

Diagnosis and prognosis are supported by integrated assessment of next-generation sequencing in chronic myeloid malignancies. A real-life study



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Haematologica 2021
Volume 106(3):701-707

ABSTRACT

Next-generation sequencing (NGS) is used to investigate the presence of somatic mutations. The utility of incorporating routine sequencing to guide diagnosis and therapeutic decisions remains unclear. We report the findings of an observational, multicenter study that aimed to assess the impact of somatic mutation testing by NGS in a real-life setting of chronic myeloid malignancies. A total of 177 patients were enrolled, partitioned into two overlapping groups. In group A (n=94), the indication was to search for clonal hematopoiesis, in a context of suspected myelodysplastic syndrome or myeloproliferative neoplasia. In group B (n=95), the therapeutic impact of somatic mutations was studied. A panel of 34 genes was used on DNA extracted from blood or bone marrow samples. Within group A, the detection of clonal hematopoiesis supported the diagnosis of chronic myeloid malignancies for 31 patients while the absence of clonal hematopoiesis ruled out the suspected diagnosis in 47 patients. Within group B, NGS identified prognostically relevant somatic mutations in 32 patients, which had a therapeutic impact in 18 cases. By determining the presence or absence of somatic mutations, the application of NGS in daily practice was found to be useful for an integrated final diagnosis in 83% of the patients. Moreover, the search for somatic mutations had a prognostic impact that led to treatment modification in 19% of the cases. This study outlines the fact that adequate implementation of new investigations may have a significant positive medico-economic impact by enabling appropriate management of patients.

Introduction

During the past decade, high-throughput sequencing has increased knowledge on the genomic landscape of hematologic malignancies. Somatic mutations have thus become new biomarkers that are useful in clinical practice to improve diagnosis, prognosis stratification and targeted treatment. In France, the implementation

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Received: November 12, 2019.

Accepted: April 1, 2020.

Pre-published: April 2, 2020.

<https://doi.org/10.3324/haematol.2019.242677>

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of next-generation sequencing (NGS) platforms by the Institut National du Cancer (INCa) has enabled physicians to investigate these markers since 2013. Non-acute myeloid malignancies, i.e., myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) represent good indications for NGS because of their relatively high incidence of mutations and the ease with which they can be assessed in peripheral blood samples¹⁻³ in time frames compatible with decision-making.

More than 40 genes are now known to be potentially recurrently mutated in MDS,¹ mixed MDS/MPN syndromes² and MPN.³ In a context of idiopathic cytopenia of undetermined significance (ICUS), the presence of somatic mutations can support the diagnosis of clonal cytopenia of undetermined significance (CCUS).^{4,5} In MDS, several mutations, including those of *TP53*, *EZH2*, *ETV6*, *RUNX1* or *ASXL1*, have been shown to be strongly associated with decreased overall survival,⁶ independently of the risk group according to the Revised International Prognostic Scoring System (IPSS-R).⁷ Other studies have shown that the addition of molecular testing allows for a better prognostication of the outcome of patients with chronic myelomonocytic leukemia (CMML)⁸ or MPN.³ It has therefore been suggested that the IPSS-R and Dynamic International Prognostic Scoring System (DIPSS)⁹ scores could be improved by incorporating information on molecular abnormalities.¹⁰ This is possibly pertinent since targeted therapies are now emerging for the treatment of MDS and MPN, which was long limited to a few drugs of moderate efficacy, making the search for somatic mutations of relevance in order to be able to propose the best available treatment.¹¹

Given the potential advantages of the broad screening capacity of NGS, this technique is now often used without proper published international guidelines. In order to explore the added value of somatic mutation testing by NGS, we conducted an observational multicenter study aimed at assessing the impact of somatic mutation testing by NGS in a real-life setting, focusing on the impact of implementation of this testing on diagnosis, prognosis and treatment in selected populations.

