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LETTER TO THE EDITOR

# Critical evaluation of current molecular MRD strategies including NGS for the management of AML patients with multiple mutations

## 1 | INTRODUCTION

Current follow-up procedures in patients with acute myeloid leukemia (AML) comprise cytomorphology, multiparameter flow cytometry (MFC), and molecular genetics by polymerase chain reaction (PCR)–based methods, Sanger, and, increasingly, next-generation sequencing (NGS).[1,2] Commercial myeloid NGS panels identify multiple mutations in most AML patients at diagnosis.[1] Before NGS, follow-up strategies using highly sensitive quantitative real-time PCR (qPCR) were limited to a few reciprocal rearrangements or hotspot mutations such as *NPM1*.[3,4] NGS offers follow-up monitoring to virtually all AML patients, including clonal and subclonal changes.[5-7] However, interpretation of NGS MRD results for individual patients remains challenging. Clinicians face uncertainty on how to proceed in patients with persisting mutation load by NGS, how to interpret divergent kinetics of various markers by NGS, and how to weigh the different sensitivities of NGS and qPCR.

## 2 | PATIENTS AND METHODS

We retrospectively evaluated 24 consecutive AML patients with greater than or equal to two mutations at diagnosis (median age, 65 y; 21 AML; three MDS-EB2). Patients received intensive regimens and consolidation with autologous or allogeneic HSCT, or palliative treatment at Bern University Hospital (01/2016-07/2018). Patients had given written informed consent to the scientific analysis of samples and data following approval of the ethics committee Bern, Switzerland. Details on treatment, responses, molecular methods, mutational profiles, and myeloid panel composition are given in Tables 1, S1, S2A,B, S3, and S4A,B.

## 3 | RESULTS

Combined molecular follow-up analysis including NGS provides a more refined assessment of the remission status at best hematologic response.

At the time of best hematologic response, molecular analyses were available in 23 of 24 cases (96%) of the cohort (Table S2A) with best hematologic response being CR (n = 7; 31%), CRi (n = 12; 52%), PR (n = 3; 13%), and one patient (4%) had refractory disease. Three patients achieved CMR (13%), 12 patients PMR (52%), seven (31%) showed molecular persistence, and one patient (4%) had clonal evolution with an emerging *TP53*mut. Comprehensive molecular analysis detected residual disease in four patients (PMR and mutation persistence in two cases each) of seven patients who had achieved hematologic CR. Residual disease was detectable in all 12 patients (100%) who had achieved CRi (PMR: n = 10; mutation persistence: n = 2). The three patients with PR showed mutation persistence (n = 2) or clonal molecular evolution (patient #23) with a new *TP53*mut subtype (n = 1) and an increasing mutational load of two additional previously identified *TP53* mutations. In the latter patient, morphologic evaluation had been challenging due to a hypocellular aspirate.

Non-intensively treated patients were more often observed with discordant dynamics of various molecular markers during follow-up than intensively treated patients.

In the subgroup of the 10 intensively treated patients, discordant mutation kinetics were observed in one patient only (patient #23; 10% of this subgroup), who carried three different *TP53* mutations. At best hematologic response, one of these mutations was increasing, whereas two other mutations were decreasing, and the new *TP53*mut indicated clonal evolution.

In the non-intensively treated patients, four of 11 (36%) patients showed discordant marker dynamics (Table S3). This was caused by persisting *ASXL1*mut in two patients (#4 and #20), probably due to refractoriness of the respective clones. In the third patient (#1), a persisting *DNMT3A*mut was probably reflecting CHIP. The fourth patient (#3) showed a persisting *IDH2*mut, which was either reflecting CHIP or a refractory clone. Thus, these data on a small number of patients suggest that discordant marker kinetics may be more common in patients with palliative treatment as compared to intensively treated patients.

