# A British Society for Haematology Good Practice Paper: Recommendations for Laboratory Testing of UK Patients with Acute Myeloid Leukaemia.

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## Methodology

This Good Practice Paper was compiled according to the BSH process at [https://b-s-h.org.uk/media/19922/bsh-guidance-development-process-july-2021.pdf]. The British Society for Haematology (BSH) produces Good Practice Papers to recommend good practice in areas where there is a limited evidence base but for which a degree of consensus or uniformity is likely to be beneficial to patient care. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org.

## Review of the manuscript

Review of the manuscript was performed by the British Society for Haematology (BSH) Guidelines Committee and the Haematology Oncology sounding board of BSH. It was also on the members section of the BSH website for comment. It has also been reviewed by National Genomic Medicine Service and The Association for Clinical Genomic Science (genetics sections consultation for 4 weeks from 20/09/2021) and the NCRI AML working party. These organisations do not necessarily approve or endorse the contents.

## Introduction

Making a diagnosis of acute myeloid leukaemia (AML) requires a multi-faceted approach bringing together clinical features of patient presentation with laboratory investigations encompassing morphological, immunophenotypic and genetic evaluation of blood, bone marrow and, when appropriate, cerebrospinal fluid (CSF). Recent years have seen updates in disease classification and risk stratification, the rapid incorporation of novel laboratory techniques into routine practice and significant changes in treatment algorithms brought about by the approval of a range of new therapeutic agents.

The revised 2022 World Health Organisation (WHO) Classification (1) subdivides AML into two categories: AML with defining genetic abnormalities (DGA) and AML defined by differentiation ( See appendix 1). A key change is the elimination of the 20% blast requirement for AML types with DGA, with the exception of *BCR::ABL1* and *CEBPA* mutation. There is also the introduction of 'AML with other defined genetic alterations' which would incorporate new and/or uncommon AML subtypes that may be identified in the future. The classification of AML has also been updated separately by the International Consensus Classification (2).

Increased emphasis is placed on genetic factors by both classifications, although morphological assessment retains importance in initial diagnosis, guiding the application of further tests and remains crucial in the emergency identification of acute promyelocytic leukaemia (APL). European Leukaemia Net (ELN) recommendations (4) were also updated in 2022 (3) and represent the current standard-of-care for AML patient risk stratification (see appendix 2), mandating multi-platform genetic evaluation that incorporates cytogenetic and molecular testing, including addition of next-

generation sequencing (NGS) analysis to detect prognostically-relevant entities such as AML with mutations in *CEBPA* and *TP53*, and those mutations associated with secondary AML (i.e. *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and *ZRSR2*).

Since 2018, ten new drugs have been approved for use in AML by the US Food and Drug Administration (FDA), with six of these now approved by the National Institute for Health and Care Excellence (NICE) (summarised in Table 1). Three of these NICE-approved agents have now been incorporated into frontline intensive treatment algorithms for newly diagnosed 'non-APL' AML, emphasising the need for timely turnaround of *FLT3* mutation testing (midostaurin), for accurate identification of AML with myelodysplasia-related cytogenetic abnormalities (CPX-351) and of CD33-positive AML with non-adverse risk karyotype (gemtuzumab ozogamicin; GO). Greater appreciation of the prognostic associations of *TP53* mutations (and interaction with cytogenetic findings) makes their early identification increasingly relevant to treatment decisions in individual patients (4, 5).

Differing rates of adoption of novel diagnostic techniques and regional variations in laboratory practice have resulted in significant heterogeneity, both in access to investigations and in laboratory turnaround times. In this BSH Good Practice paper we make recommendations on best practice in the laboratory evaluation of patients with AML at the points of initial diagnosis and relapse, including guidance on laboratory turnaround times, that allow prompt access to genetically- and disease subgroup-defined approved therapeutic agents across the UK.

