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Cytogenetics in the management of multiple Myeloma: The guidelines from the Groupe Francophone de Cytogénétique Hématologique (GFCH)

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ABSTRACT

Multiple myeloma (MM) is characterized by the accumulation of malignant plasma cells (PCs) in the bone marrow. Despite considerable advances in the treatment, MM is considered an incurable chronic disease with a very heterogeneous prognosis, mostly depending on genomic alterations whose complexity evolves over time. The cytogenetic analysis of MM is performed on CD138+ sorted PCs, in order to detect the following high risk cytogenetic abnormalities: t(4;14), 17p/*TP53* deletion, 1q21 gain/amplification, 1p32 deletion, as well as t(11;14) because of its therapeutic implication. This minimal panel can be enlarged to detect other recurrent abnormalities, according to the prognostic score chosen by the laboratory. Although the knowledge of the genetic landscape of MM is evolving rapidly with improved molecular technologies, risk scores remain to be refined as they require more time for consensual validation. The GFCH present here the overview of genomics alterations identified in MM and related PCs diseases associated with their prognostic factor, when available, and recommendations from an expert group for identification and characterization of those alterations. This work is the update of previous 2016 recommendations.

1. Introduction

Belonging to plasma cell neoplasms (PCNs), Multiple myeloma (MM), which is also the second most common hematologic malignancy, is a heterogeneous cancer disease characterized by an accumulation of abnormal plasma cells (PCs) in the bone marrow. MM is preceded by an asymptomatic expansion of clonal PCs named monoclonal gammopathy of undetermined significance (MGUS) and a more advanced phase, named smoldering myeloma (SMM). Both latter entities are observed in 2 to 3 % of the general population after 40 years of age. 1 % to 2 % of MGUS and about 10 % of SMM will progress to MM each year.

In the last two decades exceptional development in MM treatments has led to a significant improvement in overall survival (OS) and progression free survival (PFS). However clinical benefit is not uniform, and MM remains an incurable disease. Prognosis of high-risk patients remains poor with survival below 2 years. The wide heterogeneity in MM outcome is mainly driven by genetic abnormalities. The genomic landscape of MM is complex and vary among patients. The history of the disease will be punctuated by phases of treatment, remission and relapse, potentially linked to the presence of subclones that may evolve differently in time and space.

Genetically, 2 distinct entities were initially described: one showing hyperdiploid karyotype (HK-MM) and another with non hyperdiploid karyotype with translocations involving an immunoglobulin gene (t(*IG)*). These abnormalities are primary and can occur in both precursor stages. Progression to MM involves the occurrence of additional genetic abnormalities, aneuploidy, chromosomal translocations, insertions, copy number abnormalities (CNA) and mutations. Several prognostic scores have been developed to stratify MM patients and adapt treatments. The International Myeloma Working Group (IMWG) recommends the R-ISS, which combines albumin, β2-microglobulin (initial ISS score) with LDH and 3 high-risk cytogenetic abnormalities (HR-CA): del(17p), t(4;14) and t(14;16) [1,2]. However this definition appears oversimplified as an increasing number of abnormalities with a validated prognostic impact have since been characterized [3,4]. The Mayo Clinic and the Intergroupe Francophone du Myélome (IFM) have produced their own cytogenetic scores which include (or exclude) several other cytogenetics markers [5,6].

Here, we provide an overview of recurrent cytogenetic aberrations that can be detected in MM patient and we propose an update of ours previous recommendations for cytogenetic analysis of MM. New data provide in these recommendations concern complex cytogenetic events such as chromotripsis and others structural variants, as well as mutational data which are not prognostic factors used in scores at that time. The GFCH presents the new scoring systems published until 2016 about MM and an update of the different strategies used for identifying these prognostic factors. A paragraph about immunoglobulin-related (AL) amyloidosis and plasmocytoma genetic background and its characterization has been added.

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2. I-Multiple myeloma

2.1. A- Genetic landscape

2.1.1. 1-Cytogenetic abnormalities

2.1.1.1. Initiating events. The MM genome is defined by primary etiologic events that divided in two distinct groups ie the Hyperdiploid myeloma (HK-MM) group and the pseudodiploïd group with translocations. However both MM entities are not mutually exclusive 16 % of cases shared commons cytogenetic features [7].

2.1.1.1.1. Hyperdiploidy. Fifty five percent of newly diagnosed MM (NDMM) are characterized by hyperdiploidy, defined as at least two trisomies preferentially affecting odd-numbered chromosomes (chromosomes 3, 5, 7, 9, 11, 15, 19, and 21). HK-MM is associated with a favorable prognosis (standard risk). However the reality is more subtle as some studies have shown that trisomies 3, 5 and 15 had a rather positive significant impact on OS whereas trisomy 21 negatively modulated OS [6–8].