Methods

Patients

In our University Hospital, all NGS requests must be validated during regional multidisciplinary meetings. Indications for NGS analysis include challenging cases of suspected MDS or MPN in which the detection of somatic mutations could help the diagnosis, prognostic assessment or search for therapeutic markers. This occurs after morphology/pathology, flow cytometry and cytogenetics have already been performed in order to apply World Health Organization (WHO) recommendations. All patients for whom NGS testing was prescribed between October 2014 and March 2019 were included in the present study. All provided their consent according to local ethical rules. In a first cohort of patients (group A), the indication for NGS was to confirm or rule out a suspected diagnosis. In a second cohort, (group B), therapeutic decisions were expected to be supported by the detection or not of prognostic somatic mutations.

Next-generation sequencing analysis

In order to detect somatic mutations, a customized, targeted panel of 34 genes (145 kbp) was applied to DNA extracted from

peripheral blood or bone marrow samples (*Online Supplementary Table S1*). The selection of these 34 genes followed INCa recommendations published in 2013 and updated in 2016¹² and was completed by a review of the literature. DNA libraries, built with the Haloplex[®] target enrichment protocol (Agilent Technologies, Santa Clara, CA, USA), were paired-end sequenced with a MiSeq[®] Instrument (Illumina, San Diego, CA, USA). Data were analyzed using an in-house pipeline including trimmed reads alignment to the GRCh34 human reference genome (February 2009 assembly) with BWA-MEM, tumor variant detection by three variant callers (GATK HaplotypeCaller, VarScan and SAMTools) and variant annotations with public databases (gnomAD, COSMIC, dbSNP, ClinVar) and *in silico* predictors in the case of unknown variants (CADD, SIFT, PolyPhen-2, MutationTaster), using updated versions whenever available. Mutations were considered significant if they reached a good quality score (DP4), a minimum variant allele frequency of at least 1% and a minimum of 20 reads supporting the variant for hotspots or 50 reads for non-hotspot variants. Variants of undetermined significance (VUS) with no clear association to a disease were defined according to Li *et al.*¹⁵ Final reports were delivered to clinicians in real time, without mentioning variants identified as polymorphisms.

Group A: the impact of next-generation sequencing on diagnosis

Clonal hematopoiesis was defined by the presence of at least one somatic mutation. The detection of clonal hematopoiesis was integrated with suspected diagnoses of MDS or MPN according to the criteria detailed below.

Idiopathic cytopenia of undetermined significance and clonal cytopenia of undetermined significance

In the case of ICUS with morphological evidence of dysplasia but without MDS-related cytogenetic abnormalities, the detection of clonal hematopoiesis was used to retain a diagnosis of MDS. In the context of ICUS¹⁴ without morphological evidence of dysplasia and without MDS-related cytogenetic abnormalities, the presence of somatic mutations defined a diagnosis of CCUS.⁵

Chronic myelomonocytic leukemia

In cases of suspected CMML with or without minimal evidence of dysplasia, WHO criteria include the presence of acquired clonal cytogenetic or molecular genetic abnormalities (i.e., somatic mutations) as supportive of the diagnosis.¹⁵

Aplastic anemia

In a context of bone marrow hypoplasia, the absence of clonal hematopoiesis was used to exclude a diagnosis of hypoplastic MDS (hMDS). In addition, the detection of *PIGA* somatic mutations helped to make a diagnosis of aplastic anemia (AA).^{15,16}

Myeloproliferative neoplasms

In cases of suspected MPN without *BCR-ABL* fusion and without any of the three classic driver mutations (*JAK2*^{V617F}, *CALR* and *MPL*) as detected by classical methods, the WHO classification recommends searching for the most frequent accompanying mutations (e.g., *ASXL1*, *EZH2*, *TET2*, *IDH1*, *IDH2*, *SRSF2* and/or *SF3B1*) to help determine the clonal nature of the disease.¹⁵

Group B: the impact of next-generation sequencing on prognosis

In group B, the diagnoses of MDS, MPN/MDS - including CMML - and MPN were established according to the WHO classification.¹⁵

