Genetic testing in AML

An eLearning module for haematology nurses

Leukaemia Care
YOUR Blood Cancer Charity

Introduction

This eLearning module has been developed for haematology nurses. It focuses on the role of genetic testing in patients with AML.

After completing this module, you should be able to:

- Outline the importance of genetic testing in AML
- Describe the different tests that may be used and what each is used for
- Identify which tests to order and when
- Summarise the implications of test results
- Explain the significance of potential germline variants to patients and family members
- Identify when to refer a patient to clinical genetics

A self-assessment test is included so you can assess your knowledge after the module.

Financial support was provided as an Independent Medical Education Grant from Pfizer Limited. In accordance with our policies, Pfizer had no editorial input into the content.

Time to complete the module: 1 to 2 hours

Date of publication: March 2024

Reviewed and updated: March 2027

Background to genetic testing in AML

The terms ‘genetics’ and ‘genomics’ are often used interchangeably. Although they are closely related fields, there is a distinction between the two:

- Genetics is the study of individual genes, their function, and how they are inherited.
- Genomics is the study of the entire genome, or parts of it. It includes genes and non-coding sequences of DNA.

Testing for patients with acute myeloid leukaemia (AML) can involve both genetic and genomic analysis. In this module, we use the term ‘genetic testing’ to cover both genetic and genomic testing techniques.

Genetic testing involves analysis of a patient’s blood, bone marrow or other tissues to identify gene variants or chromosome abnormalities that are associated with health conditions.

Different words are used to describe genetic alterations identified by genetic testing. These include **variant**, **disease-causing alteration** or **pathogenic variant**. The most up-to-date scientific term is pathogenic variant. This describes a gene alteration that is linked to an increased health risk.

Genetic abnormalities in AML

Many gene variants and chromosome abnormalities are associated with the development of AML. Patients usually have a number of genetic abnormalities.[1] Together, these lead to the development of a population of abnormal cells that multiply uncontrollably.[2,3]

The variants can be:

- **Somatic variants**, which are acquired during a patient’s lifetime. These are present only in the abnormal cancer cells. They are not present in germ cells (sperm and ova) so they cannot be passed on to future generations. *FLT3* and *NPM1* variants are examples of somatic variants commonly associated with AML.

- **Germline variants**, which are inherited. These are present in every cell in a person's body. Because they are present in germ cells, they can be passed on to future generations. Germline variants in AML do not transmit AML directly, but they can be associated with an increased risk of developing AML. This is called a germline predisposition to AML. Pathogenic variants in the *RUNX1* gene or *CEBPA* gene are examples of variants that may be associated with a germline predisposition to AML.

Rationale for genetic testing in AML

Genetic testing to identify gene variants and chromosome abnormalities is essential in patients with AML. It can help determine:[1,4]

Diagnosis and classification

- Detection of genetic abnormalities specifically associated with AML supports an accurate diagnosis.[4]
- Genetic features are also used to classify AML according to the latest WHO classification system into:[5]
    - AML with defining genetic abnormalities, such as acute promyelocytic leukaemia with *PML::RARA* fusion.
    - AML defined by differentiation, such as acute myelomonocytic leukaemia.

Optimal treatment strategy

- Some treatment options for AML specifically target particular genetic variants, such as the FLT3 inhibitors midostaurin or gilteritinib.
- Others may be more effective in people with certain genetic profiles than others.[4]

Prognosis

- The prognosis of AML can be stratified based on the pattern of genetic abnormalities into:[2]
    - Favourable
    - Intermediate
    - Adverse

Treatment response

- Certain genetic variants can be quantified, which enables molecular monitoring of residual disease.

Potential familial predisposition

- Identifying possible germline variants allows for further testing and identification of family members who may be at risk of developing AML.