Table 1: Summary of new drug approvals in AML (2018-21)

| **Drug** | **Drug Class** | **Approved indication*** | **NICE** | **FDA** | **EMEA** |
|---|---|---|---|---|---|
| Midostaurin | Multi-targeted kinase inhibitor | Newly diagnosed *FLT3*-mutated AML (in combination with intensive induction and consolidation chemotherapy, and alone after complete response as maintenance therapy) | Recommended (June 2018) https://www.nice.org.uk/guidance/ta523 | □ | □ |
| Arsenic trioxide | Inorganic compound | Acute promyelocytic leukaemia (APL).<br>1. Untreated, low-to-intermediate risk disease (WBC<10x$10^9$/ml) when given with all-*trans*-retinoic acid (ATRA) or<br>2.Relapsed or refractory disease, after a retinoid and chemotherapy. | Recommended (June 2018) https://www.nice.org.uk/guidance/ta526 | ü | □ |
| Gemtuzumab ozogamicin | Anti-CD33 antibody-drug conjugate | Untreated *de novo* CD33-positive AML in adults. Cytogenetics favourable, intermediate, failed or not yet available | Recommended (Oct 2018) https://www.nice.org.uk/guidance/ta545 | □ | □ |
| CPX-351 | Cytotoxic (liposomal daunorubin/cytarabine) | Untreated therapy-related AML or AML with myelodysplasia-related changes in adults. | Recommended (Nov 2018) https://www.nice.org.uk/guidance/ta552 | □ | □ |
| Gilteritinib | FLT3 inhibitor | Relapsed or refractory *FLT3*-mutation-positive AML in adults | Recommended (Aug 2020) https://www.nice.org.uk/guidance/ta642 | □ | □ |
| Enasidenib | IDH2 inhibitor | Relapsed/refractory *IDH*-2 mutated AML | Not assessed (6) | □ | □ |
| Ivosidenib | IDH1 inhibitor | Relapsed/refractory *IDH*- 1 mutated AML | Not assessed (7) | □ | □ |
| Venetoclax | BCL2 inhibitor | Newly diagnosed AML (in combination with azacitidine) for patients unsuitable for intensive chemotherapy | Recommended (Feb 2022) https://www.nice.org.uk/guidance/TA765/ | □ | □ |
| Glasdegib | Smoothened inhibitor | Newly diagnosed AML (in combination with low dose | Not assessed (8) | □ | □ |

| | | | | | |
|---|---|---|---|---|---|
| | | cytarabine) for patients unsuitable for intensive chemotherapy) | | | |
| CC-486 | Oral hypomethylating agent | As maintenance treatment for adults who have achieved CR/CRi following intensive induction chemotherapy who are not proceeding to haematopoietic SCT | Under assessment (9) | □ | □ |

* Where the drug is NICE-approved, the stated indication is as per the terms of the NICE guidance. Where the drug is not yet NICE approved or has not been assessed by NICE, the stated indication is as per terms of US FDA approval.
(NICE: National Institute for Health and Care Excellence; FDA: US Food and Drug Administration; EMEA: European Medicines Agency)

**Sampling Considerations**

Patients suspected to have AML should have a bone marrow (BM) examination (aspirate and trephine biopsy). For patients with a high white blood cell count at presentation, diagnostic workup may be performed on the peripheral blood (PB) *in lieu* of a BM examination, and this may also be a suitable approach for the older/frail patient or when best supportive care is likely to be the most appropriate treatment option. Specific sampling requirements for tests are listed in Table 2. Other important considerations at the time of diagnosis are: 1) samples for trials and/or biobanking, 2) samples to allow definition of a flow cytometric or molecular measurable (minimal) residual disease (MRD) marker for future monitoring for patients treated outside clinical trials (storage of DNA and RNA is considered mandatory and a sample to assess leukaemia-associated immunophenotype [LAIP] is strongly recommended) 3) adequate consent and 4) the logistics of transport to laboratories. Integrated reporting of morphology, immunophenotyping and genetics on a sample by a Specialist Integrated Haematological Malignancy Diagnostic Service (SIHMDS) was recommended by NICE Improving Outcomes Guidance in 2016

(https://www.nice.org.uk/guidance/ng47). Integrated reports should specify the classification used in making the diagnosis of AML.

