Cytogenetically Normal Acute Myeloid Leukaemia at a single centre in South Africa

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Nicholas Jenkins, Lee-Ann Blanshard, Marian Stone, Estelle Verburgh, Jenna Oosthuizen, Karen Shires

Abstract

Background and objectives: The heterogeneous molecular landscape of cytogenetically normal acute myeloid leukemia (CN-AML) renders it an ongoing therapeutic challenge. The European LeukemiaNet (ELN) 2017 guidelines attempted to address this by guiding post-remission therapy according to six prognostically informative mutations. However, its applicability in a South African setting remains unclear due to limited local data. This retrospective study aimed to describe a South African CN-AML cohort according to clinicopathological and molecular features as well as treatment outcomes and, consequently, to investigate the local applicability of a triple-mutation testing approach for risk stratification in accordance with the ELN 2017 guidelines, using nucleophosmin 1 (NPM1), fms-related receptor tyrosine kinase 3 internal tandem duplication (FLT3-ITD), and CCAAT enhancer-binding protein alpha (CEBPA) mutation status.

Materials and methods: A review of cytogenetic results for adult de novo AML cases diagnosed at Groote Schuur Hospital between 2005 and 2018 was performed. CN-AML cases were further characterized via a review of clinical and laboratory data and additional molecular testing on stored DNA samples to allow for mutation-based risk stratification and outcome analysis.

Results: In total, 218 patients with AML were identified, of which 33% were cytogenetically normal. NPM1, FLT3-ITD, and CEBPA mutations were found in 39%, 34%, and 9% of CN-AML cases, respectively. Retrospective risk stratification according to mutations in these three genes accurately identified both patients at a high risk of induction-resistant disease and those who required an allogeneic stem cell transplant in their first complete remission.

Conclusion: Local rates of CN-AML and associated NPM1 and FLT3-ITD mutations were comparable to those of European cohorts. Limited mutation analysis in the form of triple-mutation testing proved to be an economical and therapeutically informative prognostication approach for CN-AML in a resource-limited setting.

Keywords: AML, CN-AML, NPM1, FLT3, Prognostication

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease in terms of its clinicopathological, genetic, and prognostic features. The utility of cytogenetic techniques (i.e., conventional karyotyping and fluorescence in situ hybridization (FISH)) for both classifying and prognosticating AML culminated in the development of both the World Health Organization (WHO) 2008 AML classification scheme [1] and the 2010 revised Medical Research Council (MRC) prognostication system [2]. The MRC-2010 stratified patients according to cytogenetic findings into three broad groups, namely favorable, intermediate, and adverse [2]. Prognostication ultimately allowed for a standardized approach to the choice of post-remission treatment based on the anticipated risk of relapse [3].
Unfortunately, cases without an identifiable cytogenetic abnormality, known as cytogenetically normal AML (CN-AML), remained a challenge, as they represented a large AML category [4,5] showing biological and prognostic heterogeneity [6]. Fortunately, the identification of certain gene-specific mutations assisted with further subclassification and risk stratification of these cases. The significance of these findings is highlighted by the formal incorporation of certain gene-specific mutations into the WHO 2016 AML classification scheme [7] and European LeukemiaNet (ELN) 2010 [3]/2017 [8] AML risk stratification systems. Mutations in the following genes are currently recognized as having prognostic significance in CN-AML [7,8]: nucleophosmin 1 (NPM1), fms-related receptor tyrosine kinase 3 internal tandem duplication (FLT3-ITD), CCAAT enhancer-binding protein alpha (CEBPA), runt-related transcription factor 1 (RUNX1), additional sex combs like 1 (ASXL1), and tumor protein p53 (TP53).

