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Cytogenetics in the management of myelodysplastic neoplasms (myelodysplastic syndromes, MDS): Guidelines from the groupe francophone de cytogénétique hématologique (GFCH)



#### ARTICLE INFO

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## ABSTRACT

Myelodysplastic neoplasms (MDS) are clonal hematopoietic neoplasms. Chromosomal abnormalities (CAs) are detected in 40-45% of de novo MDS and up to 80% of post-cytotoxic therapy MDS (MDS-pCT). Lately, several changes appeared in World Health Organization (WHO) classification and International Consensus Classification (ICC). The novel 'biallelic TP53 inactivation' (also called 'multi-hit TP53') MDS entity requires systematic investigation of TP53 locus (17p13.1). The ICC maintains CA allowing the diagnosis of MDS without dvsplasia (del(5q), del(7q), -7 and complex karyotype). Deletion 5q is the only CA, still representing a low blast class of its own, if isolated or associated with one additional CA other than -7 or del(7q) and without multi-hit TP53. It represents one of the most frequent aberrations in adults' MDS, with chromosome 7 aberrations, and trisomy 8. Conversely, translocations are rarer in MDS. In children, del(5q) is very rare while -7 and del(7q) are predominant. Identification of a germline predisposition is key in childhood MDS. Aberrations of chromosomes 5, 7 and 17 are the most frequent in MDS-pCT, grouped in complex karyotypes. Despite the ever-increasing importance of molecular features, cytogenetics remains a major part of diagnosis and prognosis. In 2022, a molecular international prognostic score (IPSS-M) was proposed, combining the prognostic value of mutated genes to the previous scoring parameters (IPSS-R) including cytogenetics, still essential. A karyotype on bone marrow remains mandatory at diagnosis of MDS with complementary molecular analyses now required. Analyses with FISH or other technologies providing similar information can be necessary to complete and help in case of karyotype failure, for doubtful CA, for clonality assessment, and for detection of TP53 deletion to assess TP53 biallelic alterations.

## 1. Introduction

Myelodysplastic syndromes (myelodysplastic neoplasms, MDS) are a group of clonal hematopoietic neoplasms characterized by one or more peripheral cytopenias caused by inefficient hematopoiesis and increased apoptosis. The risk of transformation into acute myeloid leukemia (AML) is about 30%. MDS mostly affect the elderly with a median age of 70 years at diagnosis. Predisposing hereditary diseases (*e.g.* Fanconi anemia) for MDS are scarce. Chromosomal aberrations (CA) are detected, by bone marrow (BM) karyotype, in 40–45% of *de novo* MDS and up to 80% of therapy-related MDS. CA are crucially informative for diagnosis, prognosis, and the choice of treatments.

## 2. Cytogenetic aberrations and description of MDS

#### 2.1. De novo MDS

Identification of CA is crucial for diagnosis from WHO-HAEM5 and ICC-2022 classifications (Table 1) [1,2].

**In WHO-HAEM5**, myelodysplastic syndromes are called myelodysplastic neoplasms but still abbreviated MDS. There are 3 types of morphologically-defined MDS and 3 types of MDS with defining genetic aberrations. Two of them are defined by CA: MDS with low blasts and isolated 5q deletion (MDS-5q) and MDS with biallelic inactivation of *TP53*, including copy number variation (CNV) of *TP53* locus on 17p13.1 (MDS-bi*TP53*). The number of lineages affected is no longer relevant. WHO-HAEM5 integrates MDS fibrosis (MDS-f) in MDS with increased blasts (MDS-IB), removes unclassifiable MDS and integrates clonal cytopenia of undetermined significance (CCUS) as clonal hematopoiesis of indeterminate potential (CHIP) (see joint article on chromosomal abnormalities of undetermined significance in hemopathies).

**ICC-2022** is similarly based on genetic alterations but provides more precision on CA than WHO-HAEM5. The terminology 'MDS with del (5q)' is considered more appropriate than MDS-5q to include the association with another CA (excluding -7/del(7q)). MDS with mutated *TP53* (MDS-multihit *TP53*) is characterized by complex karyotype (CK) often associated to del(17p). Cytopenia without dysplasia is sufficient to define MDS only for del(5q), aberrations of chromosome 7 (-7/del(7q)) or CK.

In both classifications, BM blast count boundary between MDS and AML is questioned between 10% and 20%: a 20% cutoff to define AML is maintained in WHO-HAEM5 except for AML-defining genetic alterations (see article on AML) where ICC-2022 proposes the term 'MDS/AML' for the range 10% to 20% BM blasts, except for childhood MDS with excess

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#### Table 1

Comparison between WHO-HAEM5 and ICC-2022.

	2022 WHO classification			2022 ICC					
Types			Blasts	Karyotype	Mutations	Types	Blasts	Karyotype	Mutations
MDS with defining genetic abnormalities	MDS with low bla (MDS-SF3B1) (2016 WHO: diffe	asts and SF3B1 mutation erently described)	<5% BM <2% PB	any	SF3B1*	MDS with mutated SF3B1 (MDS-SF3B1)	<5% BM <2% PB	Any, except isolated del(5q), -7/del(7q), abn3q26.2, CK	SF3B1 (> 10% VAF), without multi-hit TP53, or RUNX1
	MDS with low blasts and isolated 5q deletion (MDS-5q) (2016 WHO: MDS with isolated del(5q))		<5% BM <2% PB	del(5q) alone, or with 1 other abnormality other than -7/del(7q)	any excluding <i>SF3B1</i> or <i>TP53</i> alterations**	MDS with del(5q) [MDS-del(5q)]	<5% BM <2% PB	del(5q), with up to 1 additional, except - 7/del(7q)	Any, except multi-hit TP53
	MDS with biallelic <i>TP53</i> inactivation (MDS-bi <i>TP53</i> ) (2016 WHO: not described)		<20% BM and PB	usually CK with evidence of loss copy of TP53	**Two or more <i>TP53</i> mutations, or 1 mutation with evidence of <i>TP53</i> copy number loss or cnLOH	MDS with mutated TP53	0-9% BM or PB	CK often with del(17p)	Multi-hit TP53 mutation** or : TP53 mutation (VAF>10%) and CK often with del(17p)'***
						MDS/AML with mutated <i>TP53</i>	10-19% BM or PB	any	Any somatic <i>TP53</i> mutation (VAF > 10%)
	MDS with low blasts (MDS-LB) (2016 WHO: differently described)						<5% BM <2% PB	-7/del(7q) or CK	Any, except multi-hit <i>TP53</i> or <i>SF3B1</i> (>10% VAF)
MDS, morphologically defined			<5% BM <2% PB	excluding del(5q) alor or <i>SF3B1</i> or CK with evidence or T <i>P53</i> alt	ne or with 1 other CA, mutation, of loss copy of <i>TP53,</i> rerations**	MDS, NOS with single lineage dysplasia	<5% BM <2% PB	Any, except meeting criteria for MDS-del(5q)	Any, except multi-hit TP53;not meeting criteria for MDS-SF3B1
						MDS, NOS with multilineage dysplasia	<5% BM <2% PB	Any, except meeting criteria for MDS-del(5q)	Any, except multi-hit TP53;not meeting criteria for MDS-SF3B1
	MDS, hypoplastic (MDS-h\$) ≤25% bone marrow cellularity, age adjusted (2016 WHO: not described)		<5% BM <2% PB	excluding del(5q) alone or with 1 other CA, or SF3B1 mutation, or CK with evidence of loss copy of TP53 or TP53 alterations**		not described			
	MDS with increased blasts (MDS-IB) (2016 WHO: MDS -EB)	MDS-IB1 (2016 WHO: MDS-EB-1)	5–9% BM or 2–4% PB	excluding del(5q) alone or with 1 other CA, or <i>SF3B1</i> mutation, or CK with evidence of loss copy of <i>TP53,</i> or <i>TP53</i> alterations**		MDS with excess blasts (MDS-EB)	5-9% BM, 2-9% PB	Any	Any, except multi-hit TP53
		MDS-IB2 (2016 WHO: MDS-EB-2)	10-19% BM or 5– 19% PB or Auer rods	excluding CK with evidence of loss copy of TP53, or TP53 alterations**		MDS/AML	10-19% BM or PB	Any, except AML defining	Any, except NPM1, bZIP CEBPA or TP53
		MDS with fibrosis (MDS- f) (2016 WHO: not described)	5–19% BM; 2–19% PB	excluding CK with evider or TP53 alt	nce of loss copy of <i>TP53,</i> erations**	not described			