Table 2 Sample Requirements.

| Test | Sample Requirements | |
|---|---|---|
| **Morphology (PB & BM)** | Diagnosis, follow-up and suspected relapse | • At least 200 leucocytes in blood films and 500 nucleated cells in particulate marrow films should be counted. |
| **Trephine Biopsy** | Diagnosis and suspected relapse | • Trephine biopsy for enumeration of blasts, immunohistochemistry and evidence of any preexistent/ concurrent haematological diagnosis e.g. fibrosis, mast cells. |
| **Flow Cytometry** | Diagnosis, follow-up (specified time points) and suspected relapse – key sample characteristics | • Fresh leukocytes required - samples (kept at room temperature) should be sent immediately to the flow cytometry laboratory.<br>• First-pull bone marrow preferable to large but haemodilute samples.<br>• Clots in BM compromise results |
| | Diagnosis or suspected relapse | • BM recommended but PB may be sufficient to confirm 1) lineage and 2) PB blast percentage of ≥ 20%.<br>• PB can be used for diagnostic characterisation of leukaemic aberrant immunophenotypes (MRD target work-up) if circulating blasts.<br><br>BM: 2-5 ml in EDTA tube<br><br>PB: 10-20 ml in EDTA tube |
| | Follow-up including for MRD assessment | BM: 2-5 ml in EDTA tube |
| | CSF for suspected CNS involvement | • <2 ml CSF will not be informative unless there is a major blast infiltrate<br>• As CSF cells rapidly lose viability ex vivo, CSF should be received for processing within a few hours of sampling to prevent false negative |

| | | |
|---|---|---|
| | | results; collection into Transfix medium may also be considered.<br>FISH performed on a CSF cytospin is recommended for patients with a cytogenetic abnormality (e.g. APL, CBF AML), and is preferred to molecular analysis due to limited cell numbers. |
| **Genetics** | Cytogenetics and FISH | • BM recommended but PB may be sufficient if PB involved.<br><br>BM: in heparinised Transport Medium (supplied by Cytogenetics laboratory) or lithium heparin tube.<br>Should reach the laboratory within 24 hours. |
| | Molecular rapid single target testing<br><br>Myeloid gene panel analysis | BM: 2-3 ml in EDTA tube and/or<br>PB: 4 ml in EDTA tube |
| | Molecular fusion detection and MRD assessment | • BM preferred at diagnosis for RNA extraction.<br><br>BM: 5 ml in EDTA tube and/or<br>PB: 20 ml in EDTA tube<br>Should reach the laboratory within 48 hours |

EDTA, Ethylenediaminetetraaceticacid ; BM, bone marrow; PB, peripheral blood; CNS, Central Nervous System ; CSF, cerebrospinal fluid; FISH, Fluorescence in situ hybridisation; MRD, measurable (minimal) residual disease; RNA, Ribonucleic acid.

**Morphology**

In the 2022 revision of the WHO AML classification, a BM or PB blast count of ≥20% is required only for the diagnosis of AML without defining genetic abnormalities (DGA) (Appendix 1). For AML with DGA, a blast count of >20% is not required and a specific blast cut off has not been set, with increased emphasis on correlation between morphologic findings and molecular genetic studies. Similarly, the International Consensus Classification of AML (ICC) presented in 2022 (EHA reference) has suggested a lower blast count of ≥10% for AML with DGA, and proposes the category of MDS/AML for patients with 10-19% blasts in PB or BM with subcategories of AML

with MDS related gene mutations and AML with MDS related cytogenetic abnormalities in patients without DGA.

The new 2022 WHO classification has replaced the category of AML with myelodysplasia-related changes (AML-MRC) with AML-Myelodysplasia Related (AML-MR) and its diagnostic criteria are updated (see footnote). Key changes include removal of morphology alone to make a diagnosis of AML-MR, updating the cytogenetic criteria and introducing the mutation-based diagnosis of AML-MR. AML transformed from MDS or MDS/MPN continues to be included in AML-MR in view of the broader unifying biologic features.

Morphologically, myeloblasts, monoblasts and megakaryoblasts are included in the blast count. In AML with monocytic or myelomonocytic differentiation, monoblasts and promonocytes, but not abnormal monocytes, are counted as blast equivalents. Diagnostic criteria for Acute Erythroid Leukaemia (AEL) include erythroid predominance, usually >80% of BM elements of which >30 % are proerythrobalsts.



Footnote: Access to drugs in the treatment of AML currently approved in the UK are based on previous classification and disease definitions. Hence relevant funding approval criteria should be referred to when accessing these drugs.