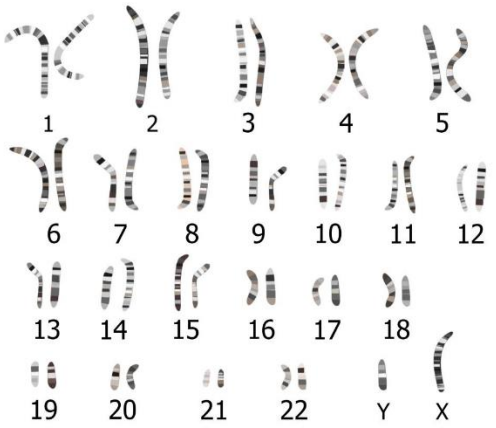
Types of genetic testing

Genetic testing can look at chromosome abnormalities or gene variants in blood or bone marrow samples. A number of different testing techniques are used, which can identify different abnormalities.

Cytogenetic tests

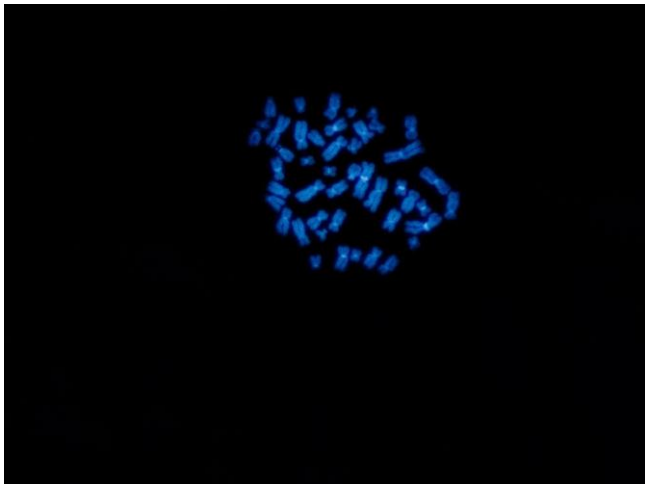
Cytogenetic tests look for chromosome abnormalities. Common cytogenetic tests include:

Karyotyping[6–8]



- A sample of dividing cells is stained so chromosomes are visible under a microscope
- Used to identify structural chromosomal abnormalities, such as:
    - Translocations (swapping of DNA between chromosomes) – for example, a translocation between chromosomes 8 and 21
    - Deletions (missing DNA in a chromosome) – for example, deletion of part of chromosome 5
    - Insertions (extra DNA within a chromosome) - for example, an insertion in the *FLT3* gene on chromosome 13
    - Inversions (sections of 'back-to-front' DNA in a chromosome) – for example, an inversion in chromosome 16
    - Extra chromosomes or missing chromosomes – for example, having only one copy of chromosome 7 or three copies of chromosome 8

Fluorescence in situ hybridisation (FISH)[6-8]