South African (SA) data describing AML and particularly CN-AML is exceedingly limited; however, the findings from the largest SA adult AML study to date [9] suggest significantly lower frequencies of CN-AML, as well as the associated NPM1 and FLT3-ITD mutations, compared to those of European cohorts [6,10,11]. In this retrospective study, we aimed to comprehensively investigate this potential discrepancy by describing an adult CN-AML population at a tertiary state hospital (Groote Schuur Hospital; GSH) in the Western Cape of SA. We wanted to not only characterize this population according to standard AML clinicopathological features but also establish the local relevance of genetic testing in this AML sub-group by reporting mutational frequencies (for NPM1, FLT3-ITD, and CEBPA mutations), as well as potential difficulties associated with the cytogenetic diagnosis and molecular prognostication of CN-AML in an SA setting. Finally, we also aimed to investigate the basic clinical outcomes of the CN-AML cohort to establish the success of post-remission strategies and determine the exigency of a more up-to-date risk stratification system (in line with the ELN 2017 system) [8] within a resource-limited hospital setting, such as those found in SA and many other developing countries.

2. Materials and methods

2.1. Patient cohort

All adults with newly diagnosed de novo AML (according to WHO criteria) [1, 7], who presented to GSH from Western Cape healthcare centers between 01/01/2005 and 31/12/2018 were included in this study. Based on cytogenetic (karyotype and/or FISH) findings, these patients were divided into three sub-groups: ‘clonal cytogenetic abnormalities’, ‘CN-AML’, and ‘cytogenetically unknown’. A retrospective review of laboratory reports and clinical folders was then performed in order to gather all clinicopathological findings on the patients with CN-AML throughout the course of their follow-up. Ethical approval for retrospective analysis was obtained from the University of Cape Town Health Faculty Human Research Ethics committee (HREC. 110/2019).

At GSH, treatment-eligible patients with CN-AML received an induction regimen consisting of ‘3 + 7’ with etoposide over the study period. All patients with CN-AML eligible for post-remission therapy were worked up for a human leukocyte antigen (HLA)-matched allogeneic (allo) stem cell transplant (SCT) in their first complete remission (CR1). Those without an HLA-matched donor were offered alternative post-remission treatments such as high-dose cytosine arabinoside (HDAC) consolidation or an autologous (auto) SCT. Auto-SCT was employed during periods of bed shortages within the GSH Hematology unit, as it generally requires a shorter cumulative hospital admission compared to HDAC consolidation [12]. HDAC consolidation was employed when patients failed to mobilize during stem cell collection for auto-SCT. It is important to note that patients with CN-AML in this cohort were not managed according to their mutation profiles.

The clinical endpoints in this study included induction response and overall survival (OS). ELN 2017 response criteria [8] were used to define induction outcomes. A morphological leukemia-free state at day 28 post-induction assessment was considered a successful induction response. Unsuccessful induction responses were classified as either induction death (death related to treatment and/or hypoplasia within 30 days of induction) or resistant disease (failure to eliminate disease, including partial remissions). OS was calculated from the date of the diagnostic bone marrow (BM) biopsy to the date of death. For patients without definitive dates of death who were not being treated with curative intent, the last day of their medical follow-up was used as the date of death. Patients in remission but lost to follow-up were censored on the last day of their medical follow-up. A comparative review of patient folders, laboratory results, and electronic hospital clerking data was used to determine the most accurate last date of medical follow-up for all relevant patients. Surviving patients were censored on 31/12/2019.
2.2. Genetic analysis

2.2.1. Cytogenetics

Diagnostic G-banded karyotyping and FISH were conducted at the National Health Laboratory Service/GSH (NHLS/GSH) using accredited methods. Karyotyping was performed on cultured BM samples in accordance with standards stipulated by the Association for Clinical Cytogenetics [13] and reported in agreement with the International System for Human Cytogenetic Nomenclature criteria [14]. At the time of diagnosis, the specific choice of FISH probes was determined on a case-by-case capacity, guided by morphology and/or immunophenotyping, and included testing for the following gene rearrangements, where appropriate: RUNX1/ RUNXIT1, CBFβ/MYH11, BCR/ABL1, PML/RARα, and KMT2A. CN-AML was defined as the absence of any karyotypic aberration in ≥10 fully analyzed metaphases with no accompanying evidence of an abnormal FISH result.