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**Morphology Recommendations:**

- **Bone marrow aspirate and trephine biopsy should be performed and at least 200 nucleated cells examined to make a diagnosis of AML.**

- **Communicating morphological findings rapidly to specialised laboratory personnel is important to aid further testing, especially if suspecting APL or core-binding factor (CBF) AML - cases with t(8;21), t(16;16) or inv(16).**

**Flow Cytometry**

Immunophenotypic profiling by multiparametric flow cytometry (MFC) is essential at diagnosis, for remission assessment and if relapse is suspected. Testing of CSF is required when CNS involvement is suspected. Eight-colour MFC panels are standard, although it is anticipated that 10-colour panels will become routine in the next few years due to the newer clinical cytometers now available.

MFC-MRD in AML applied to remission BMs provides additional prognostic information after induction chemotherapy and pre-transplant; it may also be informative at other time-points. SIHMDS are recommended to have a named reference laboratory for MFC-MRD testing. This may require additional standard operating procedures (SOPs) for send away tests.

*Sample requirements* for MFC tests are according to Table 2.

*Turnaround times*

Urgent samples (delivered and notified to the laboratory according to local guidelines):

- same working day when received before 3:30 pm

Non-urgent samples, including remission/MRD assessments:

- processed same day or next morning with results available <2-3 working days from sample receipt.

*Diagnostic panels.*

For a suspected acute leukaemia, most MFC laboratories apply a two-stage diagnostic panel

- an acute leukaemia screen (such as the Euroflow ALOT combination (10)) to confirm leukaemic blasts and allow lineage assessment
- followed immediately by an AML-specific panel if appropriate
- extended secondary testing may be required to diagnose blastic plasmacytoid dendritic cell neoplasm (BPDCN) (11) or ambiguous leukaemias or acute megakaryoblastic leukaemia (*Table 3).*

Myeloperoxidase positivity for myeloid lineage assignment can be defined as ≥20% of acute leukaemic cells exceeding a lymphocyte-based threshold (12)

APL and t(8;21)/*RUNX1::RUNX1T1* AML have typical blast immunophenotypes (Table 3); prompt reporting of these in conjunction with morphology can expedite confirmatory genetic tests.

Characterisation of diagnostic leukaemic aberrant phenotypes informs further monitoring. This requires diagnostic as well as follow-up AML samples to be tested by an AML MRD minimum marker set *(Table 3)*. Therefore, diagnostic samples may need to be routinely sent to the reference MFC-MRD laboratory.

Treatment authorisation for GO requires CD33 positivity. All AML diagnostic and relapse reports should include clear information on whether blasts are CD33 positive. There is heterogeneity in the clinical effectiveness of GO that, in part, correlates with higher CD33 expression levels, but GO can be effective in patients with partial or weak blast CD33 positivity as observed for CBF AMLs (13). MFC laboratories will have validated fluorescent thresholds for quantifying % CD33-positive blasts and maintain

a satisfactory performance for this marker in an external quality assurance scheme such as the UK NEQAS Leukaemia Immunophenotyping Part 1. Expression of other markers may also be a requirement for future immunotherapy indications such as CD123 for tagraxofusp as first line treatment in BPDCN, (FDA and EMA approved) (14, 15).

*Flow cytometric remission and MRD assessment.*

BMs may be inadequate or suboptimal for assessment of remission or MRD; this should be stated in the report, along with the cause (poor cell viability / haemodilution / insufficient leukocytes). Other critical report information includes blast percentage, markers defining the blast population and the denominator for blast percentage (leukocytes or nucleated cells).

In some cases, flow cytometric and morphological results will be discrepant for remission status; potential contributing technical and sampling factors should be considered as part of integrated SIHMDS reporting. If patients are refractory by morphology but have an MFC-MRD negative test, the latter appears to be more reliable for prognosis (16-19)

MFC-MRD assessment should be performed with a qualified assay according to ELN guidelines (20, 21), and is best achieved in a laboratory with specialist expertise in AML MFC-MRD that coordinates with genetic AML MRD laboratories for guidance on the most appropriate MRD test and result interpretation. The prognostic value of MFC-MRD may be reduced by methodological variability such as in instrument settings, panels and analysis (22, 23)

**Flow cytometry Recommendations**

- **Flow cytometric analyses are critical for diagnosis and remission assessment and require rapid reporting.**
- **Diagnostic reports should record whether AML cells are CD33 positive.**
- **Diagnostic AML subtyping by flow cytometry should include consideration of blastic plasmacytoid dendritic cell neoplasm (BPDCN)**
- **For flow cytometric MRD monitoring a diagnostic sample is preferred to identify trackable MRD targets**