2.1.1.1.2. Pseudodiploidy or hypodiploidy with immunoglobulin translocations. The second MM group shows either pseudodiploid or hypodiploid karyotype with translocations mostly involving the immunoglobulin heavy chain (*IGH*) locus on chromosome 14q32 (t(*IGH)*) which is translocated to diverse oncogenes whose expression is upregulated under the influence of the powerful *IGH* enhancer. They account for 40 % of MM and are represented by five canonical translocations: t(11;14), t(4;14), t(14;16), t(14;20) and t(6;14) [9,10].

- **t(11;14)(q13;q32) / *IGH::CCND1***

The most common translocation (15-20 %) in NDMM involves the *IGH locus* and the region covering *CCND1* at 11q13, with cyclin D1 upregulation as a result. Notably, t(11;14) occurs at a higher rate in patients with plasma cell leukemia (40 %) and light chain amyloidosis (50 %). In studies conducted before the novel agent era, t(11;14) was shown to carry standard risk for NDMM patients. More recent retrospective findings have shown that t(11;14) is associated with intermediate outcome in patients treated with novel agents, but this could be improved by autologous hematopoietic stem-cell transplantation (ASCT) [11,12]. Patients with t(11;14) have high levels of the anti-apoptotic protein B-cell lymphoma 2 (Bcl2), suggesting that *BCL2* could be a target in this subtype of MM. Venetoclax, an oral Bcl2 inhibitor, has shown remarkable effect in treating relapsed/refractory MM (RRMM) patients with t(11;14) and *BCL2* overexpression, either as monotherapy or in combination with other anti-myeloma agents [13,14]

- **t(4;14)(p16;q32)/*IGH::FGFR3(NSD2)***

The t(4;14)(p16;q32) is observed in 10 to 20 % of cases [5,10,15]. This rearrangement is quite specific for MM and leads to deregulation of two genes: *FGFR3* (fibroblast growth factor receptor 3) and *NSD2* (nuclear receptor binding SET domain protein 2) also known as *MMSET* and *WHSC1* [16,17]. The two enhancers of the *IGH* gene are implicated in this rearrangement: Eμ enhancer drives *NSD2* dysregulation on derivative chromosome 4 and *IGH* 3' enhancer dysregulates *FGFR3* gene on derivative chromosome 14. Nsd2 protein is a histone 3 lysine 36 methyltransferase which leads to epigenetic dysregulation causing transcriptional activation of oncogenic loci [17–20]. Both dysregulated genes are involved in MM oncogenesis, however *Eμ::NSD2* is more likely the definitive oncogenic factor as the derivative 14 chromosome is lost in about 25 to 30 % cases [16,21,22]. The t(4;14) has been associated with poor outcome in NDMM, with a median OS of 5 years, and is defined as a HR-CA in all risk scores [6,23]. Nevertheless, its prognostic impact can be modulated by other factors. First, the use of proteasome inhibitors (PI) in NDMM has changed its prognostic impact and PI/Immunomodulatory Drugs (IMID)/dexamethasone combination induction followed by double ASCT can overcome the poor prognosis of t(4;14) [20,21]. Secondly, the prognosis of t(4;14) can be modulated by other chromosomal abnormalities: trisomy 5 can overcome its poor prognostic value [8] while 1q21 gain or 1p32 loss can worsen it [24–26].

- **t(14;16)(q32;q23)/*IGH::MAF***

This translocation deregulates *c-MAF* (c-musculoaponeurotic fibrosarcoma oncogene *locus*) and is found in 3 %-5 % of NDMM. The R-ISS defined the t(14;16) as a HR-CA in NDMM [2]. This decision initially relied on two small cohorts where the pejorative prognostic impact of t(14;16) on PFS and OS did not persist after multivariate analysis adjustment [27,28]. However the prognostic value of t(14;16) remains highly controversial: since then at least 3 studies have been published on this topic with opposite results, mostly because of the low prevalence of the t(14;16), the incomplete panels of genetic markers inducing low statistical significance, especially for the construction of the multivariate analysis, and also because of the lack of treatment uniformity of the cohorts. In 2011, Avet-Loiseau et al. did not confirm the negative impact of t(14;16) on PFS or OS in 1033 uniformly treated NDMM patients [29]. Two studies published in 2018 and 2019 demonstrated opposite results despite both cohorts sharing similar treatments [30,31]. Finally two recent studies focusing on t(14;16) with the biggest published cohort of 123 and 223 NDMM carrying t(14;16) respectively [32,33] showed that more than 90 % of patients presented concomitant HR-CA as del17p, 1q21 gain (note that 1p32 deletion was not investigated). Both studies compared patients with single t(14;16) with patients cumulating other chromosomal abnormalities (CAs), but results were never compared to a matched control group of patients without t(14;16). Therefore today the question remains open: is t(14;16) an independent prognostic factor in MM? It is noteworthy that the IFM did no longer integrate t(14;16) into its prognostic index [6], as does the recent R2-ISS [34].

