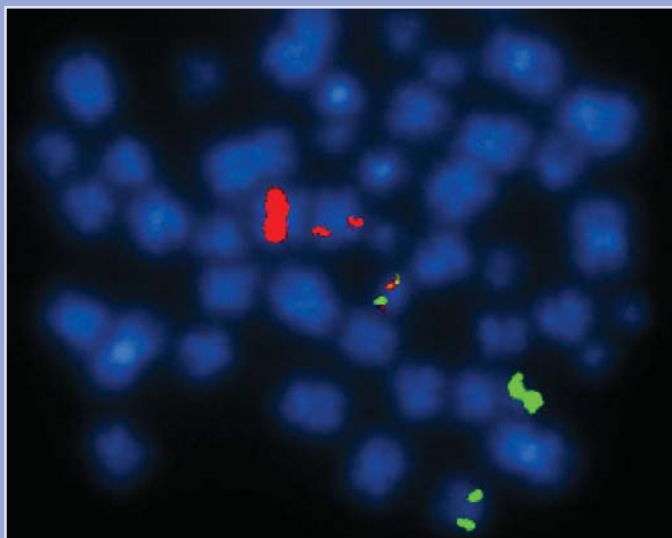
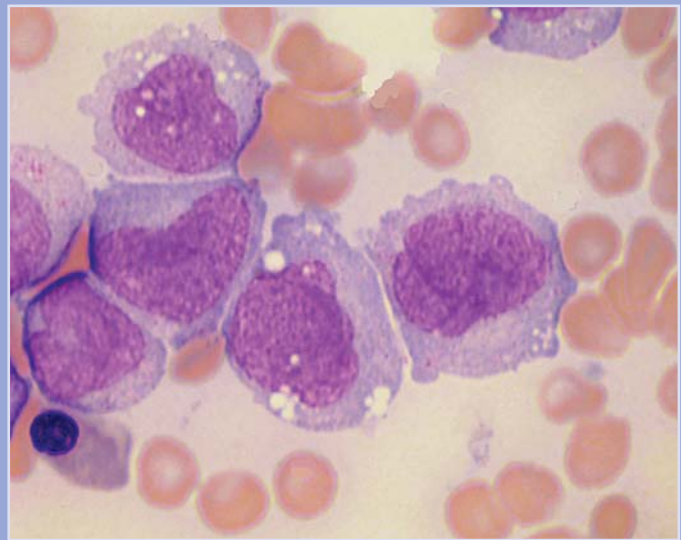
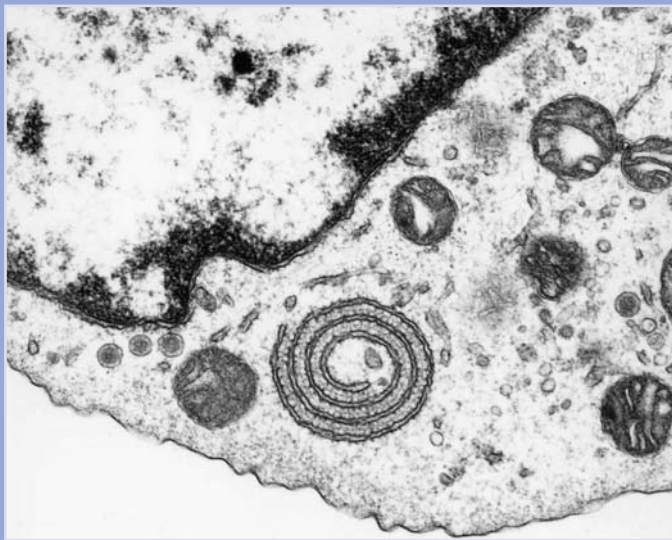


An Atlas of Investigation and Diagnosis

MYELOID MALIGNANCIES

Barbara J Bain • Estella Matutes



CLINICAL PUBLISHING

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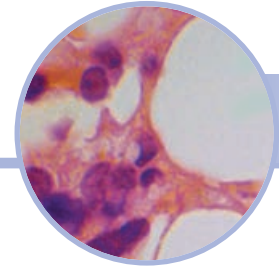
FAB group, and that of Professor John Goldman, in the field of haematological malignancy, over the last 40 years. They and other colleagues at St Mary's Hospital, Hammersmith Hospital, and the Royal Marsden Hospital have generously shared their knowledge with us.

