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Acute myeloid leukemia

***TP53* abnormalities and chromosomal aneuploidy in acute panmyelosis with myelofibrosis**

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To the Editor:

The World Health Organization (WHO) classification of acute myeloid leukemia (AML) has incorporated molecular genetic and cytogenetic aberrations in the definition of most entities [1]. The diagnosis of acute panmyelosis with myelofibrosis (APMF) is still not based on genomic changes but on clinicopathologic features and the exclusion of other myeloid malignancies, in particular AML with myelodysplasia-related changes (AML-MRC) [2]. APMF is a rapidly progressive hyperfibrotic subtype of “AML not otherwise specified” (AML NOS) and accounts for <1% of AML cases [3]. This aggressive disease is characterized by rapid onset of cytopenia and constitutional symptoms in the absence of splenomegaly, previous history of myeloproliferative neoplasm (MPN) or myelodysplastic

syndrome (MDS), and exposure to radiation or cytotoxic drugs. Blood smears are devoid of tear-drop-shaped cells but may contain dysplastic platelets and myeloid cells including rare blasts [2, 3]. Histopathologic evaluation of core bone marrow (BM) biopsies is mandatory to recognize the hallmark of this myeloid neoplasm: “panmyelosis”, i.e. trilineage myeloid proliferation with dysplastic erythropoiesis, abundant atypical megakaryocytes, and overt BM fibrosis \geq grade 2 on a 0–3 scale and \geq 20% predominantly CD34-positive precursors/blasts often arranged in clusters (Fig. 1a–d) [3–5]. As a consequence of dry tap BM aspiration and rare circulating CD34-positive precursors/blasts, genetic data are reported in a limited number of patients. An abnormal karyotype lacking recurrent aberrations was identified in most APMF cases with available cytogenetic information. Published cytogenetic data were similar to AML-MRC including aberrations of chromosome 5q, 7q/7, 8, and 11q [3, 4]. Two other studies detected different chromosomal aberrations involving chromosomes 3q, 5, 12, 13q, and 22 [6, 7]. In a single APMF patient, an amplification of *EV11/MECOM* within a derivative chromosome 8 was described [8]. In another study of four APMF patients no *JAK2* V617F, *MPL*, or *CALR* mutations were detected [9].

The first step of our study was to identify in our files de novo AML patients who presented with relevant fibrosis at initial diagnosis. Among those, we retrospectively re-evaluated cases with available BM biopsies for the presence of clinicopathologic features consistent with the 2016 WHO criteria for APMF. Cases with a previously proven MDS-type cytogenetic profile and prominent dysplasia involving \geq 50% at least in two lineages as mandatory for AML-MRC were excluded. In addition, no cases with megakaryocytic abnormalities, such as arrangement in dense clusters characteristic for primary myelofibrosis (PMF) were considered. By consequently applying WHO criteria, we retrieved a cohort of 16 patients that we could assign to the APMF category (Fig. 1a–d, Table 1, Supplementary Table 1). Briefly, the core biopsies showed BM fibrosis grade 2–3 associated with a generally rather low frequency of CD34-positive myeloblasts (median 30% in the total cohort), a proliferation of predominantly small dysplastic CD61-positive megakaryocytes and a macroblastic glycophorin C-positive and CD71-positive erythropoiesis with increased proerythroblasts. Using routine methods suitable for formalin-fixed paraffin-embedded (FFPE) material *MPL* W515L was absent in 5/5 cases, while 1/5 cases harbored a *JAK2* V617F mutation with a low (5%) mutant allele burden. Karyotyping by metaphase analysis or FISH had been unsuccessful due to BM dry tap and low circulating CD34+ cells. Thus, we performed molecular studies on genomic DNA extracted from the diagnostic FFPE and EDTA-decalcified BM trephine