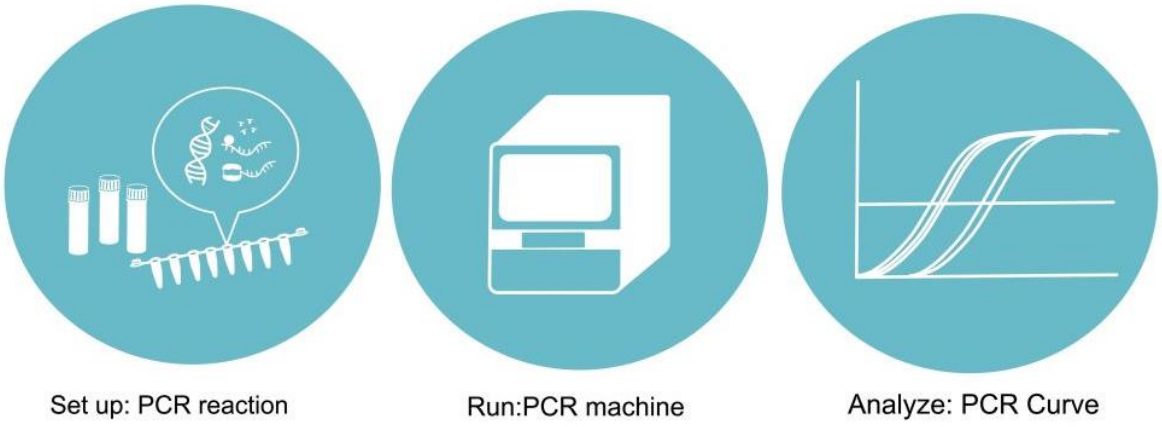


- Fluorescent probes that target particular sequences of DNA are added to a sample of patient cells
- If the target sequences are present, the probes bind to them and can be seen under a fluorescent microscope
- Looks at specific regions of interest within chromosomes
- Used to identify:
    - Chromosome abnormalities too small to be detected by karyotyping
    - Gene fusion products, where chromosome abnormalities cause two genes to fuse together to create a new abnormal gene

Molecular genetic tests

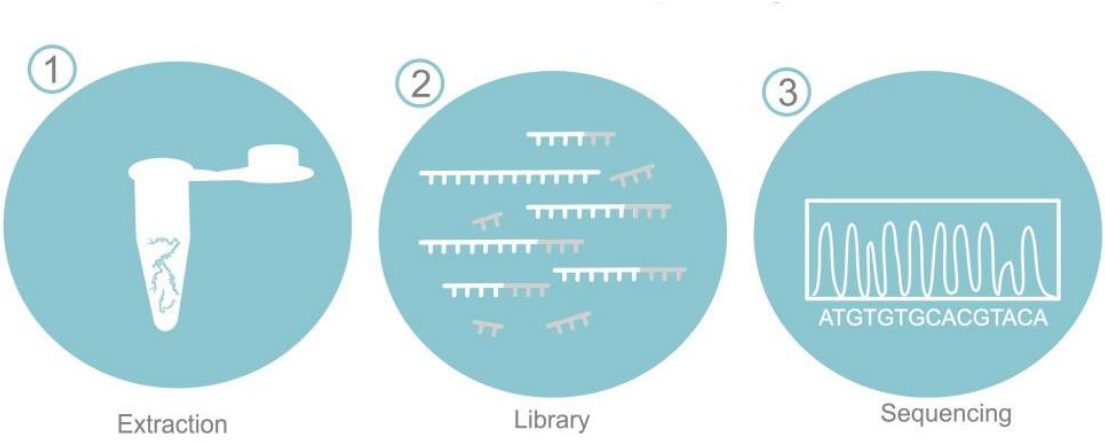
Molecular genetic tests look for abnormalities in the sequence of DNA using methods such as PCR tests, next generation sequencing or whole genome sequencing.

Polymerase chain reaction (PCR)[6–8]



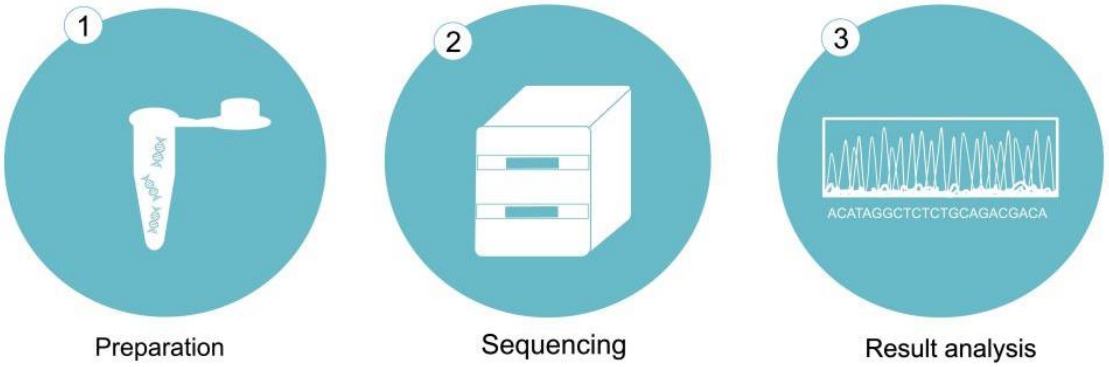
- Uses ‘primers’ to target specific sequences of DNA in a patient sample
- If present, the target DNA is amplified and can be measured (quantitative PCR, or qPCR)
- Used to:
    - Identify specific gene variants
    - Quantify target DNA, which is proportional to the number of cells with the specific variant (i.e. the number of malignant cells in the sample)
    - Monitor treatment response
- Very sensitive