- **t(14;20)(q32;q11)/*IGH::MAFB***

The t(14;20)(q32;q12) is observed in less than 1 % of NDMM and leads to an increased expression of an oncogene from the *MAFB* family. It has initially been associated with a poor prognosis. Although data for t(14;20) remain controversial. The ISS-R does not consider t(14;20) as HR-CA [2] whereas the Mayo Clinic sustains its high-risk impact [35]. Its presence in indolent MGUS and SMM suggests that additional abnormalities are responsible for a poor prognosis in MM with t(14;20) [36].

- **t(6;14)(p21;q32)/*IGH::CCND3***

Translocation t(6;14)(p21;q32) is described in 1 to 4 % of NDMM [7,37]. This translocation deregulates *CCND3* (Cyclin D3) and leads to inactivation of *RB1* (RB Transcriptional Corepressor 1) and cell-cycle progression [15]. Although its prognostic value remains difficult to estimate because of its low frequency, the t(6;14) seems to be a standard risk cytogenetic abnormality in MM with a median OS of 7 to 10 years.

2.1.2. Secondary events.

- 1q21 gain/1q21 amp

The 1q gain (3 copies) or amplification ($\geq$ 4 copies) is the most common secondary cytogenetic event being present at all stages of the disease, although more frequently at the time of progression to MM: it is detected in about 20 % of MGUS, 30 % of SMM, 50 % of NDMM, 50 to 80 % of RRMM and in about 70 % of patients with primary plasma cell leukemia [38]. The 1q21 gain is the result of chromosomal instability in the heterochromatin region (1q12) with trisomy in the long arm of

chromosome 1q, isochromosome 1q, jumping translocation or duplication 1q as a consequence. Over 500 genes are located at 1q21 region; among them *CKS1B* overexpression promotes myeloma cell proliferation and *PSMD4* is associated with resistance to PI, IL6R cell growth and survival [38–40]. 1q21 gain is associated with a poor prognosis. However, the lack of uniformity in cytogenetic data about the significant clone size remains debatable: the cut off to designate a 1q21 gain varies from less than 5 % to 30 % among studies and Ann et al. reported that the cut off of 20 % seems to be the best possible value for predicting poor outcomes [41] while the IFM score does not specify any specific threshold apart from the cut off of FISH [6]. Amplification of 1q occurs when more than 10 % of MM cells harbour at least 4 copies of 1q21: the prognostic impact of this aberration is worsened compared to duplication [42,43]. Co-occurrence of other adverse chromosomal abnormalities with 1q21 gain are likely to be associated with a worse prognosis [24,38,44].

- 1p loss

Loss of 1p is a secondary cytogenetic event with a negative prognostic impact occurring in about 11 % of NDMM but not yet considered as high risk by the IMWG. Three commonly deleted regions are described: 1p12 (*TENT5C, FAM46C*), 1p22.1(*RPL5*) and 1p32.3 (*CDKN2C* and *FAF1*) [25,45]. The deletions are often large, so that 1p deletions often encompass both 1p12 and 1p32.3 including 1p22.1. When each deletion is independently considered, only 1p32.3 loss retains a pejorative impact on OS in multivariate analysis [45]. Loss of *CDKN2C* results in cell proliferation, loss of *FAF1* in antiapoptosis. Del1p32 is the second most adverse abnormality in NDMM just after del17p and is characterized as an independent prognostic factor [3,40]. Biallelic 1p32 deletion confers a dramatically poorer outcome as does its co-occurrence with other HR-CAs [6,46].

- *TP53* defects

The genetic defects of *TP53* include either a deletion of 17p arm (8 % of NDMM), a mutation in one allele (3 %) or both (4 %) [3,42,47,48]. The 17p deletion is the most stratifying anomaly for the prognosis of MM. *TP53* is part of the minimally deleted region. Del(17p) is associated with a more aggressive presentation, reduced progression-free survival (PFS) (median just over a year) and OS (median 2-3 years) regardless of the therapy applied [49,47,50]. Accordingly, *TP53* deletion is found in 10 % to 33 % of RRMM [50–52]. The co-occurrence of *TP53* mutation and 17p loss (3 % of patients) defines a double hit category with poorer outcome [42,52,53].