<sup>\$</sup> MDS-h overlaps paroxysmal nocturnal hemoglobinuria and aplastic anemia (see article on aplastic anemia).

\*Detection of  $\geq$ 15% ring sideroblasts may substitute for *SF3B1* mutation.

\*\*Defined as 2 distinct *TP53* mutations (each VAF>10%) OR a single *TP53* mutation with (1) 17p deletion on cytogenetics, (2) VAF>50%, or (3) copy-neutral LOH at the 17p *TP53* locus.

\*\*\*If TP53 locus LOH information is not available.

AML, acute myeloid leukemia; BM, bone marrow; CA, chromosomal abnormality; cnLOH, copy neutral loss of heterozygosity; CK, complex karyotype; EB, excess blasts, NOS, non otherwise specified; PB, peripheral blood.

blasts (MDS-EB) (20% threshold).

Mixed MDS/MPN are presented in the joint article on MPN.

## 2.2. Childhood MDS (cMDS)

Childhood MDS (cMDS) is rare [3]. Juvenile myelomonocytic leukemia (JMML), myeloid leukemia associated with Down syndrome (ML-DS) and AML with defining genetic aberrations are excluded from cMDS in WHO-HAEM5. Many cMDS are germline predisposition syndromes [4,5].

WHO-HAEM5 distinguishes two cMDS types: cMDS with low blasts (cMDS-LB), previously refractory cytopenia of childhood (RCC) (blasts <5% in BM, 2% in peripheral blood (PB)) and cMDS with increased blasts (cMDS-IB), previously refractory anemia with excess of blasts (RAEB) (5–19% in BM and 2–19% in PB). In ICC-2022, cMDS-EB is defined between 10 and 19% BM blasts. cMDS and adult MDS have different cytogenetic landscapes. In cMDS-LB, BM karyotype is abnormal in 66% of cases including monosomy 7/del(7q) (30–49%), and trisomy 8 (9%) [4,6]. Deletion of 5q is rare (Table 2). In cMDS-IB, CK with 3 CA and monosomal karyotype (MK) (defined by the presence of two or more autosomal monosomies or a single autosomal monosomy associated with at least one structural CA) are frequent (50%) [7].

Germline pathogenic variants such as *GATA2* or *SAMD9* /*SAMD9L* or somatic *RAS* mutations are the hallmark of cMDS [8,9] (see joint articles on predisposition and bone marrow failure syndromes).

In cMDS, CA such as -7, del(7q) or CK are associated with an increased risk of AML transformation, whereas +8 or normal karyotype can have an indolent course [1]. The prognostic significance of -7

associated with a *SAMD9/SAMD9L* germline predisposition is difficult as -7 can represent a somatic rescue phenomenon [8,9].

## 2.3. Post-cytotoxic therapy MDS (MDS-pCT)

Previously named therapy-related myelodysplastic syndromes (t-MDS), post-cytotoxic therapy MDS (MDS-pCT) are defined as MDS occurring as a complication of chemotherapy and/or radiation and belong to the therapy-related myeloid neoplasms (t-MNs). The diagnosis of MDS-pCT excludes CCUS [10].

MDS-pCT demonstrate mostly high-risk karyotypes, mainly including chromosome 5 and 7 aberrations or CK in more than 90% of patients.

The majority of MDS-pCT is associated with *TP53* mutations. The outcomes of such patients are generally worse with biallelic (multi-hit) *TP53* alterations, manifesting  $\geq 2$  *TP53* mutations, or with concomitant del(17p)/*TP53* or copy neutral loss-of-heterozygosity (cnLOH) (refer to section on chromosome 17 aberrations). Less frequent mutations involve genes such as *PPM1D* and DNA-damage response genes that may require additional work-up for germline predisposition [1].

MDS-pCT is generally of poor prognosis, strongly influenced by CA and the primary disease [10-12].

## 3. Prognostic value of cytogenetic aberrations

## 3.1. Presentation of the cytogenetic score, IPSS-R and IPSS-M

Cytogenetic aberrations have an independent prognostic role with

## Table 2

Recurrent cytogenetic abnormalities and their frequency.

