and mutations** In addition to well established clinical staging system incorporating iFISH as outlined above, certain high-risk genomic lesions are well established. Table 1 outlines select high-risk iFISH changes detectable in routine clinical practice with supporting literature to summarize their impact on risk. In addition to having a single high-risk genomic lesion, what is frequently being referred to as double-hit myeloma (different from the double hit concept described above driving myeloma pathogenesis) includes patients who have more than 1 adverse cytogenetic lesions (such as del(17p), t(4;14), and gain of 1q) or an adverse cytogenetic lesion concurrently with a high-risk mutation. These patients display dismal survival outcomes. For example, in the Bolli et al. [ 49] experience, patients with both t(4;14) and *PRDM1* deletion had a dismal median OS of 265 days whereas data from the MGP shows that patients with bi-allelic *TP53* mutation have a PFS of only 15.4 months and OS of just 20.7 months [79].w

**Table 1** High-risk molecular and cytogenetic subgroups

| | Prevalence | High risk per R-ISS, IMWG, mSMART: | Select literature addressing prognosis | Notes |
|---|---|---|---|---|
| t(14;20)* | 1–2% | mSMART | Inconsistent due to rarity<br>• Ross et al. Haem 2010: median OS 14.4 months [35]<br>• Jurczyszyn et al. BLOOD 2018**: median PFS 30 months [73]<br>• Shah et al. Leuk 2018: HR for OS of 1.90 (*P*=0.0089) with only del(17p) showing worse prognosis [74] | • No large databases, cohorts of less than 50 patients<br>• Frequently found with other high-risk cytogenetic abnormalities: del(17p), t(4;14), t(14;16), del(13q), non-hyperdiploid karyotype |
| t(14;16) | 1–2% | R-ISS<br>mSMART | Inconsistent due to rarity:<br>• Jurczysyn et al BLOOD 2018***: median PFS 31 months; 5-year OS 55% [73]<br>• Mayo Clinic and Medical Research Council group [11] showing poor outcomes<br>• IFM studies showed neutral outcomes [75] | • Data from Jurczysyn et al. BLOOD 2018 do not vary significantly from R-ISS stage III outcomes.<br>• Of note, the R-ISS did not report specifically on outcomes of t(14;16) patients but it was present in a total of 84 or 2.7% of patients in the analyzed cohort |
| t(4;14) | 15% | IMWG<br>R-ISS | • Chan et al. CLML 2018: PFS of 33.5 months for 75 patients [76]<br>• Mayo group has demonstrated improved median OS ~ 4–5 years [77]<br>• Bolli et al Leuk 2018: on mv analyses, t(4;14) predicted both PFS and OS independently of other CAs [49] | • Compared to standard risk patients who achieve median OS ~ 10 years, t(4;14) patients remain bad actors<br>• Some frequently associated chromosomal changes worsen t(4;14) such as 1p32 and potentially 13q deletions |
| t(8;14)<br>and MYC translocations | t(8;14):<br>1–2% | None | • Myeloma IX trial: those with a MYC translocation had inferior PFS and OS on mv analyses [41]<br>• CoMMpass study [43]: only IgL-MYC translocations had worse PFS and OS**** | • Significant associations between Myc and other abnormalities highlight oncogenic dependencies<br>• Myc rearrangements can lead to deregulation of FAM46C which has been associated with hyperdiploid MM [25] |
| +1q | Overall: ~33%<br>Gain: ~21.9%<br>Amp:<br>~6.3% | None | • Giri et al. JCO 2019: 3,578 NDMM patients, any chromosome 1 abnormality inferior OS (median OS 46.6 vs 70.1 months) [78]<br>• Shah et al. Luek 2018: UK Myeloma XI and IX trials, 1q gain inferior OS (HR 1.67; $P = 3.30 \times 10- 5$); amp 1q worse (HR 2.28; $P = 2.32 \times 10- 6$) [74]<br>• MGP: both gain and amp of CKS1B associated with decreased PFS and OS but amp worse [79]<br>• Gain and amp have been shown to impact OS in other cohorts [49, 67, 74, 80] | • Cutoff for a positive test remains controversial with the EMN 20% definition frequently employed but higher CCF may impact outcomes.<br>• An et al. showed that among patients with 1q21 gains a 20% CCF predicted PFS and OS but stratifying by increasing CCF had no impact on outcomes and likely a 20% CCF cut off remains appropriate [81] |
| 1p- | ~10% | None | • Myeloma IX trial: inferior OS for both CDKN2C mutation at 1p32.3 as well as FAM46C at 1p12 [82]<br>• IFM collection: in mv analyses of 1195 patients, 1p22 and 1p32 deletions both showed inferior OS [83] | • Amplification of CKS1B is frequently associated with the deletion of the CDKN2C gene at the chromosome 1p32.3 (1p-) locus |
| del 13q/-13 | del(13q): ~5%<br>Monsomy 13: 35% | None | • Early studies showed inferior OS in NDMM patients but this may be due to co-occurring high-risk CAs [84]<br>• In 1181 NDMM patients, on mv analyses monosomy 13 leads to worse OS with a HR of 1.27 (*p*=0.022) while del(13q) was to protective with a HR of 0.48 (*p*=0.006) [85] | • Deletions and abnormalities involving chromosome 13 were one of the earliest recognized high-risk features in NDMM [86]<br>• Up to 90% of patients with t(4;14) have deletion 13q.6 [37]<br>• CCF has not been clearly defined, at CCF > 25% it is likely co-occurring CAs, particularly t(4;14) and del(17p), drive poor clinical outcomes [81] |