Next generation sequencing (NGS) panels[7–9]



- NGS panels analyse the exact sequence of particular genes from a 'gene library', rather than looking for specific abnormalities
- The panels are designed to look at the genes most likely to be abnormal in a particular disease
- Used to:
    - Identify specific variants commonly associated with AML

Whole genome sequencing (WGS)[4,7–9]



- WGS analyses the patient's entire genome
- Can detect many different genetic variations, not just pre-defined abnormalities in specific genes
- As well as genetic variants associated with the condition being investigated, WGS may find variants associated with other conditions, or variants of 'unknown significance'
- Used to:
    - Identify unusual or complex genetic variants
    - Look for variants in multiple genes
    - Investigate potential germline variants

When to order genetic tests

Genetic testing is important at the time of initial diagnosis and at relapse. It may also be used to monitor treatment response in some patients.

Tests at diagnosis

The BSH recommends the following tests at diagnosis (based on tests available in the Cancer National Genomic Test Directory).[4,10]

Tests in patients suspected of having acute promyelocytic leukaemia (APL)

Test	Why it's important	Approximate turnaround time
Urgent FISH or PCR to test for *PML::RARA* fusion gene	Rapidly diagnoses patients with APL, which is a medical emergency[11]	24 hours

Tests in all patients

Test	Why it's important	Approximate turnaround time
Simple targeted variant testing for: • *FLT3* ITD • *FLT3* TKD hotspot • *NPM1* exon 11 hotspot	Rapidly identifies AML-defining genetic variants and *FLT3* variants, which are therapeutic targets	3 days
FISH, PCR or rapid karyotype to test for: • *CBFB::MYH11* • *RUNX1::RUNX1T1*	Rapidly identifies fusion genes associated with CBF leukaemia, important for risk stratification and molecular monitoring	3 days

AML karyotype	Identifies AML-defining chromosomal abnormalities and fusion genes, important for risk stratification	Up to 7 days
AML multi-target NGS panel	Identifies AML-defining gene variants, important for risk stratification and molecular monitoring	7 to 14 days
FISH to test for *KMT2A::R* fusion gene	Identifies specific subtype of AML with *KMT2A* rearrangement, suitable for molecular monitoring	Up to 14 days
WGS of germline and tumour cells if appropriate	Consider to supplement other cytogenetic testing, or in patients with potential germline variants	Up to 42 days

Other specific genetic tests may be needed in AML patients based on clinical features or results of karyotyping.[4,10]

Tests to consider at relapse

In patients with suspected relapse, the BSH recommends repeating the following genetic tests:[4]

- Simple targeted variant testing for *FLT3* ITD and *FLT3* TKD hotspot to identify any newly-acquired variants that are potential therapeutic targets
- AML karyotyping
- AML multi-target NGS panel

Other genetic tests may be needed at relapse depending on clinical presentation and the availability of new targeted treatments.[4]

Tests to monitor molecular response

Molecular genetic tests can be used to monitor measurable residual disease (MRD). These are very sensitive tests that can detect extremely low levels of leukaemia cells. This can help monitor response to treatment and risk of relapse.