At least two questions remain open: first, the definition of a prognostic threshold associated with cancer clonal fraction (CCF) that ranged from a single positive FISH cell up to 60 % of PCs according to the studies [6,41,47,50,51]. The most recent studies have used FISH in order to set the CCF range that provides significant difference in OS and PFS at a cut-off point of 55%, which is very close to the 60% CCF set by the IFM [41,47,50]. At this level, del(17p) is validated as an independent pejorative factor on both OS and PFS in NDMM (31,37). Nevertheless, one study from the Mayo Clinic considers that all patients with del(17p) should be treated as high-risk MM irrespective of clone size [51]. Moreover the authors investigated the impact of « relative loss of 17p » defined as del(17p) in presence of trisomy 17. In their series "relative loss of 17p" was detected in 6,8% of patients: median PFS and OS were comparable for both del(17p) and relative del(17p) groups, justifying inclusion of relative del17p in the del(17p) group [51]. Second, new generation sequencing (NGS) investigations for *TP53* mutational status have highlighted discordant results and interpretations about the real impact of mono- versus biallelic *TP53* inactivation on OS and PFS. Some authors conclude that the previously found pejorative prognosis of CFF>55% may be linked to the high proportion of biallelic inactivation of *TP53* in this group [42,47]. This is not supported by the recent IFM study that demonstrates that an isolated del(17p) (CCF>55%), even if less unfavorable than a double hit, still is an indicator of poor outcome [49]. Note that, in this latter study, the percentage of mutation of the second *TP53* allele in the del(17p) group was 37% (45 of 121 cases) whereas it reached 95% (27 of 28 cases) in the study of Thakurta et al.

In brief, in the majority of studies, as long as they shared therapeutic uniformity, CCF > 55% for del(17p) detection identified high risk patients. The co-occurrence of del/mut identifies a higher double hit risk group whatever the clone size. It is worth noting that the prognostic impact of isolated *TP53* mutations is not yet established.

- *MYC* abnormalities

Aberrant *MYC* expression resulting from *MYC* amplification or translocations is a common feature of myeloma. By FISH, *MYC* is found rearranged in 15% of NDMM and up to 47% in more advanced MM [54,55]. *MYC* structural variants are detected at a higher rate by molecular techniques in 42% to 50% of NDMM [56,57].

The increased *MYC* expression levels in MM are mainly the result of the juxtaposition of a super-enhancer adjacent to *MYC* with one third involving an IG super-enhancer (*IGH, IGK* or *IGL*), one third involving another recurrent super-enhancer and the remaining third consisting of either non-recurrent or non-identified super-enhancers, or rearrangements taking place telomeric to MYC [56,58]. Most translocations involving *MYC* t(*MYC*) are complex, associated with focal *MYC* duplications, amplifications or even inversions [56,57,59]. The full repertoire of non *IG MYC* partners is not yet complete: 1p11-13, 1p21-22, 6p21, 6q21, 13q14 and 16q22 bands are recurrently involved. More than eight genes are described, the more frequently involved are *NSMCE2/TRIB1, TXNDC5, FAM46C, SNX5* and *NBEA* [56,57]. *MYC* abnormalities are the most important event promoting disease progression [60,61] and are associated with a high tumoral burden [54,61–63]. The prognostic impact of *MYC* rearrangements in MM remains controversial. A large study by the IFM group did not find significant association between the presence of a rearranged *MYC* detected by FISH and decreased survival, considering instead *MYC* remodeling as a marker of MM progression [50]. Nevertheless, the techniques used for rearrangement detection may impact the results for survival studies: initial studies conducted with FISH failed to detect about 30% of 8q24 rearrangements when compared with the use of NGS [57,64]. The use of both FISH and NGS on the same cohort confirmed differential detection level of *MYC* rearrangement between FISH (8.4%) and NGS (21%), and demonstrated an inferior survival for patient with rearrangement detected by NGS and no impact when FISH was used [57]. Finally, a recent study demonstrated that the *IGL::MYC* translocated subset is the sole *MYC* alteration predicting a poor outcome [65].

- **Translocation involving *IGL* t(*IGL*)**

T(*IGL*) are present in 10% of patients, and are indicative of poor prognosis on both PFS and OS [65]. The majority of t(*IGL*) are subclonal, indicating that these translocations are mostly secondary events. 41% involve *MYC* as a partner and the remaining occurs throughout the genome, proximal to *MAP3K14, CD40, MAFB, TXNDC5, CCND1, CCND2*, and *CCND3* albeit at much lower frequencies. Unlike t(*IGH*), t(*IGL*) are often associated with a hyperdiploid profile, and 15% of patients with a hyperdiploid profile carry an *IGL::MYC* translocation. The pathologic effects of t(*IGL*) may be directly related to the *IGL locus* and not necessarily to a gene dysregulated by *IGL* transposition. A factor intrinsic to *IGL* locus might mediate disease aggressiveness. Interestingly *IKZF1* may be involved because of its high binding affinity of *IGL* enhancer locus. It is noteworthy that IMIDs, commonly used as a front-line treatment for myeloma, target *IKZF1* for cereblon-mediated ubiquitination and proteasomal degradation [66]. This may suggest that IMIDs are less effective against t(*IGL*) MM.