2.2.2. Molecular analysis

DNA from the CN-AML patient cohort was either extracted from stored unstained BM aspirate slides using the mini-Qiagen mini-blood extraction kit (Qiagen, Maryland USA) or retrieved from archived DNA samples, extracted from diagnostic EDTA BM aspirate using the Maxwell 16 System (Promega, Madison, WI, USA). Only DNA samples that yielded >25 ng/μL with 260:280 nm ratios of >1.7 were used for mutation analysis.

For the identified patients with CN-AML, NPM1, FLT3-ITD, and CEBPα mutation analysis (triplet-mutation testing) was performed on DNA extracted from diagnostic samples, where possible. All available samples were tested for NPM1 exon 12 mutations and FLT3-ITD mutations in exons 14 and 15. These assays were performed using a PCR/capillary electrophoresis methodology capable of detecting all mutation types associated with an overall 4 bp insertion in NPM1 (mutation types A–D) and FLT3-ITDs of 3–300 bp. The FLT3-ITD allelic ratio (AR) was calculated as the ratio of the area under the curve of mutant to wild-type alleles (FLT3-ITD/FLT3wt) [8]. Testing was performed by the NHLS/GSH laboratory using validated/accredited in-house methodology, with both assays having a limit of detection of 5% mutated alleles.

CEBPα mutation analysis was subsequently performed only on cases found to be negative for both NPM1 and FLT3-ITD mutations, as the CEBPα mutation status of this specific CN-AML sub-group has been shown to be of prognostic relevance [8,15]. Testing was performed according to the PCR/Sanger sequencing methodology described by Behdad et al. [16], using the recommended reagents and PCR conditions specifically for the GC-rich amplicons. The entire CEBPα coding region was analyzed and compared to the National Center for Biotechnology Information reference genomic sequence (NG_012022.1) to identify any mutations. The assay was found to reliably detect CEBPα mutations to a sensitivity of 20% allelic load, with peak heights being used to determine single (sm) and double (dm) mutants, where appropriate.

2.2.3. Risk stratification

Following molecular mutation analysis, genetic risk stratification of patients was performed in accordance with ELN 2017 guidelines [8]. As mutation analysis of RUNX1, ASXL1, and TP53 genes was not performed, we categorized our CN-AML cohort into two risk groups, favorable and non-favorable, where the non-favorable group represented a combination of both the intermediate and adverse risk categories from the comprehensive ELN 2017 genetic stratification system [8].

2.3. Statistical analysis

Descriptive statistics, including proportions and medians, were used to summarize the distribution of variables within sub-groups. OS curves were calculated using the Kaplan–Meier survival function and compared by log-rank test. Fisher’s exact test was used to compare categorical data. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Demographic, clinical, and cytogenetic characteristics

A total of 218 patients with newly diagnosed de novo AML were identified during the 13-year assessment period (2005–2018). Of them, 154 (71%) had a successful diagnostic karyotype available and 64 (29%) did not, due to either failed karyotyping (25%, n = 50/204) or unrequested karyotyping (6%, n = 14). Further review of all additional FISH results identified 114 patients (52%) with ‘clonal cytogenetic abnormalities’, 56 patients who were ‘cytogenetically normal’ (26%), and 48 patients (22%) who were ‘cytogenetically unknown’ due to either failed karyotyping (with/without additional negative FISH results) (17%, n = 36) or no cytogenetic testing (karyotyping or FISH) requested at diagnosis (5%, n = 12).

The notable proportion of ‘cytogenetically unknown’ cases was investigated further. The only
reason documented for ‘failed karyotyping’ was ‘insufficient’ or ‘no metaphases’ being cultured due to either poor sampling (i.e., hemodiluted specimen) or a hypoproliferative specimen. Meanwhile, reasons for ‘unrequested cytogenetic testing’ (karyotyping and FISH) included: inadequate BM aspirate sample collection (dry tap) (n = 3), therapeutic insignificance due to advanced patient age (n = 5), early death (n = 1), and unknown reasons (n = 3). By excluding the ‘cytogenetically unknown’ cases, a final CN-AML frequency of 33% (n = 56/170) was identified. This is the patient cohort that was analyzed further.