biopsies by applying the OncoScan™ FFPE assay (Thermo Fisher Scientific, MA, USA) for whole genome tumor profiling [10]. Using stringent settings for gains and losses (Supplementary Materials and Methods) genomic copy number abnormalities (CNAs) were discovered in 94% (15/16) of APMF patients. Based on the number of CNAs we could distinguish two groups within our APMF cohort. Six patients harbored \leq 3 (mean 2) CNAs and were referred to as low genomic complexity group (Fig. 1e, f, Table 1, Supplementary Table 2). The low complexity group showed mostly single but heterogeneous CNAs (Table 1). In contrast, 10 patients were assigned to a high genomic complexity group since $>$ 3 (mean 15) CNAs were identified. As a part of a complex genomic profile, the most frequent CNAs were losses of 17p, 5q, and 7q (in 10, 9, and 9 patients, respectively). Other MDS-related or MPN-related CNAs occurred with lower frequency such as loss of 18p in five patients and losses of 17q, 18q, 11p, and 3p, as well as a gain of 3q in four patients, respectively (Fig. 1e, Table 1, Supplementary Table 2). The gains of chromosome 3q, 8, 12q, 17q, and 21q, as well as loss of 7q were the CNAs common to both groups and have been previously described in APMF [3, 6, 8].

DNA for mutational analysis (Supplementary Information) was only available in 5/16 patients (Table 1). Three of these patients were assigned to the high complexity group while two patients were included in the low complexity group. Molecular profiling revealed *TP53* mutations in all of these five patients. Taken together with the copy number results, 12/16 patients harbored *TP53* abnormalities (i.e. loss of 17p and/or *TP53* mutation) including 3 with inactivation of both *TP53* alleles (Table 1).

Further mutations were detected in the epigenetic regulators *DNMT3A* and *TET2* (in 2/5 samples), signaling molecule *CBL* (1/5 sample), and co-repressor *BCOR* (in 2/5 samples). Four patients did not show *TP53* abnormalities in the OncoScan assay but were not evaluable for *TP53* mutation analysis. Thus, they were considered as “*TP53* unknown”.

As compared with other AML entities, the genetic alterations underlying APMF are not well characterized. An interesting finding that has not yet been described is the presence of *TP53* aberrations in the large majority of APMF patients. In accordance, we frequently observed high levels of genomic complexity suggesting a high genomic instability universally associated with an adverse outcome in AML [11]. Patients of the large high complexity group (10/16 patients) exhibited a genetic profile overlapping with AML MRC. *TP53* alterations are rare in de novo AML (about 8–14%) but are closely associated with the presence of a complex karyotype and/or monosomal karyotype [11, 12]. In a recent study, AML with *TP53* mutations and chromosomal aneuploidies, such as deletions and monosomies of chromosomes 5, 7, 12, and 17,