**Table 1** (continued)

| | Prevalence | High risk per R-ISS, IMWG, mSMART: | Select literature addressing prognosis | Notes |
|---|---|---|---|---|
| del(17p) | 5–10% | R-ISS<br>IMWG<br>mSMART | • Shah et al. Luek 2018: UK Myeloma XI and IX trials, del(17p) inferior OS with HR 2.10 ($p$=8.86 X $10^{-14}$) [74]<br>• Perrot et al JCO 2019: 1635 patients enrolled in four trials implemented by the IFM. Cytogenetic PI developed with del(17p) being weighted the strongest: HR for OS of 3.12 (2.44–4.22; $p$<0.001) [87]<br>• Corre et al. Blood 2021: del(17p) regardless of TP53 mutational status predicts poor outcomes [88]. | • TP53 induces clonal immortalization and survival of tumor cells as well as drug resistance which is thought to drive poor prognosis [27, 28]<br>• CCF > 55% has been shown to be prognostic [88, 89]<br>• When incorporating RNA alterations and gene expression profiling, it remains predictive of both PFS and OS as well [90] |
| Non-hyperdiploid | Within NHD group, patients with hypodiploid karyotypes or hyperhaploid karyotypes are also associated with an adverse prognosis. Among HD-MM, patients with trisomy 21 are associated with a poor outcome [14]. | | | |

*While t(4;14) and translocation t(14;16) are included as high-risk chromosomal abnormalities in the R-ISS, other chromosome 14 translocations including t(14;20) are not but have been shown to be unfavorable

**5 clinical centers in Germany, Italy, and the USA

***213 patients with t(14;16) from 24 clinical centers in Germany, Italy, Spain, Israel, Poland, Romania, Czech Republic, and the USA

****These data indicate that aberrant MYC expression resulting from MYC amplification or translocation is a common feature of myeloma, but the IgL-MYC translocated subset is unique among MYC alterations in that it portends a very poor prognosis. In the CoMMpass study, patients with an IgL-translocation did not benefit from IMiD-containing therapies that target the lymphocyte-specific transcription factor Ikaros which is bound at high levels to the IgL enhancer. Also, 78% of IgL-MYC translocations co-occur with hyperdiploid disease, a marker of standard risk, suggesting that IgL-MYC-translocated myeloma is being misclassified