Table 1 demonstrates baseline characteristics for the entire CN-AML cohort. A median age of 50 years was found (44 years for patients <60 years at diagnosis (n = 39)), with no gender dominance.

3.2. Risk stratification

3.2.1. Molecular analysis

Suitable DNA was available for molecular assessment in 44 patients (78.6%) in the CN-AML cohort. These samples were assessed for the presence of NPM1 exon 12, FLT3-ITD, and CEBPα mutations according to the testing pathway illustrated in Fig. 1A. NPM1, FLT3-ITD, and CEBPα mutations were found in 39% (17/44), 34% (15/44), and 27% (6/22) of tested cases, respectively. The distribution of these clinically significant mutations is also summarized in Fig. 1A. The majority of NPM1-positive patients (71%) had co-occurring FLT3-ITD mutations, with a third (29%) having the favorable prognostic effect of the NPM1 mutations modified by a FLT3-ITD (AR >0.5) mutation. Most of the patients negative for both NPM1 and FLT3-ITD mutations with successful CEBPα mutation analysis (n = 22) were wild-type for CEBPα sequence variants (73%), with a clinically significant double (or bi-allelic) mutation found in 2/22 patients (9%).

3.2.2. ELN risk stratification

Triple mutation testing was successful in 42/44 of the patients with available DNA and allowed us to unequivocally risk stratify 50% of these CN-AML patients according to the ELN 2017 guidelines [8] (Fig. 1B). Favorable risk genotypes were found in 33% of patients, with only two patients (4.7%) falling definitively into the adverse prognostic group. According to the ELN 2017 guidelines, patients with CN-AML that are ‘NPM1wt/FLT3-ITD-negative/CEBPαwt (or sm)’ or ‘NPM1wt/FLT3-ITD (low AR)’ require additional testing for adverse risk mutations of the ASXL1, TP53, and RUNX1 (non-translocating mutations) genes in order to be definitively risk-stratified. These adverse risk mutations were not investigated in this study due to insufficient residual DNA. We were, therefore, unable to be certain that this group (n = 21/42) did not contain a few additional adverse risk mutations. Risk stratification using a simplified approach (Fig. 1B) that effectively combined the intermediate and adverse risk groups into a single ‘non-favorable risk’ group was employed. This produced larger sub-groups that allowed for further analysis of clinical outcomes.

3.3. Treatment and survival analyses

3.3.1. Treatment response

Forty-one percent (23/56) of all patients with CN-AML received supportive treatment only, with the main reason being advanced age at diagnosis (≥60 years; n = 17/23). Of the 58.9% (33/56) who were intensively treated, only a third (36.4%) achieved a successful induction treatment response. It was found that approximately half (47.6%) of all patients who failed induction therapy died during the induction phase and an almost equal proportion (52.4%) had resistant disease following the induction phase. Table 2 highlights the outcomes for the two previously defined genetic risk categories (favorable and non-favorable) (n = 42). A statistically significant difference in successful induction responses, induction deaths, and resistant disease rates was seen between these two retrospectively stratified categories (p = 0.005) (Table 2).
Fig. 1. Molecular testing and prognostication approach in the CN-AML cohort. (A) Details of how the patients with AML were initially grouped and then sequentially tested for NPM1, FLT3-ITD, and CEBPA mutations, depending upon the availability of stored DNA. A schematic summary of the co-existing mutations within the CN-AML group is supplied. (B) Subsequent molecular risk stratification of the CN-AML patients based on their NPM1, FLT3-ITD, and CEBPA mutation status according to the ELN 2017 guidelines [8] and our simplified combined intermediate/adverse risk approach. AML = acute myeloid leukemia; AR = allelic ratio; CEBPA = CCAAT enhancer-binding protein alpha gene; CN-AML = cytogenetically normal acute myeloid leukemia; dm = double mutant; ELN = European LeukemiaNet; FLT3-ITD = fms-related receptor tyrosine kinase 3 gene, internal tandem duplication; NPM1 = nucleophosmin 1 gene; sm = single mutant; wt = wild-type.
3.3.2. Survival analysis