Molecular monitoring by qPCR is recommended for AML patients with:[4,12]

- *NPM1* variants
- *PML::RARA* fusion gene (APL)
- *CBFB::MYH11* or *RUNX1::RUNX1T1* fusion genes (CBF leukaemia)
- *KMT2A::R* fusion gene

Molecular monitoring may also be considered for patients with other fusion genes.

Germline variants are not suitable for monitoring MRD because they are present in all cells.[12]

Implications of results

The results of genetic testing have important implications for treatment and prognosis. They may also have implications for family members if germline pathogenic variants are identified. Patients should be informed before they consent to testing that:

- It may find pathogenic variants that could run in families.[13]
- It may find somatic or germline pathogenic variants that are not related to AML but could be associated with other conditions.[13]
- It may find variants of 'uncertain significance'. This is when there is not enough evidence to know whether or not a variant is associated with a health condition.

Implications for treatment

Some genetic findings help guide treatment decisions for patients with AML.

- Presence of a translocation between chromosomes 15 and 17 or the *PML::RARA* fusion gene are characteristic of APL. Patients with APL require different treatment from patients with other forms of AML so the presence of either of these genetic abnormalities allows accurate diagnosis and appropriate treatment.[2,4]
- AML patients with *FLT3* variants are treated with the FLT3 inhibitor midostaurin alongside standard induction and consolidation therapy. Midostaurin monotherapy can also be used for maintenance treatment in patients with *FLT3* variants.[2,4,14]
- Patients with relapsed or refractory AML and *FLT3* variants are eligible for treatment with the FLT3 inhibitor gilteritinib.[2,4,15]
- Ivosidenib is a novel treatment that targets *IDH1* variants. In May 2024, it was approved by NICE, in combination with azacitidine, for patients with untreated *IDH1*-mutated AML who cannot have standard intensive induction chemotherapy.[4,16]

Future treatment options

There are also drugs in late-stage development that target specific variants associated with AML. These may be available in the future.

- Quizartinib is a novel treatment that targets *FLT3-ITD* variants. At the time of writing, it is being assessed by NICE, in combination with chemotherapy, for use in patients newly-diagnosed with *FLT3-ITD*-positive AML.[17]
- Enasidenib is a novel treatment that targets *IDH2* variants. It is being developed to treat relapsed or refractory *IDH2*-mutated AML but at the time of writing, it is not licensed in the UK.[2,4]

Implications for prognosis

European Leukaemia Net stratify AML by risk based on genetic abnormalities.[2]

Risk	Cytogenetic abnormalities	Molecular abnormalities
Favourable	• Translocation between chromosome 8 and 21 • Inversion in chromosome 16 • Translocation between two regions of chromosome 16 • *RUNX1::RUNX1T1* fusion gene • *CBFB::MYH11* fusion gene	• *NPM1* variant without *FLT3-ITD* • *CEBPA* variant in a region of the gene called bZIP
Intermediate	• Translocation between chromosome 9 and 11 • *MLLT3::KMT2A* fusion gene • Abnormalities not classed as adverse	• *NPM1* variant with *FLT3-ITD* • Wild-type *NPM1* with *FLT3-ITD* • Abnormalities not classed as adverse

Adverse	• Inversion in chromosome 3
	• Translocation between two regions of chromosome 3
	• Translocation between chromosome 6 and 9
	• Translocation between chromosome 9 and 22
	• Translocation involving chromosome 11
	• Missing chromosome 5 or deletion of part of chromosome 5
	• Missing chromosome 7
	• Missing or abnormal chromosome 17
	• *DEK::NUP214* fusion gene
	• *BCR::ABL1* fusion gene
	• Complex karyotype
		• *KMT2A* rearrangement
		• *GATA2, MECOM*
		• *MECOM* variant
		• *TP53* variants
		• Variants in the following, unless they co-exist with favourable-risk AML subtypes:
		  ○ *ASXL1*
		  ○ *BCOR*
		  ○ *EZH2*
		  ○ *RUNX1*
		  ○ *SF3B1*
		  ○ *SRSF2*
		  ○ *STAG2*
		  ○ *U2AF1*
		  ○ *ZRSR2*

Germline variants

Around 14% of AML patients have germline pathogenic variants,[18] which are associated with a familial predisposition to develop AML. Germline variants can be passed on to future generations.