Barbara J Bain
Estella Matutes

Abbreviations

aCML atypical chronic myeloid leukaemia
ALIP abnormal localization of immature precursors
ALL acute lymphoblastic leukaemia
AML acute myeloid leukaemia
ATRA all-*trans*-retinoic acid
BM bone marrow
c cytoplasmic
CAE chloroacetate esterase
CD cluster of differentiation
CEL chronic eosinophilic leukaemia
CML chronic myeloid leukaemia
CMML chronic myelomonocytic leukaemia
CNS central nervous system
DIC disseminated intravascular coagulation
FAB French–American–British
FISH fluorescence *in situ* hybridization
FSC forward light scatter
G-CSF granulocyte colony-stimulating factor
H&E haematoxylin and eosin
Hb haemoglobin concentration
Hct haematocrit
HIV human immunodeficiency virus
ICUS idiopathic cytopenia of undetermined significance
IPSS international prognostic scoring system
ITD internal tandem duplication
JMD juxtamembrane domain
JMML juvenile myelomonocytic leukaemia
LDC lymphoid dendritic cell

MDS myelodysplastic syndrome/syndromes
MGG May–Grünwald–Giemsa
MPD myeloproliferative disorder/disorders
MPN myeloproliferative neoplasm/neoplasms
MPO myeloperoxidase
NK natural killer
NSE non-specific esterase
PAS periodic acid-Schiff
PCR polymerase chain reaction
Ph Philadelphia
PTD partial tandem duplication
RAEB refractory anaemia with excess blasts
RARS-T refractory anaemia with ring sideroblasts and thrombocytosis
RBC red cell count
RCMD refractory cytopenia with multilineage dysplasia
RCMD-RS refractory cytopenia with multilineage dysplasia and ringed sideroblasts
RCUD refractory cytopenia with unilineage dysplasia
RT-PCR reverse transcriptase polymerase chain reaction
RQ-PCR real time quantitative polymerase chain reaction
SBB Sudan black B
SSC sideways light scatter
TdT terminal deoxynucleotidyl transferase
TKD tyrosine kinase domain
WBC white cell count
WHO World Health Organization
wt wild type



Molecular basis and classification of myeloid neoplasms

Normal haemopoiesis

In the adult, normal haemopoiesis occurs predominantly in the bone marrow, although haemopoietic stem cells circulate in the blood stream and the potential for haemopoiesis in liver, spleen or other tissues is retained. All blood cells are derived ultimately from a pluripotent haemopoietic stem cell, able to give rise to lymphoid and myeloid lineages [1]. The pluripotent stem cells are capable not only of self renewal but also of generating multipotent myeloid stem cells and the common lymphoid stem cells

(**Figure 1.1**). The multipotent stem cell gives rise in turn to committed progenitor cells from which cells of the major myeloid lineages are derived. Differentiation and maturation are controlled by a variety of cytokines which are to some extent specific for particular cell lines. In addition, the microenvironment and accessory cells such as fibroblasts and fat cells have a role in the differentiation and maturation of stem cells. Cells of haemopoietic origin include mast cells and osteoclasts.