Interpretation of NGS results during follow-up provides helpful information for most patients

The results of NGS at best hematologic response were providing crucial information for clinical guidance in nine patients with intensive treatment (90%). Difficulties of interpretation arose in one patients

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**TABLE 1** Clinical characteristics of the 24 patients in this analysis

| Parameter | All Patients n = 24 |
|---|---|
| **Gender, n (%)** | |
| Female | 6 (25%) |
| Male | 18 (75%) |
| Age, y (range) | 65 (30-85) |
| AML | n = 21 |
| Bone marrow infiltration, median (%), (range) | 50% (20-90%) |
| Origin of the AML | |
| *de novo* AML | 11 (52%) |
| s-AML | 8 (38%) |
| t-AML | 2 (10%) |
| FAB subtypes | |
| M1 | 4 (19%) |
| M2 | 5 (24%) |
| M4 | 5 (24%) |
| M5 | 3 (14%) |
| Missing | 4 (19%) |
| WHO subtypes (2016) | |
| AML with myelodysplasia-related changes | 8 (38%) |
| Therapy related neoplasms | 2 (10%) |
| AML with mutated *NPM1* | 7 (33%) |
| AML, NOS | 2 (9%) |
| AML with t(6;9)(p23;q34.1); *DEK-NUP214* | 1 (5%) |
| AML with *BCR-ABL1* | 1 (5%) |
| Genetic risk groups (ELN 2017) | |
| Favorable | 6 (29%) |
| Intermediate | 4 (19%) |
| Adverse | 11 (52%) |
| MDS | n = 3 |
| MDS-EB2 | 3 |
| Genetic risk group, IPSS-R | |
| Good | 2 |
| Very poor | 1 |
| Cytogenetic aberrations | n=24 |
| Normal karyotype | 14 (59%) |
| Complex karyotype (≥3 aberrations) | 3 (13%) |
| Independent clones | 1 (4%) |
| Chromosome 7 aberration | 1 (4%) |
| Trisomy 8 | 1 (4%) |
| *t*(6;9)/*DEK-NUP214* | 1 (4%) |
| inv(9)(p11q13) | 1 (4%) |
| del(20q) | 1 (4%) |
| Missing | 1 (4%) |
| Frequent mutations | n=24 |
| *NPM1* | 9 (37%) |
| *ASXL1* | 9 (37%) |
| *DNMT3A* | 6 (25%) |
| *IDH2* | 6 (25%) |
| *RUNX1* | 5 (21%) |
| *FLT3*-ITD | 4 (17%) |
| *SRSF2* | 3 (13%) |
| *CEBPA* | 3 (13%) |
| *IDH1* | 3 (13%) |
| *TET2* | 3 (13%) |
| *EZH2* | 2 (8%) |
| *NRAS* | 2 (8%) |
| *SF3B1* | 2 (8%) |
| *TP53* | 2 (8%) |
| *BCR-ABL1*, *CBL*, *FLT3*-TKD, *JAK2*, *KRAS*, *MPL*, *SH2B3*, *U2AF1*, *WT1*, *ZRSR2* | 1 (4%) each |

Abbreviations: AML, acute myeloid leukemia; AML, NOS, not otherwise specified; del., deletion; FAB, French-American-British classification system; inv., inversion; MDS-EB2, myelodysplastic syndrome with excess blasts 2; NOS: not otherwise specified; s-AML, secondary AML; t., translocation; t-AML, therapy-related AML.

(#9; 10%). Due to a high *EZH2* (100%) and *RUNX1* (54%) mutational load at diagnosis a germline origin needed to be excluded by saliva analysis. In the non-intensively treated patients, discordant mutation kinetics challenged the interpretation in four of 11 (36%) patients (Table S3).

Molecular MRD analyses at follow-up are predictive for the final remission status.