- Chromothripsis, and other complex structural variants (SVs)

Like other tumors, MM shows an enrichment for complex SV events (>60% of 751 NDMM tested by whole genome sequencing (WGS) that can be divided into three main complex chromosomal events [67]. Chromothripsis, found in 24% of patients, involves chromosomal shattering and random reassembly which led to oscillating copy number alteration (CNA) and localized clustering of breakpoints. Chromoplexy, found in 11% of patients, which generates novel chromosomal structures, is formed by the concatenation of multiple translocations leading to multiple loss of copy number. Templated insertions, found in 19% of patients, where the same concatenation of translocations is seen but in this case in association with focal copy number gain, usually involving key gene drivers and super-enhancers. Of these three categories, only chromothripsis seems associated with an increased risk of progression and displays an independent adverse prognostic value, the other two being neutral. The prevalence of chromothripsis is higher than previously reported (2% to 3,5% of NDMM) [67,68], in part because of the use of techniques able to integrate SV and CNA data (ie pangenomic technics, WGS and optical genome mapping (OGM) [68,69]. The presence of chromothripsis is associated with other known high-risk molecular features of MM including high APOBEC mutational activity, *TP53* inactivation, and *NSD2* and *MAF* translocations. In MM, chromothripsis mostly involves chromosomes 1, 11 and 14. Importantly, in contrast to observation in solid cancers, chromothripsis can be considered as an early event in MM pathogenesis: it is detectable as clonal event in MGUS and SMM that will progress to multiple myeloma. It is conserved over time after progression, and at relapse without any significant changes in its structure and copy number profile. It is moreover rarely acquired at relapse. Chromothripsis can be considered as a driver in MM [70]. The detection of chromothripsis seems of increasing importance. Its detection relies on the use of pangenomic techniques (WGS or OGM) that allow increasing evidence of chromothripsis-like patterns, even if the definition is sometimes unclear or even variable depending on the techniques used.

2.1.3. Mutational data

Numerous studies of mutational profiles by targeted NGS and whole exome sequencing (WES) have been carried out, revealing that over 60 mutations concerning driver genes are observed in MGUS/SMM/MM and particularly in NDMM and some of them are targetable with specific therapies [71–74]. Different pathways are involved, MAPK/ERK 50% (*NRAS, KRAS, BRAF*), NFKB 20% (*TRAF3, NFkBIA, BIRC2/3*) and DNA repair 15% (*TP53, ATM, ATR, ZFHX4*). In NDMM the most commonly mutated genes are NRAS (21%), KRAS (17%), DIS3 (9%), TENT5C (8%), BRAF (7%), and TP53 (5%) whereas most of the remaining gene mutations are less recurrent. The only gene displaying mutations associated with adverse OS by multivariate analysis was TP53 [42].

2.1.4. Prognostic scores

2.1.4.1. Multi hit Myeloma. Co-occurrence of HR-CAs (for now defined as del(17p), t(4;14), t(14;16), t(14;20), gain 1q, or 1p32 loss) is associated with a worse prognosis [23,75–77]: in the British MRC IX trial patients with two adverse cytogenetic lesions had a median overall survival of 2 years, while the survival of patients with three aberrations was only 9 months [78]. This defines double-hit and triple-hit MM. Multi-hit MM also include bi-allelic inactivation of *TP53* (mostly del/-mut) [42,76] and 1q21 amplification (>4 copies) [24,38].

2.1.4.2. Prognostic scores. Several risk scores have been published, some based solely on genetic anomalies, others incorporating clinical-biological elements, none integrating mutations (table 1).

Since the R-ISS (2015) targeting 3 HR-CA t(4;14), t(14;16) and del17p [2], many secondary abnormalities have been characterized, with assignment to prognostic value, which improve the score. The IFM proposed a new score in 2019 incorporating primary HR-CAs such as t (4;14) but eliminating t(14;16), maintaining del17p, and adding 1q21

Table 1
Prognostic score for MM management.