**Table 3** Antibody Markers for further diagnostic subtyping and MRD

| | **Antibody Markers** | **Comments** |
|---|---|---|
| **Acute megakaryoblastic leukemia** | Extended testing includes<br>CD41, CD61,<br>CD36, CD9 may also be helpful | • CD41/CD61 Interpretation confou attachment to other cells<br>• CD42 expressed on platelets bu megakaryoblasts |
| **Blastic plasmacytoid dendritic neoplasm (BPDCN)** | Extended testing includes<br>CD123, CD4, CD56 | • BPDCN always express CD123 frequently CD56<br>• CD34 and CD13 negative in almost differentiate from AML (11)<br>• Consider AML with plasmacytoid den (associated with *RUNX1* mutations) ( |
| **t(8;21)** | CD19, CD56, CD34, CD33 | Blast immunophenotype:<br>• aberrant CD19 (but not stable marker<br>• sometimes aberrant CD56<br>• typically high CD34, may be weak CD<br>• positive for myeloperoxidase |
| **t(15;17)** | CD117, CD34, CD11b, HLA-DR, CD33, CD13 | Promyelocyte immunophenotype<br>• higher side scatter (SSC) than monon classical and variant forms)<br>• positive for CD33, CD13, CD117<br>• negative for CD34, HLA-DR, CD11b [1]<br>• positive for myeloperoxidase |
| **MRD panel** | CD45 CD33 CD13 CD34 CD117 HLA-DR CD56 CD7 | • ELN recommended 8-colour antibody diagnosis and follow-up<br>• Myeloid maturation / monocyte marke e.g. CD11b, CD14 with CD13, HLA-D<br>• 'LSC' combinations may add prognos CD34, CD38 with 'LSC' aberrant mar CD45RA, CD123, CD56, CD7, TIM-3 |

1. blasts of *NPM1* mutated AML may also be negative for CD34, HLA-DR and CD11b and positive for CD117 but typically have lower side scatter and may have monocytic component.

Abbreviations MRD, measurable (minimal) residual disease

## Genetics

Comprehensive genetic profiling, comprising cytogenetic and molecular genetic testing, is integral to the diagnosis and classification of AML (Table 4; (25-27). Detection of disease-specific abnormalities supports accurate diagnosis and yields prognostic information for risk stratification and is critical at diagnosis and at relapse. If a case is discovered to be high risk MDS (e.g., high blast count), then at the time of initial request or subsequently, testing should be undertaken as per the AML pathway.

Accurate and rapid genetic characterisation allows timely deployment of specific therapeutic interventions; either according to subtypes defined by cytogenetics (e.g., CBF AML and AML with myelodysplasia-related cytogenetic abnormalities) or to specific mutations (e.g., *FLT3* and *IDH1/2*). Identification of characteristic genetic lesions also identifies patients suitable for molecular monitoring of residual disease. Storage of appropriate material for further molecular and cytogenetic studies is essential in all patients, and cell suspension, DNA and RNA should be stored, with appropriate written informed consent in place, at diagnosis and relapse where possible.

Cytogenetic testing is mandatory at both diagnosis and relapse. Conventional karyotyping may be supplemented by rapid fluorescence *in situ* hybridisation (FISH) testing to promptly identify cardinal lesions associated with various AML subtypes, however in the absence of a diagnostic finding, FISH must always be supplemented by a rapid karyotype. In the event of karyotyping failure, SNP Microarray or FISH for del(5q), del(7q) and monosomy 7 and del17p (*TP53*) will detect a significant proportion

of myelodysplasia-related abnormalities, however, it should be noted that multiple rare abnormalities and complex karyotype may be missed when using FISH alone.

Subsequent monitoring by FISH or karyotyping is not routinely recommended, however may add value in clarifying atypical or discordant results of other testing modalities or where progression or relapse are suspected.

*PML::RARA* testing is not mandatory for all cases of AML, but must be rapidly initiated in selected cases where the results of other testing (e.g. morphology) provide a high index of suspicion of APL. Where indicated, *PML::RARA* testing must be available within 24 hours. It is important to note that a small proportion of rearrangements can occur which are both chromosomally cryptic and also undetected by FISH analysis. Molecular characterisation should be offered where clinical suspicion persists, for the identification of cryptic abnormalities in difficult cases. The results of such supplementary studies must be available within 72 hours.