Scores of the IPSS-R,⁷ CMML-specific Prognostic Scoring

System (CPSS),¹⁷ DIPSS⁹ and the MYSEC prognostic model¹⁸ were used for standard prognosis assessment of MDS, MPN/MDS, CMML and primary or secondary myelofibrosis. Molecular markers considered to indicate a poor prognosis for MDS or MPN/MDS, excluding CMML, were *TP53*, *EZH2*, *ETV6*, *RUNX1* and *ASXL1* mutations, as reported by Bejar *et al.*⁶ The detection of an *SF3B1* mutation was considered a good prognostic sign.¹⁹ The Itzykson⁸ and/or CPSS-Mol²⁰ scores, which respectively integrate *ASXL1* mutation only or *RUNX1*, *NRAS*, *SETBP1* and *ASXL1*, were used for the prognostic assessment of CMML. *SRSF2*, *ASXL1*, *IDH1*, *IDH2* and *EZH2* mutations were indicative of a worse prognosis in the context of myelofibrosis.²¹ The *ASXL1* mutation was also considered to be associated with poor prognosis in patients with AA.^{16,22,23}

Results

Patients' characteristics

One hundred and seventy-seven patients (100 males and 77 females), originating from ten hospitals were included. Their median age was 60 years old (range, 10-87). These patients had ICUS or MDS (n=77), MPN/MDS (n=38), AA or a suspicion of hMDS (n=19) and MPN or suspected MPN (n=43) (Table 1).

NGS was performed for 94 patients in group A, within a median of 4 months of hematologic detection of anomalies. Group B consisted of 95 patients, with a median follow-up from molecular assessment to last news of 11 months. Twelve patients belonged to both groups.

Within group A, the most frequent blood count anomalies were cytopenia (68%: anemia [56%], thrombocytopenia [28%], neutropenia [30%] or pancytopenia [16%]) followed by thrombocytosis (16%), and monocytosis (13%). Rare patients had thrombosis with a suspicion of MPN but no blood count anomalies (3%). Bone marrow smears and/or biopsy evidenced no significant dysplastic anomalies in 53% of the cases. Cytogenetic abnormalities not specific for MDS, such as chromosome Y loss or chromosome 20 deletion, were observed in 8% of the cases. Karyotyping was normal in 72% or failed in 5% of the cases. No karyotyping was performed in seven cases of thrombocytosis, two cases of erythrocytosis, one case of thrombosis with normal blood count and one case of hypereosinophilic syndrome. Before molecular assessment, the context was ICUS (with or without morphological evidence of dysplasia) (39%), suspicion of MPN/MDS (17%) or AA/hMDS (17%) (Table 1, *Online Supplementary Table S2*). All suspected cases of MPN (27%) were negative for the three classic driver mutations.

The majority of patients in group B were diagnosed

with MDS (42%), followed by MPN/MDS (31%), MPN (21%) or hypoplasia (6%). Most patients with MDS, MPN/MDS and MPN were classified as low risk (60%) according to the respective scoring systems (Table 1).

Impact of mutations detected by next-generation sequencing on diagnosis (group A)

Thirty-three percent of the patients (31/94) had at least one mutation in favor of clonal hematopoiesis (range for the whole study cohort, 0-7). Twenty-six of them had a normal karyotype and five had a non-specific chromosomal abnormality (i.e., del(20q) or -Y). The most frequently mutated genes were *ASXL1* (12%), *TET2* (11%) and *DNMT3A* (9%) (Figure 1A, *Online Supplementary Table S2*). Patients with mutations were significantly older (median: 67 years vs. 48 years in those without mutations; $P<0.001$) but no difference in frequency of mutations was seen according to the context of suspected disease. Patients with clonal hematopoiesis also had significantly more myeloma (1.6% vs. 0.2%; $P=0.005$) and a higher red cell distribution index (16% vs. 14%; $P=0.01$). The signs of dysplasia observed on bone marrow smears and/or biopsy were not associated with the presence of a detected somatic mutation (P =not significant).