			Score R-ISS 2015	Score IFM 2019		Score R2-ISS 2022 - EMN	
Cytogenetic abnormality	Frequency	Risk level		risk category	prognostic weight	risk category	prognostic weight
del(1p32) (*CDKN2C - FAF1*)	7-17%	H		H	(+ 0.8)		
1q gain (*CKS1B*)	34-40%	H		H	(+ 0.5)	H[1]	(+ 0,5)
t(4;14)(p16;q32) *IGH::FGFR3-MMSET*	15%	H	H	H	(+ 0.4)	H	(+1)
t(6;14)(p;q32)(p21;q32) *IGH::CCND3*	2%	S					
MYC rearrangement	15-45%	C					
t(11;14)(q13;q32) *IGH::CCND1*	20%	S					
t(14;16)(q32;q23) *IGH::MAF*	2-3%	C	H				
Del(17p) (*TP53*)	5-15%	H	H	H[1]	(+ 1.2)	H[2]	(+ 1)
t(14;20)(q32;q12) *IGH::MAFB*	< 1%	H					
Hyperdiploïdy	55-60%	S		+ 5 + 21	(- 0.3) (+ 0.3)		
t(IGL)	10%	*H*					
Chromothripsis	1,3%-26%	*H*					
Additional data			ISS-I , II or III + serum LDH	[1]CCF 0.55		[1]1q gain/amp - [2]all CCF mixte with ISS-III (+1.5), ISS-II (+1) and high LDH (+1)	
Score			Low risk=ISS-I/no HR-CA/normal LDH Intermediate risk= all the rest High risk=ISS-III/HR-CA or high LDH	Low Risk <= 0 Intermediate Risk 0-1 High Risk > 1		Low risk 0 low-intermediate 0.5-1 high 3-5 points	
References			Palumbo et al - JCO 2015	Perrot et al - JCO 2019 Corre et al - Blood 2021		D'Agostino et al - JCO 2022	

H: High-risk CA; S: Standard risk; C: Controversial prognosis; *H*: High risk to be confirmed
CCF: cancer clonal fraction
EMN: European Myeloma Network; IFM: Intergroupe Francophone du Myélome

gain, 1p32 loss, trisomy 5, and 21 to their list of markers. Each risk-CA was assigned a value based on its impact on OS. Patients were then stratified into three groups based on the total additive score [6]. This was the first score incorporating additional markers with their prognostic weight.

More recently, in 2022, the European Myeloma Network (EMN) proposed a revision of the current R-ISS, including 1q gain/amplification in the risk calculation with the same integrative model as the IFM, which was called the second revision of the ISS (R2-ISS) [34]. The main purpose of this revised score was to better stratify R-ISS II patients, who represent approximately 60% of patients with considerably heterogeneous outcomes. These include t(4;14), del(17p), 1q gain/amp, high LDH and ISS II and III. Importantly, the R2-ISS no longer included t(14;16) in its high risk factors [34,77,79].

None of these scores reflects the full complexity of the risk marker landscape in MM yet. None of those included mutational data.

2.2. B-Reference technologies

PCs are characterized by a low mitotic index, which means that cell culture remains a challenge. There are no specific mitogens for in vitro PCs growth (the IL6 addition has not been shown to be effective) and the karyotype is informative in no more than 20% to 30% of cases, mostly for patients whose bone marrow is highly infiltrated. However, as most diagnoses involve patients in the early stages with low marrow infiltration, karyotype is no longer used in first intention (although if karyotyping is performed a 72h to 96h, culture without mitogen is preferred).

2.2.1. Gold standard techniques

The gold standard for studying cytogenetic abnormalities of MM requires a CD138+ sorted PCs fraction followed by prognostic marker detection.

Several techniques can be used: FISH, comparative genomic hybridization array (aCGH)/ single nucleotide polymorphism-array (aSNP), NGS, WGS and more recently OGM. These technics should provide information about prognostic abnormalities: copy number changes and translocations involving immunoglobulin genes. Nevertheless, none of them is able to give the whole pattern of abnormalities. For that, most of all have to be combined. Nowadays even if FISH is actually the most used technique in routine laboratories, the total number of FISH assays is limited by the PC enrichment of sorted samples.

2.2.1.1. Preanalytical conditions. The following criteria should be present in order to diagnose myeloma according to the IMWG: $\geq$ 10% bone marrow PCs or, in the hypothesis of bone marrow hemodilution, $<$ 10% with the presence of dystrophic PCs. There are no consensual recommendations for clinical management based on FISH abnormalities in MGUS.

Although bone marrow samples can be stored at room temperature for 48h to 72h, optimal results are obtained when PCs sorting is performed within 48h.

If the diagnosis of MM is done after the bone marrow sample has been cultured, the PCs selection can be performed after the cell culture.

Even if the infiltration is massive, PCs should be sorted because the cytogenetic sample may be hemodiluted and subclonal PCs underestimated.