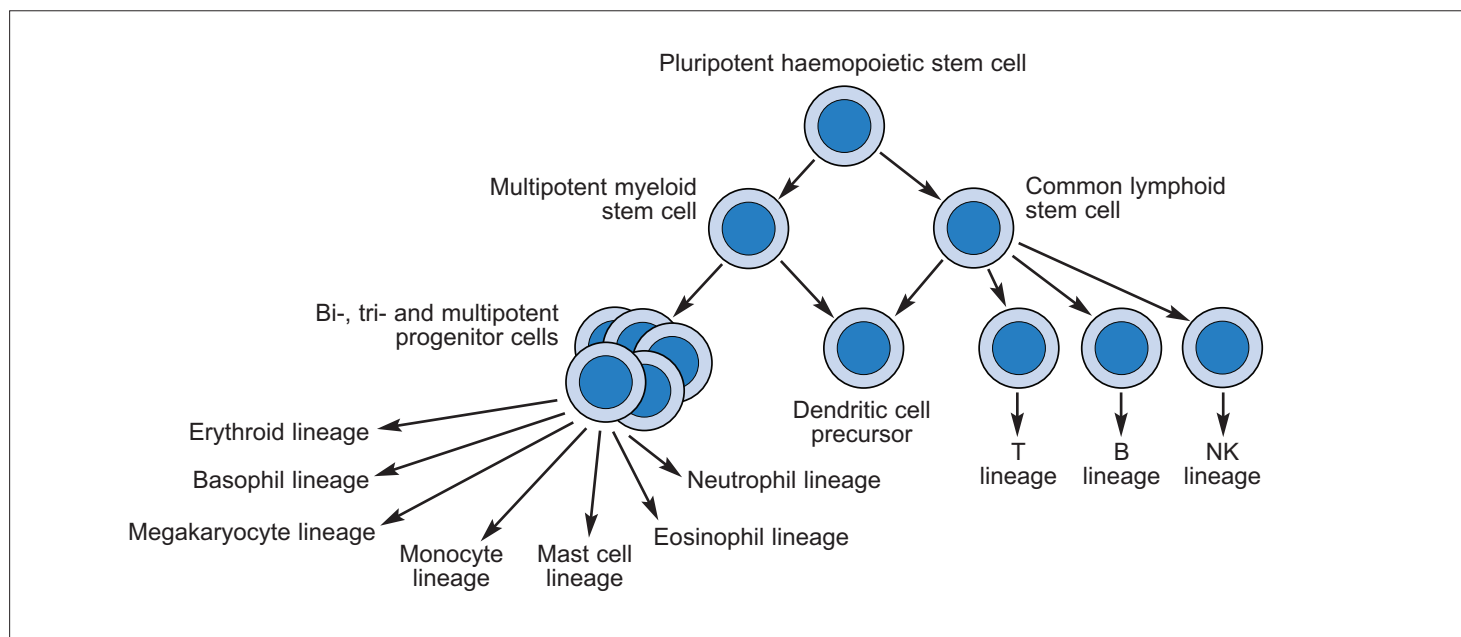


Figure 1.1 A diagram of the stem cell hierarchy and myeloid and lymphoid differentiation pathways. Abbreviation: NK, natural killer.