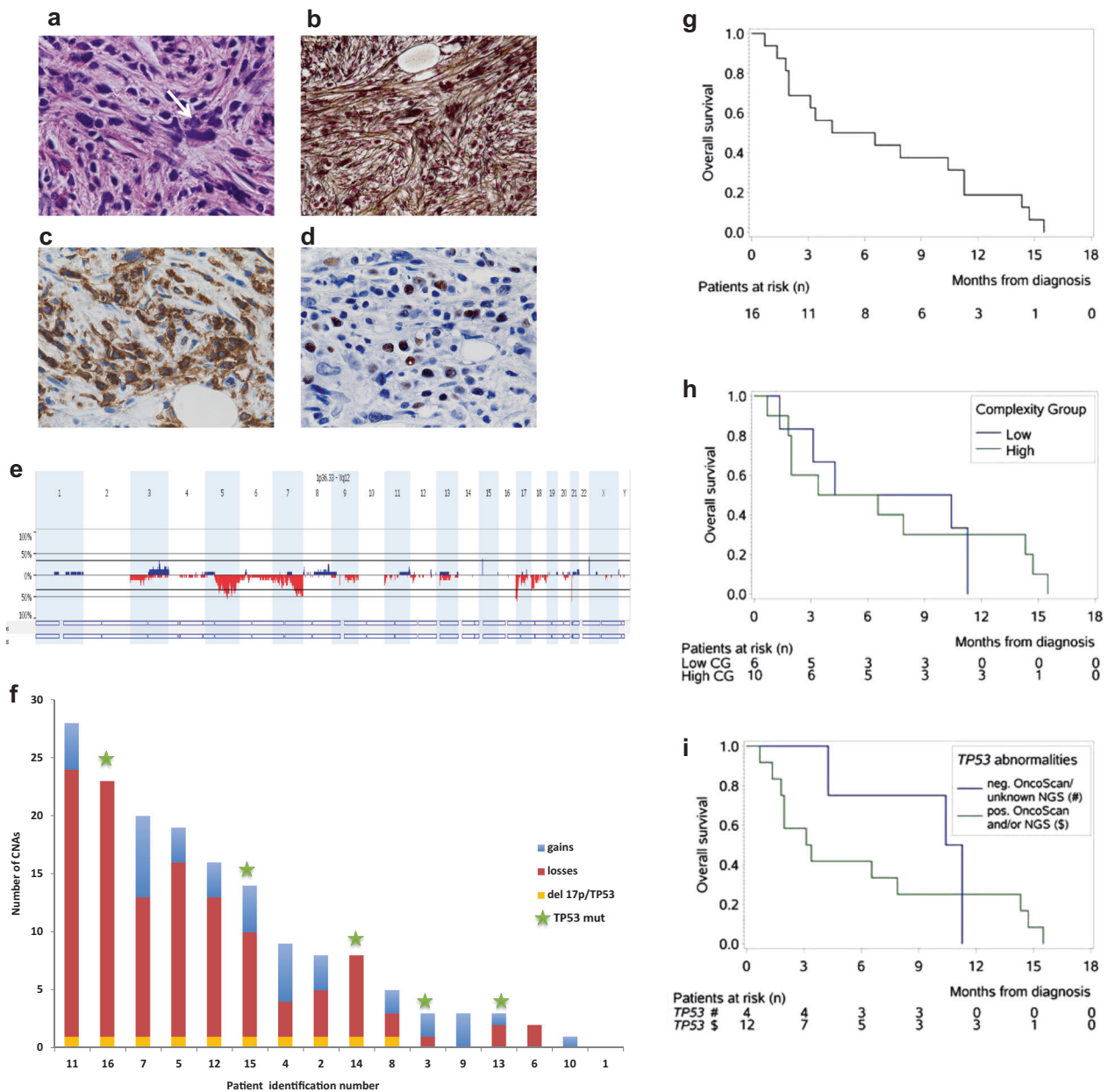


Fig. 1 Representative BM trephine biopsy, summary of the OncoScan copy number analysis and Kaplan–Meier analysis of overall survival. **a–d** BM morphology in a patient with high complexity genetic aberrations: **a** BM spaces are nearly devoid of fat cells and infiltrated by blast cells and residual dysplastic hematopoietic cells including micromegakaryocytes (arrow, hematoxylin and eosin, $\times 63$). **b** Diffuse densely increased reticulin fiber meshwork consistent with BM fibrosis grade 3 (Gomori’s reticulin stain, $\times 63$). **c** Accumulation of CD34-positive blast cells (CD34 immunohistochemistry, $\times 63$). **d** Nuclear overexpression of the p53 protein in about 30% of nucleated cells (p53 immunohistochemistry, $\times 100$). **e** Summary of the OncoScan copy

number analysis showing the type of CNAs per chromosome in all patients using Nexus Express Software for OncoScan, blue color presents copy number gains and red color copy number losses. **f** Summary of the OncoScan copy number analysis showing the number of CNAs per patient. Stars indicate the *TP53* mutations in five patients identified via panel sequencing. **g** Kaplan–Meier analysis of overall survival (OS) of the total cohort. **h** Kaplan–Meier analysis of OS according to genomic complexity group. **i** Kaplan–Meier analysis of OS according to *TP53* status as defined by: (#) no *TP53* aberrations in the OncoScan assay and unknown mutational status by NGS; (\$) *TP53* aberrations in the OncoScan assay and/or *TP53* mutations by NGS