For the entire CN-AML cohort \((n = 56)\), the median follow-up time was 239 days and the median OS was 77 days. Fig. 2A and B, respectively, show Kaplan–Meier survival estimates for the intensively treated patients as a whole \((n = 33)\) and according to the risk groups (prognosticated patients only; \(n = 29)\). The 1- and 2-year OS rates for all intensively treated patients were 36.4% (95% CI: 20.6–52.3) and 24.2% (95% CI: 11.4–39.6), respectively. No significant difference in OS was found between the two risk groups \((p = 0.114)\).

4. Discussion

CN-AML remains a particular therapeutic challenge, as it not only lacks a common molecular signature but also represents a large AML subgroup \([4,6]\) with heterogeneous clinical outcomes \([15]\). However, the prognostic importance of specific molecular mutations in CN-AML has come to light and begun to address this issue by informing the application of allo-SCTs in a risk-adapted manner \([8]\). Unfortunately, the applicability of a molecular-based stratification system for CN-AML in SA remains elusive due, primarily, to exceedingly limited and seemingly divergent local CN-AML data reported by Marshall et al. \([9]\) compared to larger European cohorts \([6,10,11]\) and, secondarily, to the limited availability of comprehensive molecular testing that is required for this stratification. With the intention of better understanding our local CN-AML population and also establishing institutional guidelines (diagnostic and therapeutic), this study aimed to describe a local CN-AML population according to demographic, clinicopathological, and molecular features as well as basic treatment outcomes and, consequently, determine the local applicability of a triple-mutation testing \((NPM1, FLT3-ITD, \text{and } CEBPα \text{ mutations})\) approach, in line with the ELN 2017 genetic prognostication system \([8]\).

The cytogenetic classification of an AML cohort is dependent upon the availability of diagnostic cytogenetic test results, particularly karyotypes. A much higher local rate of unknown diagnostic AML karyotypes (29%) was found compared to those reported in large European cohorts (approximately 16%) \([4,17]\). Additionally, the rate of failed diagnostic AML karyotyping (25%) was more than double that reported by Fischer et al. \([18]\), Grimwade et al. \([2]\), and Löwenberg et al. \((±10%)\) \([19]\). The main reasons for the lack of a karyotype at diagnosis appeared to be the lack of clear clinical and pathological guidelines (over the entire study period) regarding when and how to provide quality samples for cytogenetic analysis \([20]\). This was qualified by a reanalysis of the karyotyping data comparing an early sub-cohort \((2005–2013, n = 133)\) to a more contemporary sub-cohort \((2014–2018)\), which showed almost identical karyotyping failure rates (25.0% vs. 24.4%, respectively) despite major improvements in the actual cytogenetic testing methods over the same periods. This audit has thus highlighted the urgent requirement for the development of formal standard operating procedures (SOPs) for the collection of mandatory diagnostic samples from suspected AML patients, and we thus hope that this will improve AML management moving forward. Similarly, the notable number of CN-AML cases without available diagnostic DNA (21.4%) is troubling, given both the prognostic and treatment implications of these mutations. These mandatory