*amp* amplification, ≥4 copies

*CA* cytogenetic abnormalities

*CCF* cancer clone fraction

*EMN* European myeloma network

*HR* hazard ratio

*IFM* Institut Francophone du Mye'lome

*IMWG* international myeloma working group

*MGP* myeloma genome project

*MM* multiple myeloma

*MV* multivariate

*NDMM* newly diagnosed multiple myeloma

*OS* overall survival

*PI* prognostic index

*PFS* progression-free survival

*R-ISS* revised international staging system

**Mutations** Although the duration of follow-up is limited on large datasets of NDMM with extensive genomic sequencing, it is apparent that few solitary mutations drive prognosis. *TP53* mutation is the only consistent adverse finding in addition to a link between general mutational burden and clinical outcomes [25, 28, 49]. The impact of bi-allelic/double hit v monoallelic *TP53* inactivation or del(17p) remains unclear. The MPG had only 33 patients with monoallelic deletion/mutation of *TP53* [25]. These patients did not have an inferior outcome when compared to patients without a *TP53* abnormality. This observation could be explained by the subclonal nature of *TP53* deletions in these patients [28]. Table 1 outlines a higher cancer clone fraction (CCF) may be required to drive poor outcomes. *TP53* mutations in isolation may have additional pathological significance yet to be determined such as driving and/or propagating MM clones leading to a selective clonal advantage [28] and the co-occurrence of *TP53* mutations with other gene mutations may in the end be synergistic.

The MGP found 63 driver genes including novel previously unidentified oncogenes *PTPN11* (activator of MEK/ERK signaling), *PRKD2* (protein kinase D), *IDH1* and *IDH2* (DNA methylation), and *SF3B1* (spliceosome factor). Novel tumor suppressor genes including *UBR5* (a ubiquitin ligase) and *HUWE1* (a ubiquitin ligase that can affect MUC expression via MIZ1) were also described. Of the 63 driver genes identified in their study, only *TP53*, *TRAF3*, and *TGDS* had an impact on outcome in univariate analyses. Driver gene mutational burden did lead to worse PFS and OS ($P$=0.001). Furthermore, two markers of genomic instability were associated with outcomes including an APOBEC mutational signature and loss of heterozygosity [25]. Interestingly, the extent of LOH was positively correlated with the APOBEC signature ($P$=0.039), loss of *TP53* ($P$=0.001), and presence of mutation in at least 1 of 15 genes involved in homologous recombination deficiency ($P < 0.001$). Updated analyses focused on the key clinical problem of identifying high-risk patients destined for early relapse and death where a change in treatment strategy could result in improved outcome [79]. On multivariate modeling, only t(4;14) and bi-allelic *TP53* inactivation were predictive of PFS while bi-allelic *TP53* inactivation and amplification of *CKS1B* (1q21) were predictive of OS.

Bolli et al. [ 49] found similar results performing whole-genome sequencing of a total of 418 NDMM patients. Risk as defined by worsening survival leads to clustering of patients based on the number of mutations as well as the number/type of cytogenetic abnormalities. In multivariate analyses, they found that mutations in *SP140* and *NRAS*, t(4;14), amp(1q), and del(17p13) and deletions of *FAT1* and *PRDM1* negatively impacted PFS. Conversely, t(4;14), amp(1q), del(17p13), and del(1p) negatively impacted OS. The only mutation that negatively impacted both PFS and OS was *TP53*; *DNAH11* mutations lead to worse OS only [49]. Similar to Walker et al. [25], they discovered clusters of patients stratified based on the overall number of mutations and number/type of CNAs that lead to distinct effects on survival. Thus, individual mutations and performing an extended genotype at diagnosis likely lead to improved prognostication in NDMM. Finally, as seen from the MGP, they found distinct genomic clustering patterns leading to poor prognosis. For example, cluster 2 was enriched for IGH translocations and a high number of CNAs, enriched for amp(1q), del (13), del(17p), and deletions of *BIRC2/3* and *XBP1*, and carried more *TP53* mutations [49].

The CoMMpass study (as described above) found 55 genes that were significantly mutated and there was a 65% overlap with the MGP. After a median follow-up of 39 months, early progressive disease (PD) was detected in 191/926 (20.6%) patients. In a multivariate logistic regression model, independent factors increasing the early PD risk were *TP53* mutation (OR, 3.78, $P < 0.001$), high LDH (OR, 3.15, $P$ 0.006), IgL-chain translocation (OR, 2.25, $P$ 0.033), and *IGLL5* mutation (OR, 2.15, $P$ 0.007) [67].