as well as trisomy 8 were considered a distinct category [12]. Patients in this subgroup were older and had the worst overall survival of all AML subgroups [11]. Accordingly, the term “AML with *TP53* mutations and chromosomal aneuploidy” could also be assigned to most of our APMF patients. The

detection of a *JAK2* V617F mutation in one patient does not exclude de novo AML but may suggest a rapidly transformed MPN without a clinically evident chronic phase [13, 14]. MPN patients with *TP53* mutations have a poor prognosis with a high risk of transformation [14]. Very high risk PMF

Table 1 Clinicopathologic and molecular characteristics of patients with APMF

| Patient No. | Sex | Survival (days) | Age (years) | RBC $\times 10^{12}/L$ | Hgb g/dL | WBC $\times 10^9/L$ | Platelets $\times 10^9/L$ | Fibrosis grade | Bone marrow blasts (%) | Molecular analysis | | | Complexity group (No. of CNAs) ^c |
|-------------|-----|-----------------|-------------|------------------------|----------|---------------------|---------------------------|----------------|------------------------|--|----------------------------|---|---|
| | | | | | | | | | | NGS | JAK2/CALR/MPL ^a | OncoScan FFPE assay | |
| 1 | f | 317 | 51 | 2.5 | 8.2 | 1.4 | 31 | 2 | 30 | n.d. | wt./n.d./wt | No aberrations | 1 (0) |
| 2 | f | 60 | 78 | 1.1 | 3.5 | 4.1 | 23 | 2 | 23 | n.d. | n.d./n.d./n.d. | Losses: 5q, 7q, 11p, 17p, 18p, gains: 3 × 11q | 2 (8) |
| 3 | m | 41 | 65 | | 6.2 | 1.1 | 8 | 2 | 20 | <i>TP53</i> Exon 10 splice mutation (TP15) | V617F/n.d./wt | Loss: 7q, 2x gain on 21q | 1 (3) |
| 4 | f | 240 | 67 | 3.15 | 9.9 | 2.0 | 13 | 2 | 22 | n.d. | n.d./n.d./n.d. | Losses: 3p, 5q, 9q, 17p, gains: 3q, 6p, 8p/q ^b , 9p, 12q | 2 (9) |
| 5 | f | 199 | 79 | 3.4 | 10.3 | 2.77 | 15 | 3 | 65 | n.d. | n.d./n.d./n.d. | Losses: 3q, 5q, 5 × 6p, 4 × 7q, 3 × 11p, 12p, 17p/q ^b , gains: 8p/q ^b , 2 × 13q | 2 (19) |
| 6 | m | 343 | 61 | 2.5 | 7.0 | 3.2 | 64 | 2 | 30 | n.d. | n.d./n.d./n.d. | Loss: 2 × 7q | 1 (2) |
| 7 | f | 472 | 68 | 3.1 | 9.6 | 3.2 | 69 | 2 | 35 | n.d. | n.d./n.d./n.d. | Losses: 4q, 2 × 5p, 5q, 7q, 4 × 13q, 17p, 18q, 2 × 20q, gains: 2 × 3q, 5p, 20p 3 × 20q | 2 (20) |
| 8 | m | 103 | 68 | 3.62 | 10.4 | 0.9 | 16 | 2 | 21 | n.d. | n.d./n.d./n.d. | Losses: 7q, 17p, 18p, gains: 3q, 8q, | 2 (5) |
| 9 | f | 343 | 79 | 2.81 | 7.9 | 2.1 | 195 | 3 | 22 | n.d. | n.d./n.d./n.d. | Gains: 8q, 12q, 17q22 | 1 (3) |
| 10 | m | 130 | 83 | 3.74 | 10.8 | 11.3 | 133 | 2 | 23 | n.d. | n.d./n.d./n.d. | Gain: 8p | 1 (1) |
| 11 | f | 21 | 84 | 2.2 | 6.4 | 1.8 | 34 | 3 | 80 | n.d. | wt./n.d./wt | Losses: 2 × 3p, 2 × 3q, 2 × 5q, 2 × 6p, 7p, 7q, 11p, 2 × 11q, 4 × 13q, 17p, 5 × 17q, 18p/q ^b , gains: 6p, 7q, 11q, 17q | 2 (28) |
| 12 | m | 60 | 61 | 3.14 | 9.0 | 1.5 | 51 | 2 | 30 | n.d. | wt./n.d./wt | Losses: 3 × 3p, 5 × 5q, 7p/q ^b , 17p, 2 × 17q, 18p/q ^b , gains: 1p, 1q, 3q | 2 (16) |
| 13 | f | 95 | 56 | 3.46 | 8.5 | 2.6 | 101 | 2 | 60 | <i>TP53</i> Exon 10 splice mutation (TP15) | wt./n.d./wt | Losses: 6q, 13q, gain: 3q | 1 (3) |