Cytogenetic Abnormalities	Genes involved (deletion, fusion genes or juxtaposition)	Frequency Adult MDS (post cytotoxic adult MDS)	Childhood MDS	References
Abnormal K	/	40–50% (80%)	60–72%	Schanz et al., J Clin Oncol, 2012; Hasle, Blood, 2016; Kardos et al., Blood, 2003
+8	/	5–10%	9–15%	Solé et al., Haematologica, 2005; P. Greenberg et al., Blood, 1997; Saumell et al., BJH, 2012; Kardos et al., Blood, 2003; Hasle, Blood, 2016
del(20q)	MYBL2 (20q13), TP53RK (20q13) and TP53TG5 (20q13)	3,6–7%	rare (SDS)	Haase D, Blood, 2007; Bacher, Br J Haematol, 2014
del(5q)	EGR1 (5q31), RPS14 (5q33), CSNK1A1 (5q32), miR- 145 (5q32), miR-146a (5q33), CSF1R (5q32), UBE2D2 (5q31) and CTNNA1 (5q31)	10–15% (40%)	1–2%	Haase D et al., Blood, 2007; Solé F et al., Br J Heam, 2000; Stengel, GCC, 2016; Bernard et al., Nat Med, 2020; Adema et al., ebiomed, 2022; Hasle, Blood, 2016
-7/del(7q)	CUX1 (7q22), SAMD9 (7q21), SAMD9L (7q21), EZH2 (7q36) and MLL3 (7q36)	5–10% (40%)	30-40%	Olney and Le Beau, Leukemia Resarch, 2007; Inaba et al., Blood, 2018; Hasle, Blood, 2016; Kardos, Blood, 2003
-Y*	/	3–5%	rare	Haase, Ann Hematol, 2008
-X	/	0,2–1,5%		Abruzzese E, Cancer Genet Cytognet, 1997
+21	/	0,3–0,8%	3–4%	Schanz J et al., J Clin Oncol, 2012; Grimwade D et al., Blood, 2010; Sole F et al., Haematologica, 2005
-21	/	0,3–0,5%		Haase D, Blood, 2007; Schanz J, J Clin Oncol, 2012
+11	/	0,2–0,3%		Schanz J et al., Journal of clinical oncology, 2012; Wang SA et al., Leukemia, 2010; Pozdnyakova O et al., Cancer, 2008
+13	/	0,2–0,8%		Mehta AB et al., British journal of hematology, 1998; Haase D, Blood, 2007; Mesa RA et al., Blood, 2009; Fehniger TA et al., Blood, 2009
+19	/	Rare		Johansson et al., Cancer Genet Cytogenet, 1994; Mitelman, Johansson and Mertens, 2011
i(17q)/del(17p)	<i>TP53</i> (17p13)	2–5% (25–30%)		Haase D, Blood, 2007; Bejar, Curr Opin Hematol, 2017
-13/del(13q)	/	2%	rare	Hosokawa et al., Hematologica, 2012; Haase, Ann Hematol, 2008; Pedersen-Bjergaard et al., Blood, 1995
+14/i(14q)	/	Rare		Tumewu and Royle, Cancer Genet Cytogenet, 1992; Haase, Ann Hematol, 2008
del(11q)	ATM (11q22), KMT2A (11q23), CADM1 (11q23)	0,6–3%	not described	Wang et al., Leukemia res, 2011; Stengel et al., Leukemia, 2017; Lafage-Pochitaloff et al., Blood Adv, 2022
del(12p)/t(12p)	ETV6 (12p13)	0,6–7,6%/<2%		Braulke et al., Genes Chromosomes Cancer, 2015; Haferlach et al., Genes chromosome cancer, 2012
del(9q)	/	1–2%		Haase, Ann Hematol, 2008
idic(X)(q13)	/	1%	not described	Dierlamm J et al.,Br J Haematol. 1995; Penther et al., Am J Hematol, 2019
t(11;16)(q23;p13)	KMT2A::CREBBP	(3%)		Tria et al., Diagnostics, 2022
t(3;21)(q26;q22)	RUNX1::MECOM	2–4%	rare	Haase D, Blood, 2007; Groschel et al., cell, 2014
inv(3)(q21q26)/ t(3;3)(q21;q26) **	GATA2;MECOM			
t(1;3)(p36;q21)	/	1%		Tria et al., Diagnostics, 2022
t(2;11)(p21;q23)	/	1%		Tria et al., Diagnostics, 2022
t(X;20)(q13;q13)	inactivation of 20q genes?	Rare		Michaux L. et al., Cancer Genet Cytognet, 1995
complex karyotype (CK)	/	10% (30–45%)	18%	Greenberg et al., Blood, 2012; Mauritzson et al., Leukemia, 2002; Kuendgen et al., Leukemia, 2021; Gohring G et al., Blood,
very complex karyotype (VCK)		7% (30–45%)		2010

CK: 3 unrelated chromosomal abnormalities classically associating 5q and 7q abnormalities.

VCK: 4 or more chromosomal abnormalities.

\* Loss of the Y in a small quota of mitoses may be due to age.

\*\* In WHO-HAEM5, in case of MECOM rearrangements, a blast count under 20% is acceptable to define AML.

consequences for the management of MDS patients.

The **international prognostic scoring system (IPSS)** has been widely used for clinical and therapeutic management of patients. CA were classified in 3 prognosis groups according to overall survival (OS) and risk of transformation to AML [13]. In the **revised IPSS (IPSS-R)** of 2012, the BM blast percentage, cytopenias, and a number of less common CA were included resulting in 5 prognostic groups (Table 3) [14, 15]. The IPSS-R can be applied to MDS-pCT [10].

In 2022, Bernard et al. proposed a molecular IPSS (IPSS-M) for MDS that includes the IPSS-R cytogenetic score [16] (Table 4). Deletion of 5q and aberrations of *TP53* locus are considered in the cytogenetic and the genetic components. A calculator is available at https://mds-risk-model. com.

#### 3.2. Unbalanced aberrations

General remark: For all the listed aberrations, please refer to Section 5 and Table 6 for the recommendations on karyotype, FISH and other complementary techniques.

3.2.1. Chromosome 5 aberrations: 5q deletion (del(5q)) or loss of 5q due to unbalanced translocation

Del(5q) is the most frequent CA, found in 15% of *de novo* MDS [17–20] -less in Asians [21] - and 40% of MDS-pCT (Table 2). Half of del (5q) are found isolated, 17% with ACA, and 36% with CK [22].

- del(5q) is found alone or with only 1 ACA other than -7/del(7q) and <5% BM and <2% PB (defining 'MDS-5q' in WHO-HAEM5 or 'MDS with del(5q)' in ICC-2022). It is the only MDS defined by a CA. The 5q- syndrome, characterized by abnormal megakaryocytes, was historically a specific subtype of MDS with del(5q). A distal common deleted region (CDR) 5q32-5q33.2 characterizes isolated del(5q) and 5q- syndrome.
- del(5q) is observed with ACA including chromosome 7 or multihit *TP53* alterations [20,23] and higher blast counts. A proximal CDR 5q31.2–5q31.3 is frequently observed with ACA or CK. This CDR is of poor prognosis [24] and is also altered in AML [25]. Here, the presence of specific ACA allows for classifying in MDS types other that MDS-5q (Table 5).

We do not recommend to systematically distinguish both CDRs by FISH because many patients display a larger deletion encompassing both CDRs [24,26].

Many genes of the del(5q) are haploinsufficient and involved in del (5q) pathogenicity [23,24,27–31]. Among them, *CSNK1A1, EGR1, miR-145* and *miR-146a* that are implicated in thrombocytosis, neutropenia and megakaryocytic dysplasia, *RPS14* that is a major player of the failed erythropoiesis characterizing 5q- syndrome, or *UBE2D2, CTNNA1*.

#### Table 4

IPSS-M prognostic molecular score parameters. Four variables are considered to calculate IPSS-M (from A/ to D/). The IPSS-R cytogenetic score can be found in Table 3. IPSS-M according to Bernard E et al., NEJM Evid, 2022. The score can be calculated at https://mds-risk-model.com.