The detection of clonal hematopoiesis by NGS allowed us to retain the diagnosis of myeloid malignancy for 17 patients (18%) (3 MDS, 7 MPN/MDS and 7 MPN), eight cases of CCUS and six cases of bone marrow hypoplasia with clonal hematopoiesis (Table 2). In most patients with AA/hMDS (n=10/16), the absence of clonal hematopoiesis favored a diagnosis of idiopathic AA, while clonal hematopoiesis detected in six cases supported a diagnosis of hMDS. Thirteen mutations were observed in seven patients with suspected MPN. Among those, four had *MPL* mutations, respectively two subclonal hotspot p.Trp515Leu (not previously detected by Sanger sequencing) and three non-canonical mutations (p.Trp515Ser, p.His499Valfs*46, p.Tyr591Asp).

Considering the patients without detected clonal hematopoiesis (n=63), the initial suspected diagnosis of myeloid malignancy was ruled out in 47/63 (75%) of the cases including 25/26 suspected MDS (96%), 8/9 suspected MPN/MDS (89%), all suspected hMDS (10/10) and 4/18 suspected MPN (22%). In these patients, cytopenias were ultimately mainly considered as of peripheral origin or ICUS and monocytosis as reactive. In the absence of clonal hematopoiesis, no diagnosis could be ruled out for most suspected cases of MPN (14/18) (Table 2, *Online Supplementary Table S2*) because of morphological anomalies on bone marrow biopsy (*data not shown*).

VUS were found in 26 cases (28%) (Figure 1A, *Online*

Table 1. Partition of the patients according to diagnosis or suspected diagnosis.

	ICUS n=37	MDS n=40	MPN/MDS or suspicion n=38	AA/hMDS or suspicion n=19	MPN or suspicion n=43	Total n=177
Age, years: median (range)	53 (18-84)	63 (10-87)	63 (32-73)	58 (14-78)	57 (16-80)	60 (10-87)
Group A, n	37	NA	16	16	25	94
Group B, n	0	40	29	6	20	95
Both groups, n	0	NA	7	3	2	12

ICUS: idiopathic cytopenia of undetermined significance without dysplasia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; AA: aplastic anemia; hMDS: hypoplastic myelodysplasia.