Table 1 (continued)

| Patient No. | Sex | Survival (days) | Age (years) | RBC $\times 10^{12}/L$ | Hgb g/dL | WBC $\times 10^9/L$ | Platelets $\times 10^9/L$ | Fibrosis grade | Bone marrow blasts (%) | Molecular analysis | | | Complexity group (No. of CNAs) ^c |
|-------------|-----|-----------------|-------------|------------------------|----------|---------------------|---------------------------|----------------|------------------------|---|----------------------------|---|---|
| | | | | | | | | | | NGS | JAK2/CALR/MPL ^a | OncoScan FFPE assay | |
| 14 | f | 55 | 82 | 2.29 | 7.7 | 3.1 | 28 | 2 | 30 | <i>BCOR</i> Exon 11, <i>CBLB</i> Exon 9, <i>TP53</i> Exon 8 (MP) | n.e./wt/wt | Losses: 5q, 7p, 7q, 2 \times 11p, 12p, 12q, 17p | 2 (8) |
| 15 | m | 436 | 65 | 1.9 | 6.2 | 1.7 | 14 | 2 | 50 | <i>BCOR</i> Exon 4, <i>TET2</i> Exon 6, <i>TP53</i> Exon 7 splice mutation (MP, TP15) | n.e./wt/wt | Losses: 5q, 7p, 3 \times 7q, 8p, 2 \times 9q, 17p, 19p, gains: 8p, 19p, 2 \times 21q | 2 (14) |
| 16 | m | 448 | 66 | 3.88 | 11.4 | 3.1 | 34 | 2 | 21 | <i>TP53</i> Exon 10 splice mutation, <i>DNMT3A</i> Exon 19 (MP) | wt/wt/wt | Losses: 3p, 2 \times 3q, 4 \times 5q, 6p/q ^b , 7p, 3 \times 8q, 9p, 9q, 12p, 3 \times 12q, 17p, 17q, 18p, 18q, 20q | 2 (23) |

f = female, *m* = male, age at initial diagnosis, *RBC* red blood cell count, *Hgb* hemoglobin, *WBC* white blood cell count, *n.d.* not done, *NGS* next generation sequencing, *MP* Illumina TruSight™ Myeloid Panel, *TP15* Illumina TruSight™ Tumor 15 sequencing panel, *n.e.* not evaluable, *wt* wildtype, *wt/wt/wt* number of copy number abnormalities, see supplement

^aMutational status from routine diagnostics or NGS

^bAssumed monosomy/trisomy

^cgenetic complexity group: 1 low complexity, 2 high complexity

overlap at least in loss of 17p with APMF but the most frequent CNAs reported in PMF affect different chromosomal regions: 20q, 17q, 7p, 9p, 13q, or 1q [15]. Therefore, our results could be helpful in distinguishing APMF from PMF.