2.2.1.2. Quality criteria for sorted PCs. To assess the yield of the obtained CD138+ fraction, a May Grunwald Giemsa stained cytospin or a flow cytometry analysis should be performed: an efficiency of $\geq$ 80% of PCs in the sorted fraction is considered a good performance. The amount of PCs in the CD138+ fraction should be stated on the report. If the efficiency is $<$ 80%, it is worth pointing out that the detection of subclones may be of sensitivity, and/or that their related allelic frequency (VAF) must be recalculated on this basis for each detection technic used.

2.2.2. Recommendations of the GFCH

The GFCH does not recommend one technology over another, as each has its own limitations, advantages and disadvantages, including economic and financial considerations (table 2).

Testing for only the three historic genetic markers (t(4;14), del17p and t(14;16)) recommended by the R-ISS is now obsolete, too restrictive and may lead to misclassification. The prognostic score developed by the IFM based on 6 cytogenetics markers, *ie* t(4;14), del17p, 1g gain, 1p loss, +5, +21, confers a better discriminatory performance and can be carried out by FISH in many routine laboratories. Indeed, this score integrates the notion of multi hit MM and introduces a nuance linked to a specific prognostic weight for each anomaly tested. In so doing, it describes anomalies that can negatively or positively modulate the high-risk weight inherent in historical HR-CA, such as t(4;14), delTP53. Nevertheless, the panel of markers to be tested in this disease should be regularly reviewed and adapted according to new data in the literature and the therapeutic protocols.

2.2.2.1. FISH strategy. At diagnosis and for all patients requiring treatment, at least 17p deletion, *IGH::FGFR3(NSD2)* and *IGH::CCND1,* 1q21 gain and 1p32 deletion should be tested. If possible (according to the sample richness) searching for aneuploidy of chromosomes 5 and 21 can be performed to follow the IFM prognostic score index. In case of sample depletion, as minimum *IGH::FGFR3(NSD2)* and *TP53* should be tested but analysis should be carried out again.

For t(*IGH*) detection, we recommend the use of translocation rather than breakapart probes. We recommend first searching for *IGH::FGFR3 (NSD2)*, and, if a negative result is obtained, searching for *IGH::CCND1:* indeed there is an increased evidence of complex t(*IGH*) in MM that generates abnormal FISH patterns as 3'*IGH* loss, loss of der(4)t(4;14) or der(11)t(11;14), atypical breakpoints and multiple translocation partners. A normal FISH pattern with t(4;14) probe does not presume absence of t(11;14).

Technical thresholds or cut-offs must be established in each laboratory for the validation of FISH. It is noteworthy that technical thresholds are not always identical to the prognostic thresholds established in the various clinical studies. These significant differences do not preclude the description of minor clones and subclones as they exist in the sample studied. The GFCH recommends that the published prognostic threshold for 17p/*TP53* deletion should be mentioned in the report.

2.2.2.2. Alternative technologies. Alternative techniques include aSNP and aCGH, NGS, WGS and OGM. All these technologies need DNA extraction from sorted PCs. Each technique presents its advantages and disadvantages that need to be put in the balance to choose the best strategy (table 2).

Nevertheless, for now, FISH appears to be the best technique for accurate quantification of subclone size (del*TP53*) and for discrimination of concomitant subclones (1q21 gain /1q21 amp) at cellular level. Of note, recent studies have demonstrated that the combination of the present prognostic scores with minimal residual disease (MRD) measured by very sensitive techniques like flow cytometry or NGS should also be investigated as MRD negativity seems to overcome the poor prognosis conferred by baseline prognostic risk factor [80–82].

3. II- Other related diseases with significant cytogenetics

3.1. A- Immunoglobulin-related (AL) amyloidosis

Several hematologic malignancies producing paraproteins (M-proteins) define diseases with monoclonal immunoglobulin deposition that could require treatment. The non-IGM types tend to be associated with

Table 2
Comparative description of available genetic testing technics for the management of MM.

	Biological material	coverage	Detection threshold	Detection of subclone	Precise quantification of subclone	SV	CNA	CTH	LOH	Mutation
FISH	Cells	targeted, limited assays	3 to 7 %	+	+	+	+	-	-	-
SNParray	DNA	pangenomic	25 %	-	-	-	+	-	+	-
CGHarray	DNA	pangenomic	25 %	-	-	-	+	-	-	-
NGS	DNA	Targeted +/-large coverage	1 %	*	*	+	+	+/-	-	+
OGM	HMW DNA	pangenomic	SV 8- 10 % CNV 20 %	-	-	+	+	+	+	-
WGS	ADN	pangenomic	1 %	*	*	+	+	+	+	+

CSV: structural variant, CNA: copy number alteration, CTH: chromotripsis, LOH: loss of heterozygoty.
HMW DNA: high molecular weight DNA, *according percentage of cells.