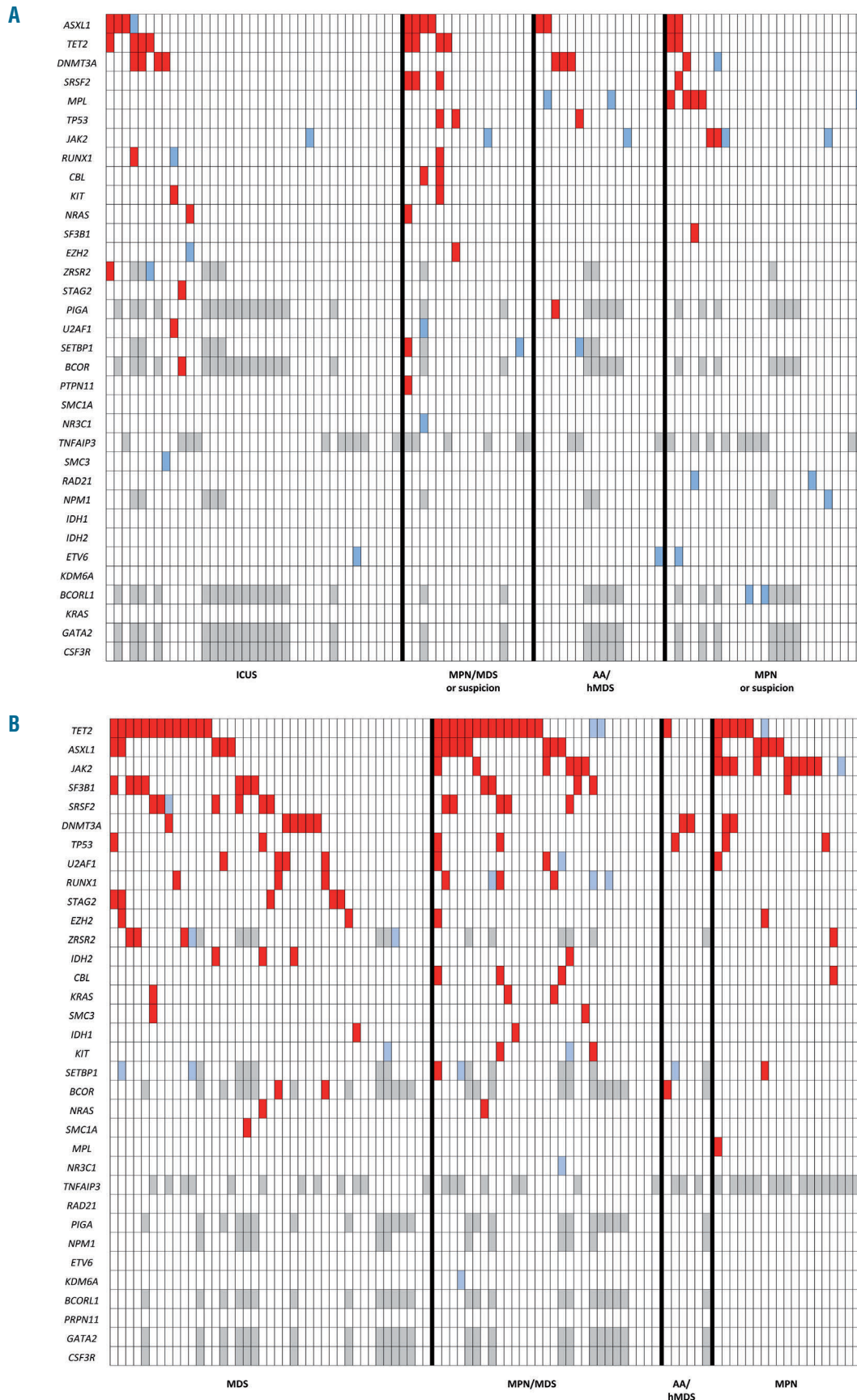


Figure 1. Profile and frequency of mutations in the two study groups. (A) Profile of mutations according to the suspected context in diagnosis group A. Red: somatic mutations; blue: variant of undetermined significance (VUS); gray: not evaluated. (B) Profile of mutations according to the proven pathology in prognosis group B. Red: somatic mutations; blue: VUS, gray: not evaluated. ICUS: idiopathic cytopenia of undetermined significance without dysplasia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; AA: aplastic anemia; hMDS: hypoplastic myelodysplasia.

Table 2. Diagnostic assessment.

Suspected diagnosis before NGS testing	Clonal hematopoiesis		No clonal hematopoiesis (N=63)	
			Diagnosis ruled out	Diagnosis not ruled out
ICUSwd (n=21)	n=8 (CCUS)		n=13 (MDS)	n=0
ICUSwdt (n=16)	n=3 (MDS)		n=12 (MDS)	n=1
MPN/MDS (n=16)	n=7 (CMML)		n=8 (MPN/MDS)	n=1
Aplasia/hypoplasia (n=16)	n=6 (clonal hypoplasia or hMDS)		n=10 (MDS)	NA
Suspicion of MPN (n=25)	n=7 (MPN)		n=4 (MPN)	n=14 (MPN)
Total n=94	31 (33%)		47 (50%)	16 (17%)

NGS: next-generation sequencing; ICUSwd: idiopathic cytopenia of undetermined significance without dysplasia; ICUSwdt: idiopathic cytopenia of undetermined significance with dysplasia; CCUS: clonal cytopenia of undetermined significance; MPN/MDS: myeloproliferative neoplasm/myelodysplastic syndrome; hMDS: hypoplastic myelodysplasia; MPN: myeloproliferative neoplasm.