Despite the molecular heterogeneity, all patients of our APMF cohort had a dismal outcome with a median survival of 5.4 months (range 1.8–11.3 months, Fig. 1g–i, Supplementary Table 1). No patient was treated by allogeneic hematopoietic stem cell transplantation. Three patients received standard induction chemotherapy, one patient thalidomide and another patient decitabine. Statistical analyses comparing low versus high complexity cases or those with known or unknown *TP53* abnormalities did not reveal any significant differences with regard to overall survival and hematologic pretreatment parameters, except with regard to platelet counts and number of CNAs (Fig. 1, Supplementary Table 1). Patients negative for *TP53* abnormalities in the OncoScan assay and unknown *TP53* mutation status had significantly higher platelet counts ($p = 0.045$) and lower numbers of CNAs ($p = 0.006$) than those with *TP53* abnormalities. It is worth noting that the superior survival of 11.3 months was observed in a patient of the high complexity group with a *TP53* exon 10 splice site mutation receiving hypomethylating therapy (decitabine). Interestingly, the frequency of *TP53* splice mutations (4/5, 80%) in our cohort is high, compared to the splice mutation frequency of around 5% in myeloid neoplasms (11/201, see Suppl.) estimated from the IARC *TP53* database. Whether this observation can be confirmed and may even be linked to the fibrotic phenotype needs to be shown in a larger cohort.

The mechanisms that contribute to the typical morphologic and clinical features, and the rapidly progressive disease that entailed the designation as APMF still remain to be elucidated. Previously, we have observed an increased inflammatory T-cell rich background in APMF core biopsies [5]. Inflammatory microenvironment changes of the niche may contribute to genomic instability of hematopoietic stem cells and disease evolution. Cytokines and chemokines released from inflammatory cells and abundant megakaryocytes may contribute to the fibrotic modulation of the BM that is a hallmark of APMF. Although the number of our patients is small and the spectrum of methods that we could apply is limited, the data presented here provide further insights into the molecular basis of APMF and highlight the high prevalence of *TP53* abnormalities and chromosomal aneuploidy.

Author contributions All authors have reviewed and approved the manuscript. MP and DP designed the study, performed the OncoScan FFPE assay, the mutational analysis, the interpretation of results and contributed to manuscript writing. AS-G designed the study, performed the histopathological evaluation, and wrote the initial draft of the manuscript. SK-S retrieved the cohort, collected clinicopathologic, and molecular data, performed immunohistochemical stains and

contributed to the histopathologic evaluation. GI performed the statistical analysis. HB, RZ, and JD contributed with analysis and interpretation of data, reviewed and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Acute myeloid leukemia

Phorbol ester induced ex vivo expansion of rigorously-defined phenotypic but not functional human cord blood hematopoietic stem cells: a cautionary tale demonstrating that phenotype does not always recapitulate stem cell function

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To the Editor:

Hematopoietic stem cells (HSCs) are a rare population residing at the apex of the hematopoietic hierarchy [1]. HSCs have the capacity to self-renew and differentiate into all blood cell types, thus they play a key role in hematopoietic cell transplantation (HCT) [2]. HCT is widely used as a curative therapy for numerous malignant and non-malignant hematological and even non-hematological diseases [3]. The fast developing field of gene editing techniques, including ZFNs, TALENs and CRISPR-Cas9, broaden usage of HCT in clinical therapy of diseases caused by genetic mutations [4]. i.e. β -thalassaemia or Sickle Cell Disease (SCD) may possibly be interrogated by CRISPR based gene editing of β -globin in HSCs from patients [5]. However, efficient gene editing and infusion of the gene edited HSC into patients requires sufficient numbers of donor HSC. In addition, when cord blood (CB) is used as the source of HSC for HCT, rare HSC numbers in single CB units may lead to delayed hematopoietic recovery in recipients [6]. It is thus important in some cases to develop efficient means that can overcome limited HSC numbers to enhance the efficacy of HCT.