<table>
<thead>
<tr>
<th>Key treatment points</th>
<th>Favorable risk (no. (%))</th>
<th>Non-favorable risk (no. (%))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial treatment</strong></td>
<td>(n = 14)</td>
<td>(n = 28)</td>
<td>0.019</td>
</tr>
<tr>
<td>Supportive treatment only</td>
<td>3 (21.4%)</td>
<td>10 (35.7%)</td>
<td>0.019</td>
</tr>
<tr>
<td>Induction treatment</td>
<td>11 (78.6%)</td>
<td>18 (64.3%)</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>Induction treatment response (Day 28)</strong></td>
<td>(n = 11)</td>
<td>(n = 18)</td>
<td>0.005</td>
</tr>
<tr>
<td>Induction death</td>
<td>5 (45.5%)</td>
<td>3 (16.7%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Refractory disease</td>
<td>0</td>
<td>11 (61.1%)</td>
<td>0.005</td>
</tr>
<tr>
<td>CR1 achieved</td>
<td>6 (54.6%)</td>
<td>4 (22.2%)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Post-remission strategy in CR1</strong></td>
<td>(n = 6)</td>
<td>(n = 4)</td>
<td></td>
</tr>
<tr>
<td>HDAC</td>
<td>2 successful – both in CR on 31/12/19</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Allo-SCT</td>
<td>1 successful and in CR on 31/12/19</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Auto-SCT</td>
<td>1 relapsed and reached CR2</td>
<td>1 successful and in CR on 31/12/19</td>
<td></td>
</tr>
<tr>
<td>CR2</td>
<td>1 relapsed and died</td>
<td>3 relapsed and died</td>
<td></td>
</tr>
<tr>
<td><strong>Post-remission strategy in CR2</strong></td>
<td>(n = 2)</td>
<td>(n = 0)</td>
<td></td>
</tr>
<tr>
<td>Haplo-identical SCT</td>
<td>2 successful – both in CR on 31/12/19</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

Note. HDAC = high-dose cytosine arabinoside.
samples are now also being incorporated into local SOPs, and we hope that this has a positive impact on patient outcomes in the future.

No significant differences in median age, sex, and cell counts (peripheral blood and BM-derived) were observed between our CN-AML cohort and other international cohorts \[6,15,21,22\]. However, noteworthy variances in FAB findings were demonstrated, with higher rates of M0 and M1 cases and lower rates of M5 cases found compared to

Fig. 2. Kaplan–Meier survival estimates for the intensively treated patients with CN-AML. (A) Survival curve for all intensively treated patients with CN-AML in the cohort (n = 33). (B) Comparative analysis of the survival curves of the favorable risk (blue curve) (n = 11) versus non-favorable risk (red curve) (n = 18) stratified patients. A p-value of 0.114 was determined between the two risk groups. CN-AML = cytogenetically normal acute myeloid leukemia.
international CN-AML cohorts [6,21–23]. These dissimilarities may be attributed to differences in population ethnicity, which deserves? further investigation, and may also reflect subtle differences in laboratory diagnostic methodology and cytomorphological judgment [24–29].

The frequency of CN-AML in our local cohort (33% of AML cases) was slightly lower compared to European findings, especially those of Grimwade et al. (41%) [2] and Schlenk et al. (45%) [6]. The slightly lower observed rate of CN-AML may be explained by a difference in local AML driver mutation frequencies, a finding which was reported by Blanshard et al. [30], who found marginally higher rates of t(8;21) (q22; q22) and t(15;17) (q24; q21) re-arrangements compared to others [2,31]. Additionally, as was proposed by Marshall et al. [9], the comparatively higher proportion of non-Caucasian patients in the SA healthcare system [32,33] may have contributed to this, a hypothesis supported by Sekeres et al. [34], who found lower frequencies of CN-AML in African-American patients compared to Caucasian American patients. The observed frequencies of NPM1 and FLT3-ITD mutations in our cohort, at 39% and 34%, respectively, were akin to findings from numerous large European CN-AML cohorts (approximately 50% and 31% respectively) [6,10,11,15]. Unexpectedly, the rates of CN-AML and associated NPM1 and FLT3-ITD mutations in our cohort were almost double those reported by Marshall et al. (which were 18%, 23.5%, and 17.6%, respectively) [9]. This may be attributed to the almost three-fold difference between our cohort sizes, with our larger cohort probably giving a more accurate indication of these rates in this disease group. Comparing the rate of NPM1 (sm and dm) mutations found in our cohort to those in European CN-AML cohorts [15,35–37] would be misleading, primarily because of the more targeted approach by which we performed NPM1 mutation testing.