Table 3. Prognostic evaluation according to the pathology.

Diagnosis	MDS n	MPN/MDS n	AA n	MPN n	Total n (%)
Prognosis assessment	40	29	6	20	95 (100)
Prognostic somatic mutation detected	14*	11 [†]	1 [‡]	5**	31 (33)
Theranostic modification	8	6	0	4	18 (19)

*Eight patients with high-risk mutations (HR) and six patients with low risk mutations (LR); †Ten HR and one LR; ‡One HR; **Five HR. MDS: myelodysplastic syndrome, MPN: myeloproliferative neoplasm; AA: aplastic anemia.

Table 4. Patients in whom therapy was changed in the light of the next-generation sequencing data.

Patient	Pathology	Sex	Age (years)	Risk*	Prognostic mutations	Other mutations	VUS	Therapeutic impact
#71	CMML-0	F	67	Int	<i>ASXL1</i>	<i>CBL</i>	<i>U2AF1</i> ,	ASCT
#2	CMML	M	39	Low	<i>ASXL1</i>	<i>RUNX1, KRAS</i>	<i>NR3C1</i>	Second ASCT
#4	CMML	M	63	Low	<i>ASXL1</i>	<i>TET2, U2AF1, TP53, CBL, EZH2[‡], JAK2 and SETBP1</i>		ASCT
#65	CMML	M	58	Int	<i>SF3B1</i>	<i>KIT D816V</i>	<i>RUNX1, TET2</i>	Therapeutic abstention
#32	CMML-2	M	70	High	<i>ASXL1</i>	<i>RUNX1, SRSF2, TET2[†]</i>		ASCT after partial response to HMA and high-risk mutation
#42	CMML-2	F	65	Low	<i>ASXL1</i>	<i>TET2</i>	<i>SETBP1, KDM6A</i>	ASCT
#51	PMF	M	58	Int	<i>ASXL1, EZH2</i>	<i>SETBP1</i>		ASCT
#7	SMF	F	51	Int		<i>JAK2</i>		Switch from ASCT to the association of ruxolitinib and hydroxyurea
#17	SMF	F	45	Int		<i>TET2</i>		Switch from ASCT to hydroxyurea followed by anagrelide then interferon
#161	SMF	M	67	Low	<i>ASXL1</i>	<i>JAK2 V617F</i>		ASCT
#16	MDS-MLD	M	71	Int	<i>ASXL1</i>	<i>ASXL1</i>		Switch from lenalidomide to HMA
#120	MDS-MLD	F	69	Low	<i>EZH2, ASXL1</i>	<i>STAG2, TET2[†]</i>	<i>SETBP1</i>	ASCT
#145	MDS-MLD	M	67	Low	<i>SF3B1</i>	<i>TET2 and ZRSR2</i>		Therapeutic abstention
#90	MDS-RS-MLD	M	57	Low	<i>EZH2</i>			ASCT
#104	MDS-RS-MLD	F	68	Low	<i>RUNX1</i>	<i>BCOR, U2AF1</i>		ASCT after evolution to MDS-EB1
#93	MDS-RS-SLD	M	44	Low	<i>ASXL1</i>	<i>U2AF1</i>		ASCT
#5	MDS-SLD	F	55	Int	<i>TP53, ASXL1, SF3B1</i>	<i>TET2</i>		ASCT
#124	MDS-SLD	F	57	Int		<i>DNMT3A</i>		Therapeutic abstention