## Perspectives moving forward

Our ability to define risk in NDMM has vastly improved as our knowledge of the pathogenesis, biology, and genomics of MM has rapidly expanded. However, we continue to struggle to standardize risk as is evident by the routine use of several risk staging systems that do not completely align including the R-ISS, IMWG, and mSMART [77]. With the R-ISS being now nearly a decade old, it is likely time to formally revisit and develop an updated risk stratification system wielding the power of our increased knowledge of myeloma genomics. The biggest barrier to implementing our improved knowledge of mutations and other genomic factors impacting risk is the ability to perform such assays (advanced mutational profiling, whole-genome/exome sequencing, and even gene expression profiling (GEP) as outlined below) in routine clinical practice outside of clinical trials or major academic centers where the vast majority of NDMM patients are treated. Furthermore, payer reimbursement for such testing is likely to continue to be a problem.

This is particularly important given the rapid expansion and Food and Drug Administration (FDA) approval of new therapeutics since the R-ISS 2014 publication including but not limited to ixazomib (2015), carfilzomib (in combination with lenalidomide and dexamethasone; 2015), daratumumab (2015), elotuzumab (2015), selinexor (2019), belantamab mafodotin-blmf (2020), isatuximab (2020), and idecabtagene vicleucel (2021). These therapeutics have improved MM outcomes and may influence the impact of genomic risk

in MM patients. It is critical to accurately identify high-risk patients at diagnosis and move toward the establishment of risk-adapted treatments. While many studies are targeting improved outcomes in high-risk myeloma [91], there has yet to be a randomized trial that has demonstrated a definitive preferred approach in this challenging patient population with the most recent negative study being the SWOG1211 (bortezomib, dexamethasone, and lenalidomide with or without elotuzumab in treating patients with newly diagnosed high-risk multiple myeloma) [92].

Is there a way to incorporate whole-genome/exome-level genomic data into routine clinical use? Gene expression profiling may very well be the answer. Gene expression profiling measures which genes are being expressed in a myeloma cell and specifically measures mRNA levels, showing the pattern of genes expressed by a cell at the transcription level. Given that DNA-based assays such as whole genomic sequencing are able to identify individual lesions and markers of global genomic instability and ultimately prognosis, it is not surprising that the development and now validation of several GEP score systems that have prognostic value have increased interest in incorporating GEP into routine clinical practice. Various reports, using distinct approaches, have identified gene expression signatures capable of predicting event-free survival and OS in multiple myeloma [93–95]. Although a variety of other GEP have been developed, only two have matured into validated clinical tests: MMprofiler (EMC92/SYK92) and MyPRS (UAMS GEP 70). Despite growing evidence of the prognostic value of GEP, its application to routine clinical care remains challenging. There is not a consensus on a universal adaptation of a particular GEP system. GEP remains an investigational tool and is not yet validated by the FDA.

In order to best define risk in NDMM patients, we would recommend the following cytogenetic and mutational assessments be obtained at diagnosis prior to treatment initiation whenever possible. We understand that currently the scope of advanced genomic sequencing is limited thus many of the mutations listed below. Most recommended cytogenetic studies below are readily available in MM FISH panels:

- High risk:
  - Cytogenetics:

    t(14;16)
    t(4;14)
    IgL-MYC translocation
    +1q amplification (≥4 copies)
    1p-
    del(17p)

  - Mutations/advanced genomic sequencing

    *TP53 deletion*
    LOH and APOEBEC signature
    *CKS1B* amplification

- Potentially high risk:
  - Cytogenetics:

    t(14;20)
    t(8;14) and other MYC translocations
    +1q gain (3 copies)
    del 13q/-13

  - Mutations/advanced genomic sequencing

    *TRAF3*
    *TGDS*
    *PRDM1*
    *DNAH11*
    *FAT1*
    *NRAS*
    *SP140*
    *IGLL5*
    Driver gene mutational burden

In conclusion, as we move forward, there is a need for improved longitudinal genomic data in addition to that already described above. This will require large-scale collaboration between researchers and institutions but will help further elucidate key genomics driving pathogenesis and risk. Importantly, this may translate into an improved understanding of poor outcomes, drug resistance, and potentially targetable pathways for new therapeutics.

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## Declarations

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