The characteristic lesions associated with CBF leukaemia (i.e. *CBFB::MYH11* and *RUNX1::RUNX1T1*) should be identified within 72 hours of sample receipt. This can be undertaken by FISH, molecular characterisation or a rapid karyotype. It is important to note that cytogenetically cryptic *CBFB* rearrangements must be excluded by FISH or molecular characterisation where a diagnostic suspicion of CBF leukaemia persists.

Testing for *KMT2A* (*MLL*) rearrangement is essential, but less time critical than CBF, or *FLT3*, as it is less likely to impact initial therapy decisions. As *KMT2A* rearrangements may be cytogenetically cryptic (28), and are recognised as a disease

defining entity in the updated WHO classification, *KMT2A* testing is mandatory for cases where no other DGA is identified. Given the well documented heterogeneity of *KMT2A* fusions, a gene partner agnostic approach such as “break-apart” FISH testing is essential. Supplementation by an RNA-based NGS fusion panel may be helpful. Where a *KMT2A* rearrangement is identified by a partner agnostic approach, it is essential for appropriate risk categorisation that further testing is undertaken (3); this may be by karyotype, metaphase FISH or molecular characterisation. With the continuing development of genomically derived disease classifications(1, 2) expanded molecular profiling, for example through rapid whole genome sequencing or RNA-based NGS fusion panels, will play an increasingly important role in AML diagnosis.

Internal tandem duplications (ITD) and tyrosine kinase domain (TKD) driver variants in *FLT3* have been shown to respond to FLT3 inhibitors and as such their rapid identification is critical. Although the *FLT3*-ITD allelic ratio (AR) is no longer required for assignment of ELN risk group, it is recommended that this is still reported if the assay is appropriately standardised and validated for this purpose (29), as AR provides additional prognostic information which may be valuable in some situations . Whilst rapid identification of the recurrent driver variants in exon 11 of *NPM1* is not mandated, in practice they are often identified in tandem with *FLT3* analyses for prognostic purposes and can also aid interpretation of cases of suspected APL without *PML-RARA* rearrangement as well as cases with morphological evidence of dysplasia (25)

NGS panel testing for the identification of pathogenic variants within *CEBPA* , *TP53*, *FLT3*, *IDH1*, *IDH2*, *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*,

*DNMT3a, WT1* and *ZRSR2* is mandatory and will aid in using a genomic classification (32) of AML and ELN 2022 risk stratification. There is no standard NGS panel in use by UK Genetics Laboratories; panel design, target enrichment techniques, and sequencing are available from a variety of commercial and in house methods. They must meet minimum standards of practice and requirements as defined in local or National testing algorithms, for example the National Genomic Test Directory (https://www.england.nhs.uk/publication/national-genomic-test-directories/) for services commissioned by NHS England, and remain flexible to incorporate changes in practice. NGS methods can have variable performance, particularly for detection of mutations in key genes that are difficult to sequence, such as CEBPA and quantification of FLT3 ITD. It is the responsibility of the testing laboratories to have robust NGS methods for the detection of typical mutations of mandatory targets or to provide alternative, quality assured single target tests. The majority of NGS panels available for use in AML will also contain a range of genes associated with other myeloid diagnoses and the reporting of these is considered desirable, but not mandatory at this time. In the future, rapid diagnostics for these and other abnormalities may be required for selection of upfront therapy, however at present this is only relevant within specific clinical trials and should be provided by the respective trials laboratory where needed.

It is important to recognise that driver variants identified by somatic-only testing may be of germline origin, and protocols to robustly identify and confirm such variants in high-actionability germline cancer predisposition genes (i.e., *RUNX1, CEBPA, DDX41, ANKRD26, ETV6, GATA2*) are essential (Table 5). Laboratories must have processes in place to identify and report potential germline findings (30) in high-

actionability genes from somatic only sequencing. Where a potential germline finding has been identified, a detailed clinical and family history is essential in determining whether to pursue further testing to confirm the aetiology of the variant. Should germline testing be indicated then a skin biopsy to obtain cultured skin fibroblasts will be required for further characterisation. Discussion and, depending upon local practice, referral of such cases to Clinical Genetics should be considered.