*Risk assessed according to the relevant scoring systems: the Revised International Prognostic Scoring System (R-IPSS) for myelodysplastic syndromes, the chronic myelomonocytic leukemia (CMML)-specific Prognostic Scoring System (CPSS) or Itzykson score for CMML, the Dynamic International Prognostic Scoring System (DIPSS) for primary myelofibrosis and MYSEC for secondary myelofibrosis. †Double mutations of the gene.VUS: variant of undetermined significance; F: female; M: male; Int: intermediate risk; ASCT: allogeneic stem cell transplantation; HMA: hypomethylating agent; PMF: primary myelofibrosis; SMF: secondary myelofibrosis; MDS-MLD: myelodysplastic syndrome with multilineage dysplasia; MDS-RS-MLD: myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia; MDS-RS-SLD: myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia; MDS-SLD: myelodysplastic syndrome with single lineage dysplasia; MDS-EB1: myelodysplastic syndrome with excess blasts 1.

Supplementary Table S2). The most frequent ones were observed in *JAK2* (p.Asn1108Ser, p.Arg1063His, p.Ile35Thr), *MPL* (c.1565+5C>T, p.Pro70Leu, p.Gln433Arg, p.Ala622Pro), *ETV6* (p.Ala329Thr, p.Pro223Leu, p.Ala329Thr) and *BCORL1* (p.Arg21His, p.Ala612Thr, p.Ile1022Thr, p.Arg1183Gln). Among patients with VUS, 14 had no clear somatic mutations, no proof of clonal hematopoiesis and no significant association with a given suspected context. Of note, identification of a poor prognostic mutation led to intensification of therapy in 3/12 patients in group A.

Prognostic/theranostic impact of mutations detected by next-generation sequencing (group B)

The median number of mutations in group B was two (range for the whole cohort, 0-9) and 75% of these patients had at least one mutation (Figure 1B, *Online Supplementary Table S2*). NGS identified prognostic mutations in 31/95 (33%) patients at different proportions according to the pathology, i.e., 14/40 (35%) in MDS, 11/29 (38%) in MPN/MDS, 5/17 (29%) in myelofibrosis and 1/6 (17%) in AA. Among the 24 patients with poor prognostic mutations, the conclusion was to proceed to an allogeneic stem cell transplantation for 12 of them and to start a hypomethylating agent in another one (Table 4). Among these patients, *ASXL1* was the most frequent poor prognostic marker with therapeutic impact (n=11). Conversely, *SF3B1*, a mutation associated with a good prognosis, was identified in six cases of MDS and one of MPN/MDS. In five patients, this information led to a de-escalation of treatment intensity, i.e., allogeneic stem cell transplantation was postponed because of the presence of a good prognostic mutation (*SF3B1*) or absence of poor prognostic mutations.

Of note, VUS were observed in 18/95 cases, three being detected in *RUNX1*, thereby potentially conveying a poor prognosis.

Discussion

The interest of high-throughput NGS has been widely demonstrated in onco-hematology, mostly in clinical trials, but evaluation of these new molecular data outside clinical research is lacking. In the same way as for the usage of a drug, it appears essential to evaluate the medico-economic impact on clinical decisions of the use of these new tools, which are modifying the workload and costs in diagnostic laboratories.²⁴ Nevertheless, the use of high-throughput sequencing to aid diagnosis and treatment decisions in chronic myeloid malignancies has seldom been evaluated in “real life”. Here, we retrospectively examined the impact of NGS assays on a series of 177 patients from ten centers. The impact on diagnosis and therapeutic decisions were assessed separately by dividing the patients into two groups.

The main objective of NGS for diagnostic purposes is the search for somatic mutations to provide or not proof of clonal hematopoiesis in favor of myeloid malignancies when standard diagnostic criteria for the disease have not been reached with classical tests (cytology, cytogenetics, pathology). *ASXL1*, *DNMT3A* and *TET2* mutations are the most frequent but can be age-related²⁵ and observed in the hematopoietic cells of apparently healthy older individuals without MDS i.e., clonal hematopoiesis of indetermi-