PCN. Primary amyloidosis defined in the WHO2017 is now called Immunoglobulin-related (AL) amyloidosis in the WHOHAEM5 [83,84]. In cases of AL amyloidosis (immunoglobulin light chains) extracellular deposits are produced by PCs in various organs and lead to organ dysfunction requiring early diagnosis, cardiac and renal damage being the most important prognostic factors. The median survival is about 5 years and very poor prognosis is observed in case of cardiac involvement (30-40% at 6 months). Therapeutic strategies are similar to those used in PCN depending on organ involvement and new therapeutic classes improve survival. In cases with t(11;14), BCL2 inhibitors such as venetoclax considerably improve the response rate and could be proposed as 1st line therapy. Morphological PC invasion is usually moderate (9%), the cytological and the phenotypical aspects are those described in PCN, intramedullary amyloid deposits are observed in 60% of cases [85].

3.1.1. Cytogenetics

The spectrum of cytogenetic abnormalities in AL amyloidosis has profound similarities with other monoclonal gammopathies from MGUS to PCN with different frequencies of abnormalities. The t(11;14)(q13;q32)*/IGH::CCND1* is the most frequent, described in 40-60% of patients. The procedures for studying cytogenetic abnormalities are identical to those used in PCN, studies by interphasic FISH after enrichment in PCs by CD138 selection. In case of suspected amyloidosis, CD138 sorting should be performed if the flow cytometry (CMF) shows monoclonal PCs, even if the percentage of plasma cells in the bone marrow is less than 10%. Anomalies are detected in 80% of cases. Hyperdiploid forms with more than 10% medullary invasion would be associated with a negative evolution. As in PCNs, subclones are described that appear during the course of the disease. The t(11;14) is most often associated with low plasma cell count and high free light chains. AL with t(11;14) have a lower rate of subclones reflecting a certain stability of the t(11;14) clone; on the contrary hyperdiploid forms have more subclones [13,85–87].

3.1.2. Mutations

WES techniques reveal mutations affecting the same genes as in PCN but at very low rates and without significance on the evolution.

In conclusion the cytogenetic abnormalities of AL-amyloidosis are the same as those observed in PCN but with different distributions, in particular for t(11;14) which responds particularly well to Venetoclax [13,85–87].

3.2. B- Plasmocytoma

Plasmacytoma is a solidary neoplasm of clonal PCs without evidence of bone marrow involvement or end-organ damage or CRAB criteria. Plasmacytoma must be differentiated from proven extramedullary myeloma. Two types of plasmacytoma are defined according to the WHOHAEM5: i) Solitary plasmacytoma of bone (SPB) corresponding to a single lytic lesion, observed in 4% of PCN and representing 70% of plasmacytomas. It mainly arises in bones with active haematopoiesis affecting the axial skeleton; ii) Extramedullary plasmacytoma (EMP) corresponding to a soft tissue mass without contact with the bone and more likely localised in the upper respiratory tract observed in 2% of PCN and representing 30% of plasmacytomas. About half of patients have a serum paraprotein, IgG more often than IgA or light chain. Both entities meet specific diagnostic criteria based on a tissue biopsy with histological and immunohistochemical analysis.

Cytogenetic abnormalities, mainly in EMP, could be studied by formalin-fixed paraffin-embedded (FFPE) FISH or aCGH and are the same as in PCN, but do not seem to have the prognostic significance they have in PCN. It is worth noting that no t(11;14) was observed.

The evolution of these entities is linked to the presence or absence of abnormal clonal PCs in the bone marrow with risk of progression to MM of 50% at 10 years for SPB and 30% at 10 years for EMP[88,89].

4. Conclusion

The main aim of MM Cytogenetics is to identify high-risk or ultra high-risk patients with a very poor prognosis whatever the therapeutic. While FISH on sorted plasma cells remains the most widely used tool, new genetic technologies may also be investigated, and each laboratory will need to implement the technical strategy best suited to identifying new anomalies in order to meet future prognostic scores. These prognostic scores are likely to evolve in the future as « risk is a dynamic concept » according to J Corre [3]. The integration of mutational data and latest described genetic anomalies could improve theses scores. Finally, the combination of the present prognostic scores with minimal residual disease measured by very sensitive techniques should also be investigated as MRD negativity seems to overcome the poor prognosis conferred by baseline prognostic risk factors.

CRediT authorship contribution statement

Agnès Daudignon: Writing – original draft, Conceptualization. **Wendy Cuccuini:** Writing – original draft, Conceptualization. **Claire Bracquemart:** Writing – original draft, Conceptualization. **Catherine Godon:** Writing – original draft, Conceptualization. **Benoit Quilichini:** Writing – original draft, Conceptualization. **Dominique Penther:** .

Declaration of Competing Interest

We have no Conflict of Interest.

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