It is important to stress the identification of familial predisposition to haematological cancer, particularly to enable wider testing when considering transplantation from a related donor. A family history of MDS/acute leukaemia/aplastic anaemia, early onset of cancers of any type, or multiple close relatives with cancer should always be sought. In addition, a personal or family history of cytopenias, abnormal bleeding, skin/nail abnormalities, idiopathic liver disease, immune defects, atypical infections, lymphoedema, limb abnormalities or pulmonary fibrosis should be elicited. Index patients reporting this history should be referred to Clinical Genetics for counselling and expert advice.

Conversely, in patients with a clinical or family history suggestive of an AML predisposition syndrome or inherited bone marrow failure disorder, up-front germline testing may be warranted. Testing will typically be undertaken by large constitutional NGS panel analysis although, where there is a suspicion of Fanconi anaemia, then functional cytogenetic studies of mutagen (e.g. Diepoxybutane DEB, Mitomycin C MMC) sensitivity may be appropriate, with further characterisation of any positive findings by molecular studies to inform wider family studies.

Whole genome sequencing (WGS) is an emerging technique in UK clinical practice which at present may be used to supplement standard-of-care studies. WGS offers the potential to replace and enhance standard-of-care diagnostics and as WGS pathways embed and expand, and as the turnaround times for testing improve, it is likely that this technique will supplant some of the current testing modalities.

Genetic lesions currently suitable for molecular MRD monitoring by quantitative polymerase chain reaction (PCR) include *NPM1* mutation, *PML::RARA* and CBF and *KMT2A* fusion genes, with a turnaround time of 3-14 days. Urgent samples requiring a faster turnaround time (e.g., 3-7 days) include:

- *NPM1*-mutated cases at post-course 2 time point (PB sample essential for risk stratification) (20, 31).

- Repeat sample following a previous concerning result (e.g., suspicious for molecular relapse or molecular progression).

- Clinical suspicion of relapse (e.g., falling counts).

As these assays are RNA-based, it is essential that RNA is stored at diagnosis for an accurate baseline assessment, and that follow-up samples reach the molecular MRD laboratory within two days of sampling. Further recommendations on molecular MRD testing will become available in NHS England guidance documents in due course. Other molecular MRD monitoring techniques such as digital droplet PCR and NGS are currently under investigation and not yet recommended for routine clinical care.

**Table 4** Tests at diagnosis (all patients)

| Test Name | TAT** (days) |
|---|---|
| *FLT3* ITD § | 3 |
| *NPM1* exon 11 | 3 |
| *FLT3* TKD hotspot § | 3 |
| FISH/PCR or Karyotype *CBFB::MYH11* [inv(16)] | 3 |
| FISH/PCR or Karyotype *RUNX1::RUNX1T1* [t(8;21)] | 3 |
| AML Karyotype §+ | 7 |
| *KMT2A*::*R* FISH | 14 |
| AML NGS Panel §* | 14 |
| WGS Germline and Tumour # | 42 |

§ It is essential that these tests are repeated at relapse. Other tests may need to be repeated at relapse, depending on the clinical situation.

\+ Cytogenetically cryptic CBF should be excluded where diagnostic suspicion persists

* NGS panel should include as a minimum, *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2*, *TP53*, *FLT3*, *IDH1*, *IDH2, DNMT3a* and *WT1*.

** TAT Turnaround time

\# May be used to supplement standard-of-care studies, and considered in patients being investigated for germline predisposition

**Table 5** Tests to be considered for specific diagnostic indications

| Test Name | Indication | TAT (days) |
|---|---|---|
| FISH *PML::RARA* [t(15;17)] | Suspicion of APL from morphology or flow | 1 |
| RT-PCR *PML::RARA* [t(15;17)] | Suspicion of APL from morphology or flow | 3 |

| | | |
|---|---|---|
| MyeChild01 FISH panel§ | Children and young adults, if no other primary genetic changes | 7 |
| MLDS NGS panel (*GATA 1*) | Children with known trisomy 21 | 21 |
| Fanconi breakage testing | Suspected Fanconi anaemia pre-transplant | 14 |
| Inherited bone marrow failure syndrome panel | Suspected familial predisposition syndrome | 84 |

§ MyeChild01 FISH panel: t(5;11)(q35;p15.5)/*NUP98::NSD1*, t(7;12)(q36;p13)/*MNX1::ETV6*, inv(16)(p13.3q24.3)/*CBFA2T3::GLIS2*