Several genes, if they have a pathogenic variant, are known to be associated with a germline predisposition to AML. Some examples of these include:[13]

- *RUNX1*
- *CEBPA*
- *DDX41*
- *ANKRD26*
- *ETV6*
- *GATA2*

Initial genetic testing of blood or bone marrow samples (i.e. tumour tissue) cannot determine if a variant is somatic or germline.[13] However, genetic test reports should flag up significant variants that are **potentially** germline in origin.

Potential germline variants

Potential germline variants require additional investigation by:[13]

- Taking a detailed medical and family history to look for a possible familial link and confirm if confirmatory germline testing is required.
- If indicated, genetic testing of non-tumour tissue (typically a skin biopsy) to determine if the variant is somatic or germline. Depending on local arrangements and clinical urgency, this may be arranged by the haematology team or after referral to clinical genetics.

Talking to patients

Patients should be informed of any potential germline pathogenic variants, and any further testing they can be offered to investigate this.[13] They are likely to be anxious and concerned about the potential impact on their family. It's important to explain that:

- If a germline pathogenic variant is confirmed, it does not mean that other family members will definitely have it. They **may** have it, and they will be offered genetic counselling to decide if they'd like to be tested for it.
- Having the pathogenic variant does not mean that people will definitely get AML. But it does mean they have a higher chance of getting it than people without the variant.
- Some people with the pathogenic variant may get AML, others may not. It is not possible to predict what will happen in individual cases.
- Having the pathogenic variant is not due to anything the patient has or has not done.
- It is the patient's choice whether or not to have confirmatory testing.

The UK Cancer Genetics Group has developed resources for health professionals and for patients with germline variants associated with AML. They are available at UKCGG leaflets and guidelines - Cancer Genetics Group.

Confirmed germline variants

Patients with confirmed germline variants should be referred to the local clinical genetics service. They will explain the potential significance of the variant, and implications for close family members.

If a patient has a confirmed germline pathogenic variant, other close family members (parents, children and siblings) may also carry it. This means they may be at increased risk of developing AML during their lifetime.

- It is recommended that patients with germline pathogenic variants tell close family members about the possible risk. The clinical genetics team can help support patients to talk to their family.
- Adult family members can be referred to the clinical genetics team for genetic counselling to help them decide if they wish to be tested for the variant.[13]
- Predictive testing in children should be decided on a case-by-case basis depending on the clinical circumstances.[13]
- Family members who are found to have the variant may be referred to a haematologist for follow-up.[13]
- They should be made aware of symptoms to look out for.
- They may be offered screening blood tests, but this is not routinely available and there is little evidence to support its use.[13]

Leukaemia Care has developed a patient booklet about genetics in AML that covers germline variants. We have also developed a factsheet for people with AML and a separate factsheet for family members.

Germline variants and stem cell transplants

Germline variants can complicate donor selection in patients who need an allogeneic stem cell transplant (SCT).

- Stem cells from a close family member could also carry the germline pathogenic variant, which has a risk of leading to a donor-derived leukaemia in the future.[19]
- Potential related donors of AML patients with germline pathogenic variants should be offered urgent genetic counselling and potential predictive testing in a time-sensitive way.
    - A family member who has the germline pathogenic variant would not usually be considered as a potential stem cell donor unless other options were limited.[19]
    - If a potential related donor declines testing, the risks and benefits of using their stem cells should be discussed at a multidisciplinary team meeting (MDT).[19]
    - If all potential related donors carry the germline pathogenic variant, the risks and benefits of using their stem cells over those of an unrelated donor should be discussed at an MDT.[19]
- A search for an unrelated donor should also be conducted to allow different options to be considered.[19]

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