The notable prevalence of these three prognostically relevant mutations (NPM1, FLT3-ITD, and CEBPα) in our CN-AML cohort indicated their potential local use in risk stratification. However, prevalence alone does not justify the testing cost; instead, the expense must be justified by the ability of these mutations to inform therapy in an evidence-based manner. Post-remission strategies in eligible patients, including HDAC consolidation, auto-SCT, and allo-SCT, were employed to good effect in the patients who we retrospectively stratified as ‘favorable risk’, supporting the utility of the triple-mutation testing approach for this sub-group. However, the high rate of induction deaths we observed in the ‘favorable risk’ patients is concerning and may be due to delayed presentation, poor performance status, co-morbidities, and low socio-economic status amongst SA state patients.

In contrast, the induction and post-remission strategies in the patients retrospectively assigned as ‘non-favorable risk’ were mostly unsuccessful, with only one of the 18 treated patients achieving a long-term remission. This was due to high rates of resistant disease and a lack in the availability of HLA-matched donors. Nonetheless, the fact that the triple-mutation testing approach was retrospectively capable of clearly identifying patients at a high risk of resistant disease supports its utility in the development of novel induction strategies for this group. The finding that three-quarters of these patients who were managed with an auto-SCT strategy had unfavorable outcomes highlights the need for upfront allo-SCT in CR1 for non-favorable risk disease. Unfortunately, GSH is still the sole publicly funded center in sub-Saharan Africa offering a full SCT program. Moreover, HLA-matched donor options are hard to come by for SA patients due to two factors: restricted sibling donor availability due to the HIV epidemic, and restricted unrelated donor availability due to both an underdeveloped African stem cell registry and the profound ethnic disparity in HLA within the world marrow donor registries [38]. Haplo-identical (haplo) SCT is now considered as a viable post-remission therapy for both patients with intermediate and adverse risk AML without an HLA-matched donor [39], and since 2016, has been implemented in the GSH unit as an alternative post-remission treatment option for these two AML risk groups. We recently confirmed the feasibility of unmanipulated haplo-SCT as a valid treatment strategy for adults with acute leukemia lacking a suitable unrelated donor [40]; two patients in this CN-AML cohort were successfully salvaged in CR2 using this strategy.

Despite the clear utility of the triple-mutation testing approach, which was retrospectively apparent in our cohort, it is also important to discuss the large proportion (50%; n = 21/42) of our cohort who remained inconclusively stratified (either intermediate or adverse risk) using the triple-mutation testing approach. As per ELN 2017 guidelines [8], these patients require additional mutation analysis for adverse risk mutations (RUNXI, ASXL1, and TP53 specifically) in order to be conclusively stratified as either intermediate or adverse risk. The biological complexity of mutations in these three genes (particularly RUNXI and TP53) means that modern molecular methods such as next-generation sequencing (NGS) are more suited to their analysis. However, the accessibility and expense of such
technology remains the primary obstacle to its comprehensive introduction in a local state healthcare setting. Furthermore, investigation for these mutations would currently be regarded as academic, as the differentiation between intermediate and adverse risk disease would not influence the choice of post-remission treatment strategy for patients with stem cell donors who are being treated with curative intent (both risk groups require allo-SCT). Nonetheless, as the accessibility and cost of NGS steadily improves over time, it would be prudent for us to investigate the genetic landscape of our local treatment-eligible AML population as it may provide insight into the high rates of resistant disease that we observed.

The concerning number of ‘cytogenetically unknown’ AML and CN-AML cases without available DNA emphasizes the need for a detailed institutional diagnostic AML SOP to ensure that relevant genetic results are obtained. While a triple-mutation testing approach was not able to conclusively risk-stratify all CN-AML cases as per the full ELN 2017 approach [8], this cost-friendly approach was retrospectively capable of identifying ‘favorable risk’ patients, patients at a high risk of induction-resistant disease, and patients who unequivocally required an allo-SCT post-remission strategy in CR1. Moving forward, this mutation-testing strategy could be used as a guide for choosing CN-AML therapy in a resource-limited setting.

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