A/Clinical variables	B/ Cytogenetics
bone marrow blasts platelet count hemoglobin	IPSS-R cytogenetic score
C/ Prognostic genes (in decreasing order of pejorative weigh)	D/ Number of mutated genes within this list:
TP53 <sup>multihit</sup> MLL <sup>PTD</sup> FLT3 <sup>ITD+TKD</sup> SF3B1 <sup>5q</sup> NPM1 RUNX1 NRAS ETV6 IDH2 CBL EZH2 U2AF1 SRSF2 DNMT3A ASXL1 KRAS	BCOR BCORL1 CEBPA ETNK1 GATA2 GNB1 IDH1 NF1 PHF6 PPM1D PRPF8 PTPN11 SETBP1 STAG2 WT1
SF3B1 <sup>a</sup>	

 $SF3B1^{5q} = SF3B1$  mutation in presence of isolated del5(q).

 $SF3B1^{\alpha} = SF3B1$  mutation without comutations in *BCOR*, *BCORL1*, *RUNX1*, *NRAS*, *STAG2*, *SRSF2*, and del(5q).

 $SF3B1^{\alpha}$  is the only gene of the list with a favorable prognostic weight.

## Table 3

Cytogenetic score for IPSS-R according to Greenberg PL et al. Revised International Prognostic Scoring System for MDS; Blood (2012).

Cytogenetic score	Prognosis	Cytogenetic abnormalities	Frequency (%)	Median survival (years)	IPSS (1997)
0	Very good	-Y del(11q)	3–4	5,4	
1	Good	Normal karyotype del(5q) isolated or with 1 other abn del(12p) del(20q)	66–72	4,8	Normal karyotype -Y del(5q) del(20q)
2	Intermediate	del(7q) i(17q) +8 +19 +21 Any single or double independent clones* (except with del(5q) or -7/del(7q))	13–19	2,7	Others
3	Poor	3q abn —7 Double abn. including —7/del(7q) Complex karyotype (3 abn)	4-5	1,5	Complex karyotype (3 abn) Chr7 abn
4	Very poor	Complex karyotype >3 abn**	7	0,7	
	-				

abn: abnormalities; chr: chromosome.

\* all as single abnormalities.

\*\* most MDS-biTP53 are in this case (Khoury et al., 2022).

Patients with del(5q) present less mutations than those without del (5q) [24,32]. Two distinct co-mutation patterns associated to del(5q) are identified [32]:

- Isolated del(5q): 70% present at least 1 mutation, mostly in *SF3B1* (18%), *ASXL1*, *TET2*, *TP53* (13%), etc.
- Non-isolated del(5q): 85% harbor at least 1 mutation, including 45% with *TP53* mutations [24]. Del(5q) is observed in 85% of *TP53* multi-hit patients [20]. MDS with unbalanced 5q translocations (*i.e.* with loss of telomeric 5q region and/or -5) show more CK [33].

Del(5q) appears as founder (dominant clone) more frequently in isolated than non-isolated del(5q) [24].

Patients with isolated del(5q) have a better prognosis and a lower risk of transformation to AML [14,24,34]. Patients with a dominant del (5q) clone have a better overall survival (OS) than those with del(5q) in a co-dominant or subclonal configuration [24]. Presence of *TP53* mutations correlates with adverse prognosis [20].

Patients with MDS-5q can be treated with lenalidomide, whose mechanism of action relies on 5q genes haploinsufficiency. This treatment can lead to a complete cytogenetic remission. Of note, in cases with del(5q) plus other CAs, the lenalidomide therapy response is reduced and *TP53* mutations lead to a resistance to lenalidomide.

## 3.2.2. Chromosome 7 aberrations: monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation

Chromosome 7 aberrations are recurrent in MDS, frequently found in cMDS (see Section 2.2), and, in adults, in 5–10% of *de novo* MDS and in 40% of MDS-pCT [35], mainly in MDS-EB2 (MDS-IB2 in WHO-HAEM5) [32].

Chromosome 7 aberrations are often -7 or del(7q). The translocation der(1;7)(q10;p10) and other unbalanced translocations can induce a del(7q). Several CDRs are described in 7q22, 7q32–33 and 7q34–36 [36].

An increased prevalence of -7 or other unbalanced rearrangements are described in constitutional predisposition to myeloid malignancies (read article on Aplastic Anemia).

Chromosome 7 aberrations are often associated with +21, del(5q), +8, inv(3), del(12p)/ETV6, 11q23 rearrangements, del(17p)/*TP53* alterations and CK [22,37].

Haploinsufficiency of 7q genes seems to be the pathogenic mechanism [36]. *SAMD9* (7q21.2), *SAMD9L* (7q21.2), *CUX1* (7q22.1), *LUC7L2* (7q34), *EZH2* (7q36.1) and *KMT2C* (7q36.1) may be key players in the disease phenotype and pathogenesis or progression to AML [38,39].

*U2AF1, ASXL1* and *TET2* mutations and *MECOM* overexpression are associated with chromosome 7 abnormalities [32,40]. *IDH1* and *IDH2* mutations is reported in MDS-pCT/AML patients with der(1;7) [41].

Isolated del(7q) is associated with an intermediate score in IPPS-R, whereas -7 or del(7q) has a worse prognosis related to ACA and/or gene alterations like 3q26 / *MECOM* abnormalities [38].

Campelo et al. confirm a time-dependent benefit of azacitidine on the outcome in patients with high-risk-MDS and CA involving chromosome 7, especially with CK [42].

## 3.2.3. del(9q)

Del(9q) is more common in AML than in MDS, and is no longer in the list of MDS-defining cytogenetic aberrations [22,43]. Del(9q) is associated with *TET2* mutations [44].

## 3.2.4. del(11q)

Del(11q) is a rare clonal abnormality found in 0.6% to 3% of adult MDS and MDS/MPN [45,46]. Dysmegakaryocytopoiesis with frequent presence of multinucleated megakaryocytes is observed [47]. In WHO-2008 classification, del(11q) represents a hallmark of MDS in a context of unexplained cytopenia [48].

Del(11q) appears mainly as an interstitial deletion extending from 11q13 to 11q24 (*CCND1* and subtelomeric sequences being retained), but the deletion can range from large (11q shorter than 11p), medium (11q equal in size to 11p) or to a less frequent small deletion (11q larger than 11p).

ACA are present in half of cases, mainly as a single ACA or less frequently as multiple ACA: del(5q) (20%), +8 (10%) and -Y (14%). Del (11p) seems to be mutually exclusive with -7/del(7q) or 3q26 aberrations [47].

The del(11q) is mainly a primary abnormality [15,17,45,47].

Del(11q) pathogenesis could result from multiple haploinsufficiencies. The CDR is in 11q23.2. *ATM* and/or *KMT2A* are always deleted; *CBL* is deleted or mutated in most of cases. *CADM1* and *NXPE2* genes are also on 11q23.2, the former being expressed in myeloid cells. Experimental *CADM1* deletion results in hematopoietic features reminiscent of MDS.