Table S2B provides the hematologic and molecular remission status in all 21 patients with available molecular MRD results at the last follow-up after a median follow-up of 8 months since diagnosis (range, 1-29). In the patients with intensive therapies, six of 10 (60%) patients were in hematologic CR, and four of 10 (40%) showed refractory/relapsed disease. Three patients achieved CMR, three PMR, and four had molecular progression. Molecular follow-up investigations were predictive for the remission status at the last follow-up in eight of 10 (80%). In four patients, CMR (n = 3) or PMR (n = 1) was followed by hematologic CR with intervals between 1 and 14 months after molecular response was documented. In the remaining four patients, hematologic relapse/progression was preceded by PMR or clonal evolution (#23). In two of 10 patients, the molecular remission status was not predictive or could not be evaluated.

In the non-intensive group, molecular follow-up was predictive for the remission status in eight of 11 (73%) patients with available results. CMR (n = 2) and PMR (n = 1) were predictive for hematologic CR at the last follow-up in three patients, and CRi was preceded by PMR in one patient. The interval from molecular response to the achievement of CR or CRi in these four patients

ranged between 1 and 5 months. Four patients with molecular persistence/progression showed hematologic relapse/refractory disease at the last follow-up. In contrast, in the remaining three patients (27%), the final remission status was not clearly predictable by the molecular response (probably due to CHIP in two cases).

All five patients with hematologic remission who relapsed during subsequent follow-up had achieved previous MRD positivity. In the sixth patient, pre-emptive allogeneic SCT following molecular relapse by qPCR prohibited overt hematologic relapse. In summary, molecular follow-up assessments including NGS correlated with the remission status in 16 of 21 (76%) patients considering both intensively and non-intensively treated groups.

Comprehensive molecular follow-up analyses allow an early modification of therapeutic strategies.

Three patients (#8, #10, and #20) received enasidenib for IDH2 inhibition during follow-up, being successful in two patients, by bridging to allo-HSCT in an intensively treated patient (#10) and inducing CR after azacitidine failure in a second patient (#20). FLT3 inhibition during follow-up was successful in one of three patients; this patient (#15) with *FLT3*-ITD, *KRAS* and *NRAS*mut AML developed azacitidine-refractory molecular relapse after allo-HSCT. Midostaurin combined with DLI and continued azacytidine therapy induced durable CMR and hematologic CR. In summary, molecular analysis during follow-up was helpful to trigger targeted treatment in six of 24 patients (25%).

## 4 | DISCUSSION

This report suggests that response assessment combining cytomorphology with comprehensive molecular MRD results provides significant additional information. Molecular follow-up analysis identified residual disease in four of seven patients with CR and in all 12 patients with CRi at best hematologic response. In one patient with MDS-EB2 and unclear cytomorphologic response, NGS detected an increasing mutation load of one of the previously known *TP53*mut and additionally clonal evolution with a new *TP53*mut emerging after induction therapy.

In 76% of the patients without clinical relapse at last follow-up and in all patients with refractory/relapsing disease, molecular MRD studies including NGS allowed earlier prediction of impending relapse. Molecular MRD guidance triggered allocation of six patients to IDH2 or FLT3 inhibitors during follow-up, and one patient was admitted for pre-emptive allogeneic SCT.

Discordant dynamics of various molecular markers were more frequent in patients with non-intensive therapies than in intensively treated patients. Single mutation persistence was observed in 36% of the patients in the non-intensive therapy group indicating either CHIP or refractory clones. In the above patient from the intensive group, a new *TP53*mut was emerging after induction therapy, while the mutation load was growing within another previous clone and declining within the other two earlier known *TP53*mut. Therefore, a combined follow-up of various mutations by NGS seems to be justified.

In conclusion, NGS allows follow-up monitoring in virtually all AML patients.[8,9] Considering the possibility of discordant dynamics of simultaneous mutations, MRD strategies should include more than one mutation, if available in a given patient. Prospective multicenter studies should aim to define the most appropriate time points and thresholds for the different genes/hotspots investigated by NGS. The effects of targeted compounds need to be studied using NGS follow-up assessments considering also interclonal developments under treatment.

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## CONFLICT OF INTEREST

The authors report no relevant conflicts of interest.

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