**Genetics Recommendations**

- **Cytogenetic and molecular genetic analyses are critical at diagnosis and relapse**
- **Where indicated, *PML::RARA* testing must be completed within 24 hours of sample receipt**
- **Rapid identification of core binding factor leukaemia, *FLT3* internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations is essential**
- **A complete conventional cytogenetic analysis is required within 7 days of sample receipt**

- **Molecular MRD assessment should be performed for patients with an *NPM1* mutation, *CBFB::MYH11, RUNX1::RUNX1T1* and *PML::RARA* fusions, and considered for other fusion genes.**
- **Where a suspected germline finding in a cancer susceptibility gene is identified, it should be clearly highlighted in the genetic report.**


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## Declaration of Interests

The BSH paid the expenses incurred during the writing of this good practice paper. All authors have made a declaration of interests to the BSH and Task Force Chairs which may be viewed on request. PM has undertaken advisory board, educational grant or speakers fees with Pfizer, Jazz Pharmaceuticals, Astellas and Abbvie. SK: Advisory board: Astellas, Jazz, Servier, Abbvie, Novartis. Speakers fees: Astellas, Novartis, Jazz. Research funding: Novartis. AH: Advisory Board Novartis; Speakers' Bureau Gilead, Roche, Pfizer, Jazz, AbbVie; Data analysis CTI. SF: Speakers Bureau: Jazz, Novartis, Research Funding: Jazz, BMS, Advisory Board: Novartis, Neogenomics, IMPAAC.

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## Review Process

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BSH guidelines website (www.b-s-h.org.uk).

## Disclaimer

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## Appendix 1

2022 WHO Classification Acute Myeloid Leukaemia (1)

**Acute myeloid leukaemia with defining genetic abnormalities**

- Acute promyelocytic leukaemia with *PML*::*RARA* fusion
- Acute myeloid leukaemia with *RUNX1*::*RUNX1T1* fusion
- Acute myeloid leukaemia with *CBFB*::*MYH11* fusion
- Acute myeloid leukaemia with *DEK*::*NUP214* fusion
- Acute myeloid leukaemia with *RBM15*::*MRTFA* fusion
- Acute myeloid leukaemia with *BCR*::*ABL1* fusion
- Acute myeloid leukaemia with *KMT2A* rearrangement
- Acute myeloid leukaemia with *MECOM* rearrangement
- Acute myeloid leukaemia with *NUP98* rearrangement
- Acute myeloid leukaemia with *NPM1* mutation
- Acute myeloid leukaemia with *CEBPA* mutation
- Acute myeloid leukaemia, myelodysplasia-related
- Acute myeloid leukaemia with other defined genetic alterations

**Acute myeloid leukaemia, defined by differentiation**

- Acute myeloid leukaemia with minimal differentiation
- Acute myeloid leukaemia without maturation
- Acute myeloid leukaemia with maturation
- Acute basophilic leukaemia
- Acute myelomonocytic leukaemia
- Acute monocytic leukaemia
- Acute erythroid leukaemia
- Acute megakaryoblastic leukaemia

## Appendix 2

2022 European Leukaemia Net Risk Stratification of AML by Genetics

| Genetic group | Subsets |
|---|---|
| **Favourable** | t(8;21)(q22;q22); *RUNX1*::*RUNX1T1*<br>inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB*::*MYH11*<br>Mutated *NPM1* without *FLT3*-ITD<br>bZIP in-frame mutated *CEBPA* |
| **Intermediate** | Mutated *NPM1* with *FLT3*-ITD<br>Wild-type *NPM1* with *FLT3*-ITD<br>t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A* |

| | |
|---|---|
| | Cytogenetic and/or molecular abnormalities not classified as favourable or adverse |
| **Adverse** | inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *GATA2, MECOM (EVI1)*<br>t(6;9)(p23;q34)/ *DEK*::*NUP214*<br>t(v;11)(q23.3)/*KMT2A*-rearranged<br>t(9;22)(q34.1;q11.2)/ *BCR::ABL1*<br>-5 or del(5q); -7; -17/abn(17p)<br>Complex karyotype (≥ 3 abnormalities) / monosomal karyotype<br>Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1* or *ZRSR2*<br>Mutated *TP53* |

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336 NB Initial risk assignment for favourable/ intermediate by genetics may change during the treatment
337 based on MRD results