Del(11q) is associated with SF3B1 and ASXL1 mutations [46,47].

#### 3.2.5. 12p13 aberrations

The 12p aberrations are interstitial deletion, unbalanced translocation or balanced translocation.

## • 12p13 deletion

Del(12p) is found in 0,6 to 7,6% MDS, is often cryptic, and can be associated with CK and -7 [49].

*ETV6* (*ETS variant 6*) located on 12p13.2 is frequently involved. It encodes a transcription factor crucial for hematopoiesis. Other candidate genes are present on 12p13 such as *CDKN1B* (*KIP1, p27*), which plays a role in inhibiting cell proliferation.

## • 12p13 structural rearrangement

12p13 structural rearrangements involving *ETV6* gene are rare events in MDS (less than 2%) and involved more than 20 different translocated partners [50].

*ETV6* rearrangements are often associated with other genetic events [51].

*ETV6* rearrangements as a sole aberration are associated with an intermediate prognosis, unless the partner is of poorer prognosis.

#### Table 5

MDS types associated to presence of del(5q).

	del(5q): isolated	del(5q) associated with 1 ACA other than -7/del(7q)	del(5q) associated with 2 or more ACA (= CK)
without multi-hit TP53	MDS-del(5q)	MDS-del(5q)	other MDS types
with multi-hit TP53*	MDS-bi <i>TP53</i> **	MDS-bi <i>TP53</i> **	MDS-biTP53 **

ACA, additional chromosomal abnormality; CK>=3 chromosomal abnormalities.

\* multi-hit (or biallelic) TP53 inactivation: Two or more TP53 mutations, or 1 mutation associated to evidence of TP53 copy number loss or cnLOH.

\*\*MDS-bi7P53 (=biallelic TP53) or MDS with mutated TP53 (=multi-hit TP53) respectively according to WHO-HAEM5 and ICC-2022.

## 3.2.6. Monosomy 13 or del(13q)

Monosomy 13/del(13q) are rare in adult and children MDS including MDS-pCT [22,52].

Del(13q), involving the band 13q14 [53], contains *RB1* gene, a tumor suppressor.

These abnormalities are considered as intermediate risk in IPSS-R. However, some studies have associated isolated del(13q) with good response to treatment [52,54].

# 3.2.7. Chromosome 17 aberrations: del(17p), monosomy 17 and isochromosome i(17q)

Chromosome 17 aberrations are recurrent in MDS, found in 2 to 5% of *de novo* cases [17], and in 30% of MDS-pCT. Isolated isochromosome 17q occurs in about 1% of MDS [55].

The 17p deletions result mainly from unbalanced translocation, and less frequently from -17, i (17q) and partial del(17p). Chromosome 5 is the most frequent partner chromosome followed by chromosomes 7, 12, 18, 21 and 22.

Chromosome 17 aberrations are frequently associated (80%) to at least 2 other CA mostly chromosomes 5 and/or 7. Some cases of i(17q) are isolated or associated with few ACA [55].

The breakpoint on chromosome 17 is variable, but always proximal to *TP53* gene (17p13.1). This gene plays a fundamental role in MDS and AML [56–58].

Chromosome 17 aberrations are associated to few mutations in genes other than *TP53: TET2* (15%), *DNMT3A* (11%), and *ASXL1* and *SF3B1* (7% both) [59]. MDS with isolated i(17q) shows higher frequency of mutations (*SETBP1* (69%), *ASXL1* (67%), *SRSF2* (63%)) [55].

'MDS with biallelic *TP53* (MDS-bi*TP53*)' in WHO-HEAM5 and its equivalent 'MDS with mutated *TP53* (=multihit *TP53*) in ICC-2022 (Table 1) consists in two or more *TP53* mutations, or *TP53* mutation with concurrent deletion of the other allele, or mutation combined with cnLOH. It supersedes MDS-5q and MDS-*SF3B1* types.

Isochromosome 17q is associated with intermediate risk in IPSS-R. As multihit *TP53* aberrations are strongly associated with CK (=3CAs) and mostly with very CK (>3CAs) (90% to 100%) [1,59], they can be considered as very high risk in IPSS-R.

MDS with multihit *TP53* may be regarded as AML-equivalent for therapeutic considerations [60]. Drugs that specifically target mutant p53 (like APR-246) or members of the p53 pathway are under clinical investigation.

Altogether, assessment of biallelic status of *TP53* is required for diagnosis classifications whatever the techniques. Cases where TP53

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FISH [61](or by other techniques) must be performed are listed in Table 6 and Section 5.

## 3.2.8. del(20q)

Deletion of the long arm of chromosome 20 (del(20q)) is found in 3.6% to 7% of MDS [17]. Its findings in the absence of sufficient morphologic dysplasia is currently not considered MDS-defining.

Del(20q) appears mainly as an interstitial deletion extending from 20q11 to 20q13 with a median size of 21,7 Mb. The minimal CDR is 4,6 Mb in size and includes 96 genes [62].

The CDR includes genes such as *MYBL2* (encoding a nuclear protein involved in cell cycle progression), *TP53RK* (TP53 regulating kinase) and *TP53TG5* (TP53-target gene 5), which may be involved in the TP53-mediating signaling pathway. *ASXL1* gene on 20q11.2, frequently mutated in MDS, is deleted in 33,3% cases [62].

In a third of cases, del(20q) is associated with one CA; CK are less frequent. Recurrent ACAs are -Y, del(5q), -7, del(7q) and +8.

The most frequent mutations are in *U2AF1* and *SRSF2* (20% each), *ASXL1* (16,3%), *RUNX1* (9%) and *SF3B1* (5%). *ASXL1* mutations are more frequent in advanced MDS with increased blasts.

Isolated del(20q) is associated with good risk according to IPSS-R; the 2-year OS is 85%, and the majority of patients have MDS without increased blasts. Del(20q) can evolve into ider(20q) with possibly a poorer prognosis [63,64].

## 3.3. Balanced aberrations

3.3.1. Chromosome 3q aberrations, including inv(3)/t(3;3)

CA of 3q with *MECOM* (3q26, *EVI1/MDS1*) rearrangements are found in 2–4% of MDS [17].

WHO-HAEM5 now better reflects the MDS to AML continuum: a blast count below 20% is acceptable to define AML with *MECOM*-rearrangement, similarly to rearrangements involving *KMT2A*, and *NUP98* (read article on AML).

The most common (classic) *MECOM* rearrangements are inv(3) (q21q26) and t(3;3)(q21;q26) (partner: *GATA2*). Other CA involving 3q26 include t(3;21)(q26;q22) (partner *RUNX1*), t(3;12)(q26;p13) (partner *ETV6*), and other rare forms ('other' *MECOM*-rearrangements). ACA are seen in 30–50% of patients: monosomy 7/del(7q) (50%), CK (30%), +8, -Y, +21, del(20q) [65,66].