nate potential (CHIP).²⁶ Consequently, the interpretation of such molecular abnormalities in subjects over 70 years of age requires a multidisciplinary discussion. Clonal hematopoiesis was identified here in 33% of the patients and a firm diagnosis of myeloid malignancy was retained in 18%. The diagnosis of CCUS was reached in 9% of the patients and had an impact on the continuation of clinical follow-up because there was a higher risk that these patients would develop a myeloid neoplasm.^{5,27} Conversely, because more than 85% patients with MDS have one or more somatic mutations that can be detected using a minimal panel of recurrently mutated genes,¹ the absence of mutation provided significant help to exclude a diagnosis of MDS in the vast majority of cases. In the context of aplasia, the absence of clonal hematopoiesis and the presence of a *PIGA* mutation (mostly associated with AA) helped to exclude a diagnosis of hMDS.^{16,23}

Overall, in this series, the search for clonal hematopoiesis by NGS was useful for 83% of the patients, allowing a diagnosis to be confirmed (33%) or excluded (50%). Nevertheless, it is essential to integrate these results with clear diagnostic criteria.²⁸ The absence of detection of clonal hematopoiesis could interestingly help to exclude MDS, hMDS or mixed MPN/MDS but not MPN.

Molecular studies are also interesting to guide treatment decisions, especially through prognostic evaluation. In group B, 33% of the patients had a molecular anomaly with a reported prognostic impact. Treatment was modified for 19%, in most cases with a reinforcement of therapy and/or a decision to perform allografting.

Integration of a search for molecular abnormalities by NGS is thus shown here to have a clear impact on patients' therapeutic management provided that it complements a thorough diagnostic algorithm and multidisciplinary indication. Currently, decisions on therapeutic intensification are rarely based on the integration of molecular data given the lack of international consensus.

These new tools also reveal the presence of VUS for which the somatic or constitutional origin as well as the pathogenicity are still unknown. This may be problematic when such VUS are observed in genes whose mutations carry a prognostic impact. Our data show that in the cases of VUS in *RUNX1*, patients were not ultimately considered as being at high risk. Non-hematopoietic DNA testing and updating of international databases for the interpretation of these VUS are required. Among patients for whom treatment has not been modified, a minority was found to harbor a high-risk mutation and a majority of cases lacked risk-conferring mutations. Again, NGS results cannot be considered individually but should be integrated with the usual IPSS-R markers.²⁹

In summary, we report a benefit in terms of diagnosis and therapeutic impact for, respectively, 83% and 19% of the patients in a large real-life cohort. This outcome is interesting given that decisions to run these tests were made in a critically explored context and not in a systematic fashion. The medico-economic implications of such integrative and multidisciplinary prescription and analysis deserve specific, thorough investigations. The latter would involve a calculation of the savings generated by promptly stopping investigations and alleviating treatment after ruling out a neoplasm, including the reduced costs generated by the decision of not performing an allograft. This should be balanced by the cost of adapted therapy resulting from the positive identification of a neoplastic disease. Such a

large scale microcosting evaluation was not the aim of this study. It can however already be suspected, from the results of this work, that a proper use of relatively expensive NGS assays is largely balanced by an improved and more reasoned therapeutic management of patients.

Disclosures

No conflicts of interest to disclose.

Contributions

PP and LBY conceived the study; VS, PP, BMC and LBY wrote the manuscript; DC, WS, EM, LPD, UV and LBY performed

cytological analyses; GC and TO performed cytogenetic analyses; AM and LBY performed molecular analyses; PP, TS, DV, GT, GA, LBA, DJ, MH, MN, VB, VS, LE, LM, PA, GP, MM, LLR, LCL, MP, LGS and PC prescribed NGS analyses in multidisciplinary concertation and collected data from their patients.

Acknowledgments

The authors would like to thank Laetitia Aubert, Marie-Christine Boursier, Emilie Brangoulo, Cécile Lagarde, Veronique Chenais and Amandine Sebie for their excellent technical help with molecular analyses. We would also like to thank all the members of the HUGO group involved in this work.

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