MECOM is a proto-oncogenic transcription factor [67,68].

In inv(3)/t(3;3), *GATA2* enhancer is close to *MECOM* gene, resulting in *MECOM* expression and *GATA2* haploinsufficiency [69,70].

#### Table 6

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Mandatory karyotype on bone marrow <b>Informative karyotype</b> Normal $\geq$ 20 mitoses; Karyotype showing abnormalities compatible with MDS $\geq$ 20 mitoses	First failure or not sufficiently informative karyotype < 20 mitoses w/o clonal CA or w/o CK (=3 CA) <sup>e</sup>	Second failure or still not sufficiently informative karyotype
<ul> <li>FISH to determine <i>TP53</i> biallelic status<sup>#,b,c</sup></li> <li>Other FISH<sup>b,c</sup> in cases of:</li> <li>Doubt in the karyotype (to confirm involvement of MDS/myeloid-related locus and in particular CA that change the prognostic group<sup>d</sup>, or to provide details on breakpoint)</li> <li>Assessment of clonality of CA found in less than 2 (or 3 for chromosome losses) metaphases</li> <li>Discordance with an evocative cytology</li> </ul>	If possible, perform a new attempt of bone marrow karyotype. If fibrosis and circulating precursor cells, a blood karyotype is possible.	<ul> <li>FISH to determine <i>TP53</i> biallelic status<sup>a,b,c</sup></li> <li>FISH mandatory<sup>b,c</sup>: 7q (7q22 (CdX1),7q36) +/- cen7 (D7Z1) 5q31 (EGR1), 3q26 (MECOM), cen8 (D8Z1) or 8q probe</li> </ul>

CA, chromosomal abnormality; CK, complex karyotype; OGM, optical genome mapping.

1. Normal or abnormal karyotype (excluding complex karyotype) with TP53 mutation.

2. Abnormal karyotype with involvement or suspicion of 17p rearrangement without data about TP53 mutation.

3. Complex karyotype without data about TP53 mutation.

<sup>a</sup> Cases where FISH TP53 is required for determination of biallelic status.

<sup>b</sup> Other techniques than FISH can be used. For example, SNP-arrays for CNV and cnLOH and/or OGM for CNV and structural CAs can be alternative.

<sup>c</sup> Deletion probe must be associated with a control probe; gene-specific probes are given as examples.

<sup>d</sup> As an example, FISH is highly recommended when doubt on del(7q): perform FISH 7q (7q22 (EX: CUX1),7q36) +/- cen7 (D7Z1).

<sup>e</sup> In cases with CK>3 CA, karyotype can be considered sufficiently informative even with < 20 mitoses.

*MECOM*-rearrangements cause MECOM overexpression leading to alteration of hematopoiesis [71].

The most frequent mutations are *SF3B1* (28%), *NRAS* (24%), *PTPN11* (22%), *ASXL1* (19%), *SRSF2* (16%), *RUNX1* (14%), *DNMT3A* (12%), and *TP53* (10%).

MECOM-rearrangements are prognostically adverse.

#### 3.3.2. Other translocations

Acquired translocations are not specific but recurrent in MDS and observed in 2–9% of MDS [72] such as the unbalanced der(1;7)(q10; p10) [73,74]. Recurrent breakpoints are 1q10, 3q21, 3q26, 7p10, 7q22, 12q13 and 17q22 [50].

It is important to differentiate balanced translocations - associated or not with CK – from unbalanced translocations.

In IPSS-R, isolated balanced reciprocal translocations excluding 3q translocations are classified in the intermediate group (read section on 3q). However with more than half of patients with CK, presence of translocations could stratify a subgroup of MDS patients with a more aggressive clinical presentation and higher scores in IPSS-R [75]. Some translocations (*e.g.* t(11;16) or t(3;21)) orientate to therapy-related MDS.

We exclude CA in 3q26, 11q23 and 11p15 bands described in previous sections or in the AML paper.

## 3.4. Numerical aberrations

## 3.4.1. Trisomy 8 (+8)

Trisomy 8 is a common CA in MDS, but its findings in the absence of sufficient morphologic dysplasia is currently not considered MDS-defining.

Isolated +8 occurs in 5–13% of *de novo* MDS patients [13,15,76,77]. Trisomy 8 occurs more frequently in Asian compared to Western populations [21].

Of note, trisomy 8 can be detected as germinal mosaicism and more frequently in patients with Behçet syndrome [78].

Trisomy 8 occurs late during disease pathogenesis [79].

The most common ACA are del(5q) and del(11q) [77].

Dosage of chromosome 8 genes is proposed to explain myeloproliferation. Higher expression of chromosome 8 genes is described in AML with trisomy 8.

Trisomy 8 cells are resistant to apoptosis by upregulation of the antiapoptotic survivin, c-myc, and CD1.

Recurrent mutations are found such as ASXL1, U2AF1, TP53, DNMT3A, RUNX1, and TET2 [80].

Conflicting data exist about OS from 5.9 to 26 months [15,81,82] depending on the ethnic background and the association with ACA [21, 83].

Trisomy 8 MDS patients show remarkable response rates to immunosuppressive therapies (up to 67%).

#### 3.4.2. Trisomy 11

Isolated trisomy 11 (+11) is found in 0.2-0.3% of MDS [15]. The ACA are trisomies of chromosomes 2, 8, 10, 19 or 22 without poor-risk chromosome 7 abnormalities.

Chromosome 11 carries *KMT2A* on 11q23. Fifty percent of MDS patients with +11 show *KMT2A* partial tandem duplications (*MLL*-PTDs) [45].

Trisomy 11 does not show any specific association with WHO or FAB MDS subtypes or secondary or MDS-pCT. In IPSS and IPSS-R, isolated +11 is assigned to the intermediate risk category, but seems to be associated with an aggressive course of the disease, short OS, and rapid progression or leukemic transformation. Within a median interval of 5 months 69% of the patients develop secondary AML, the remaining patients progress to advanced MDS stages [84,85].

#### 3.4.3. Trisomy 13

Trisomy 13 (+13) is very rare but recurrently observed in myeloid neoplasia, mostly in AML, primary myelofibrosis, atypical CML and MDS where it is found mostly as isolated abnormality [17,86,87].

Typically, the MDS is advanced with blast excess and moderate pancytopenia and dysgranulopoiesis with hypogranulation. Trisomy 13 is in the intermediate group in IPSS-R, although the prognosis is bad and mostly related to AML cases (median survival: 6–12 months) [86,87].

Hypomethylating agents for MDS patients with +13 might be inefficient. High-dose lenalidomide could be an option.

## 3.4.4. Trisomy 14 or isochromosome 14q

Trisomy 14 is extremely rare in hematologic malignancies but shows a clear association with myeloid neoplasia [88]. Trisomy 14/14q is mainly observed in MDS and CMML, more rarely in AML and atypical MDS/MPN overlap syndromes [88–90]. This abnormality is typically seen in older male MDS patients.

The trisomy results from an additional chromosome 14 or from an i (14q) [17].

Trisomy 14 has an intermediate prognosis in IPSS-R.

The abnormality seems to be an early event. The affected cell clones seem to be genetically stable with few acquisition of additional changes and a low leukemic transformation [89].

## 3.4.5. Trisomy 19

Isolated trisomy 19 (+19) is strongly associated with myeloid disorder, and specifically to a majority of MDS (or AML) [91]. Isolated +19 is a very rare CA, most of +19 are associated with CK [50]. It has an intermediate prognosis in IPSS-R.

#### 3.4.6. Trisomy 21

Constitutional trisomy 21 (Down syndrome) is associated with an increased risk of AML and ALL [92]. Trisomy 21 also occurs as a somatic CA in hematologic malignancies. Acquired trisomy 21 is a recurrent and quite frequent CA in myeloid malignancies (3%–6% in MDS, MPN, MDS/MPN) [50]. But, isolated +21 is rare in MDS, occurring between 0.3 and 0.8% [15,76,93]. MDS patients with +21 classically show low absolute neutrophil counts with mild anemia, thrombocytopenia, and a median blast count at 6%, associated with higher risk MDS [15].

Experimental models of +21, mimicking Down syndrome, show increased levels of several genes of chromosome 21, for example *ERG* and *RUNX1*, major players of hematopoiesis [94,95].

*RUNX1* mutations, which are often duplicated as a consequence of the trisomy, are frequent in various hematologic malignancies with +21 [96,97].

Patients with isolated +21 are classified in the intermediate risk group in IPSS-R, though they could be better fit in the poor-risk group [76].

#### 3.4.7. Monosomy 21

Monosomy 21 is a rare cytogenetic finding in MDS [15,17]. This CA is more frequent in AML. Like +21, the impact of isolated -21 on the prognosis is limited due to insufficient data and is categorized under the intermediate-risk group [14].

As a general rule, as monosomies can be due to technical bias, it is recommended to confirm those findings with additional techniques such as FISH, especially for monosomies in chromosomes with high prognostic and diagnostic values (ex: -7) when they are found in less than 3 metaphases according to ISCN-2022 (see Section 5 on FISH recommendations).

## 3.5. Aberrations of sexual chromosomes

#### 3.5.1. Y loss and X loss

See the joint article on "Chromosomal abnormalities of undetermined significance in hemopathies". Acquired loss of a sex-chromosome (–Y in male, –X in female) is an age-related event, but also occurs in hematological malignancies with aberrant metaphases [11,92].

Isolated -Y is a frequent cytogenetic finding in MDS [92]. Almost 10% of patients with -Y developed MDS; the risk to develop MDS is increased to 3.8-fold with -Y [98].

Trisomy 15 may occur concurrently with –Y; however, in presence of +15, –Y seems benign [98]. It is still unclear how to count the number of abnormalities when –Y is associated with another CA. However, it seems that a percentage of –Y cells  $\geq$  75% correspond most likely to a disease-associated clonal population [99,100].

Isolated -Y is in the very good prognosis group in IPSS-R.

Loss of Y potentially drives myeloid disorders.

Loss of the X chromosome in female is a relatively rare defect (isolated –X: 0.2–0.3% MDS; –X with ACA: 1.5% MDS) and correlates with an intermediate prognosis [15]. Patients with Turner's syndrome (45,X) do not show an increased risk of developing MDS and other hematologic malignancies [101]. As a general rule, constitutional abnormalities are not included in the 'abnormality count'; it also applies to loss of X in case of Turner syndrome. However, it must be determined whether –X is or is not constitutional.

### 3.5.2. idic(X)(q13)

An isodicentric X chromosome, with a breakpoint in Xq13 named idic(X)(q13) is a rare recurrent abnormality.

It is observed mostly in MDS and in AML, usually secondary to MDS or in lymphoid malignancies. It is found as an unique abnormality, suggesting an early implication in the disease process, or sometimes as part of CK [102]. It is found in 1% of MDS, frequently in old female [102, 103]. The majority of patients present marked dysplastic features (>10%) in one or more lineages [102].

The activity of idic(X)(q13) is controversial.

There is no specific gene involved in the myelodysplastic or leukemic process in the Xq13.5 region. In idic(X)(q13), the mitochondrial iron transporter gene and ATP-binding cassette transporter (*ABCB7* located in Xq21), implicated in X-linked sideroblastic anemia and spinocerebellar ataxia, is lost.

The most frequent mutations are in *TET2* (74%), *SRSF2* (68%) and *ASXL1* (47%). Biallelic inactivation of *TET2* is observed in half of the patients [102,104].

Idic(X)(q13) is in the intermediate group in IPSS-R.

The cytogenetic distinction between del(Xq) and i(Xp) is difficult due to the similarity of X p-arm and q-arm banding patterns; therefore data from advanced cytogenetic techniques as locus-specific FISH and CGH-array are needed [105].

In previous classification, its clonal detection by karyotype was sufficient to diagnose MDS in case of cytopenias without morphological dysplasia [11].

## 3.5.3. t(X;20)(q13;q13.3)

t(X;20) represent 0.4% of translocations involving chromosome X in MDS [106] and 1% of structurally abnormal chromosome X in hematological malignancies [106,107].

All t(X;20) cases are female with myeloid malignancies, mostly MDS. Spreading of X inactivation into the autosomal 20q region could provide an alternate mechanism to chromosomal deletion for the loss of function of tumor suppressor genes on 20q [107,108].

#### 3.6. Complex karyotype

#### 3.6.1. Complex karyotype

Complex karyotypes (CK=3 CA) and very CK (>3 CA) occur respectively in  $\approx$ 10% and 7% of *de novo* MDS [14,15]. CK is found in 30–45% MDS-pCT patients, most of them being very CK [10,109].

Counting rules are proposed [63,110,111]. In IPSS-R and ISCN-2022, CA are counted only in the clone presenting the highest number of CA,

even if some articles demonstrate that counting all CA in the sample and not only in the highest clone is more accurate [110,111].

MDS with CK have relatively few mutations in genes other than *TP53* [59,60]. About 55% of MDS with CK harbor *TP53* mutations, 86% of which have highly CK (>=5 CA) [60,112]. CK is associated to *TP53* multi-hit mutations (mutation, deletion, cnLOH affecting *TP53* locus) [20].

Multi-hit *TP53* mutations are found predominantly in the dominant clone with CK and with few other mutations, reflecting early genetic events in MDS pathogenesis [20].

In IPSS-R, in *de novo* or MDS-pCT, patients with CK have poor cytogenetic risk; those with a very CK have a very poor cytogenetic risk [10,13]. Only multi-hit TP53 mutation, but not monoallelic TP53 mutation, is associated with CK and poorer OS [20,32,59,113].

In ICC-2022, in MDS with *TP53* mutations, in the absence of *TP53* locus LOH information, the presence of a single TP53 mutation in the context of any CK is considered equivalent to a multi-hit *TP53*. Cases where TP53 FISH (or other techniques) must be performed when CK is present are listed in Table 6.

## 3.7. Aberrations of unknown significance

See the joint article on the topic. Some aberrations, even though frequent in MDS, are not presumptive of MDS. It includes trisomy 8 or del(20q), and -Y. Trisomy 15 as the sole autosomal CA or in combination with -Y, even uncommon in hematological malignancies is preferentially associated with MDS. In ICC-2022, at the exception of del(5q), -7/del (7q), CK and *SF3B1* and *TP53* mutations, other CA abnormalities are not sufficient to define MDS in case of cytopenia without dysplasia. They define CCUS.

#### 4. Molecular aberrations

Many genetic aberrations are identified in MDS. Two MDS types are specifically defined by genetic variants (*SF3B1* and *TP53*-multihit). Major mutations are included for their prognostic impact in the IPSS-M along with cytogenetic aberrations (see section 3.1) (Table 4) [16].

## 4.1. Predisposition

See the joint article on predisposition. Predisposition syndromes are integrated into the subgroup of secondary MN in WHO-HAEM5. These syndromes are not only present in children and their detection is important for the management of the patients and their family.

## 5. Techniques for analyzing chromosome aberrations and guidelines

#### 5.1. Karyotype

In MDS, karyotype (chromosome banding analysis, CBA) is mandatory on BM (1 to 3 ml per sampling) to define WHO-HAEM5 or ICC-2022 MDS types and to address IPSS-R and IPSS-M scores. PB, if it contains blasts or immature myeloid cells, is used as a rescue, mainly in fibrosis. At least 2 million cells are necessary but 10–20 million cells is optimal. Culture times are 1 day (at least overnight) and 2 days. Myeloid growth factors such as G-CSF enhance the mitotic index and enable a 3- or 4-day culture time. Cell concentration is 0.5–2 million cells per ml of culture, decreasing with culture time and presence of myeloid growth factors.

At least 20 metaphases must be fully analyzed to assess a normal karyotype or to exclude CK or very CK. When karyotype failed, which is mainly due to insufficient and/or hemodiluted sample, the cytogeneticist should quickly request a new sample for karyotyping and a new attempt of karyotype should be performed. In case of repetitive failure, techniques such as FISH or chromosomal microarray or optical genome mapping can be valuable.

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Karyotype must be defined and reported according to the last ISCN recommendations.

## 5.2. FISH

FISH is complementary to CBA to support a correct assessment of IPSS-R scoring and WHO or ICC classifications.

- FISH is recommended for:
- Clarification of a subtle CA suspected by CBA,
  - $\circ\,$  in the case of doubt in the karyotype to confirm the involvement of a MDS/myeloid-related locus, in particular those which may change the prognostic group,
  - $\circ\,$  or to provide details on a breakpoint.
- Assessment of clonality of a CA, in particular CA with diagnostic or prognostic impact, found in less than 2 (or 3 for chromosome losses) metaphases according to ISCN-2022 (ex: -7; +8; +13;-21...)
- Discordance with an evocative cytology
- Detection of *TP53* deletion to assess *TP53* biallelic alterations, more especially if *TP53* mono- or biallelic *TP53* alteration are found or suspected by sequencing (*e.g.* NGS). Indeed, FISH sensitivity can be superior to NGS for detection of small clones (Table 6).

As most CAs are losses, and gains less frequent, FISH probes should be located in the critical chromosomal regions.

In order to detect small clones and to avoid false negative or false positive results, the FISH probes used for deletions or gains should be double or triple color probes combining a control probe on the same chromosome and 1 or 2 probes located in the critical regions such as:

- TP53 (17p13) probe and, as a control a chromosome 17 centromeric or 17q probe for deletion of *TP53*,
- EGR1 (5q31) probe often combined with a CSF1R (5q32) probe and, as a control, a chromosome 5p probe for del(5q),
- CUX1 (7q22) probe often combined with a 7q36 probe and, as a control a chromosome 7 centromeric probe for del(7q),
- $\circ$  MYC (8q24) probe and a chromosome 8 centromeric probe for trisomy 8.

International recommendations do not specify a threshold for defining a *TP53* deletion. We recommend a 5% threshold of nuclei with a typical deleted pattern (and at least 90% of nuclei hybridized and 100 counted nuclei).

## 5.3. Molecular techniques

See the joint article on this topic. Briefly:

## Comparative Genomic Hybridization (CGH) and Single Nucleotide Polymorphisms (SNP) arrays

Due to their low sensitivity (20%), they may miss rare subclones. Balanced structural abnormalities are not detected. However, detection of CNV, by CGH- or SNP-arrays, demonstrates a prognostic utility in case of failed or non-informative karyotype [114]. Detection of LOH greater than 25Mb, by SNP-arrays, demonstrates a prognostic impact in MDS with normal karyotype [115]. SNP-arrays are valuable techniques, especially for peripheral blood samples, in cases of failure of conventional karyotype due to fibrotic or hypocellular bone marrows [116, 117]. SNP-array is an appropriate technique to detect CNV and cnLOH of 17p13 to determine the allelic status of *TP53*.

Their worldwide adoption has been hampered by financial constraints and reimbursement practices.

## High Throughput Sequencing including NGS

MDS diagnosis (WHO-HAEM5, ICC-2022) and prognosis (in particular IPSS-M) currently requires the evaluation of genomic alterations other than CA, including single nucleotide variants. Gene panel by NGS detects somatic mutation in a targeted and limited with high sensitivity (1%).

## **Optical Genome Mapping (OGM)**

In MDS, OGM provides additional information compared to karyotype in 34% cases and changes the comprehensive cytogenetic scoring system and the IPSS-R risk groups in respectively 21% and 17% of patients [118]. Combination of OGM and NGS enables refinement of prognosis [118,119]. OGM can be recommended to reduce the number of FISH, it provides details on a breakpoint and precision on abnormal chromosome regions.

## 6. Conclusion and guidelines

Details on the guidelines are provided in Table 6.

Karyotype on BM is mandatory in MDS. FISH is complementary to CBA to support a correct assessment of IPSS-R scoring and WHO or ICC classifications. FISH is recommended in cases of karyotype failure or insufficiently informative karyotype.

*TP53* multi-hit alteration must be assessed whatever the technique is. Therefore, unless 2 or more *TP53* alterations are already identified (for example by NGS or a combination of techniques, ex: NGS and karyotype), or unless one *TP53* mutation in a context of CK is found, *TP53* FISH is mandatory; other available techniques are possible.

Responses at diagnosis should be optimally given within 14 days for MDS-IB and 21 days for other MDS [120].

## **Declaration of Competing Interest**

None.

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