2 Molecular basis and classification of myeloid neoplasms

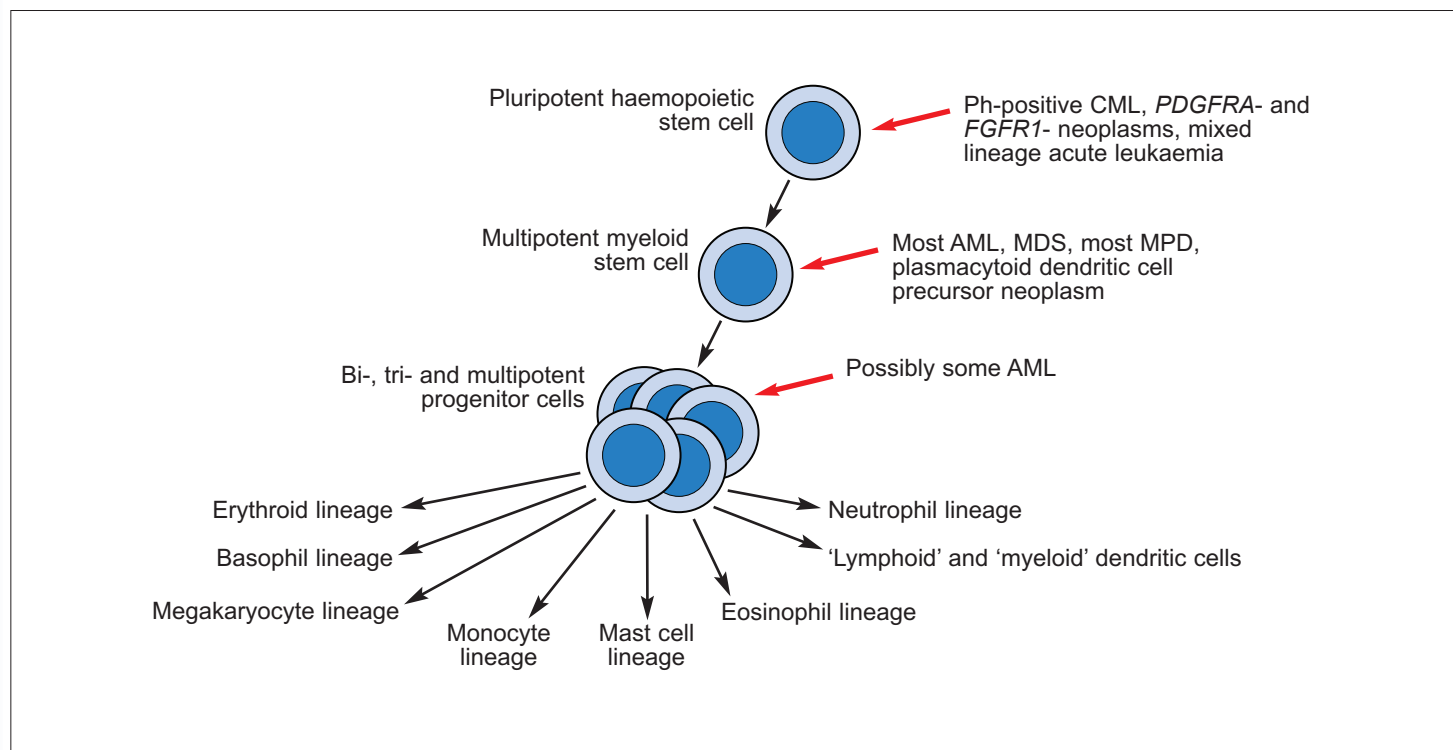


Figure 1.2 A diagram of the stem cell hierarchy and myeloid differentiation pathways showing the cell in which the causative mutation appears to occur in various haematological neoplasms. Abbreviations: AML acute myeloid leukaemia; CML chronic myeloid leukaemia; MDS myelodysplastic syndrome(s); MPD myeloproliferative disorder(s).

Myeloid neoplasms arise from mutation in a haemopoietic stem cell or progenitor cell (Figure 1.2). Many neoplasms, including most types of acute myeloid leukaemia (AML) and the myelodysplastic syndromes (MDS) arise from a mutated multipotent stem cell. Some chronic myeloid leukaemias arise from mutation in a pluripotent stem cell so that at one stage of the disease the leukaemia may manifest itself as a lymphoid leukaemia or lymphoma. This is true of Philadelphia (Ph)-positive chronic myeloid leukaemia associated with a *BCR-ABL1* fusion gene (in which B-lineage and less often T-lineage blast transformation can occur) and of *FGFR1*-related neoplasms, which at various stages of the disease may be manifest as chronic eosinophilic leukaemia, T-lineage lymphoblastic leukaemia/lymphoma, B-lineage lymphoblastic leukaemia/lymphoma or AML. It is possible that some subtypes of AML arise in a mutated committed progenitor cell without the capacity to differentiate into cells of erythroid or megakaryocyte lineages.

The molecular basis of haematological neoplasms

In common with other neoplasms, haematological neoplasms can be viewed as acquired genetic diseases in the sense that they result from genetic alteration in a stem cell that gives rise to an abnormal clone of cells, the behaviour of which is responsible for the disease phenotype. The host immune response also has a role in disease development since the body's immune response includes some ability to recognize tumour cells and destroy them.

Classification of haematological neoplasms

Classification of haematological neoplasms is moving from a period when classification was largely based on clinicopathological features, including morphology and, to a

lesser extent, immunophenotype, to a period when definitions are based to some extent on identified molecular abnormalities. Although certain syndromes are defined mainly on the basis of the genetic abnormality these must be interpreted in the light of the clinicopathological features. Thus $t(9;22)(q34;q11)$ and *BCR-ABL1* fusion are the hallmark of chronic myelogenous leukaemia (CML) but they can also be observed in acute lymphoblastic leukaemia (ALL) and, uncommonly, AML. Similarly $t(15;17)(q22;q12)$ is the hallmark of acute promyelocytic leukaemia, including its variant form but can be observed, albeit rarely, in transformation of a chronic myeloproliferative neoplasm (MPN). The conditions that are defined largely on a molecular basis are CML, the *FIP1L1-PDGFRB* syndrome and MPD associated with rearrangement of *PDGFRB* and *FGFR1* genes. A second group of disorders are currently defined on the basis of clinicopathological/morphological features supplemented by cytogenetic/molecular genetic information. This applies to AML, MDS, polycythaemia vera, essential thrombocythaemia, primary myelofibrosis, systemic mastocytosis and juvenile myelomonocytic leukaemia (JMML). There remains a third group of disorders where the disease definition is essentially based on clinicopathological/morphological features, even though relevant cytogenetic/molecular genetic abnormalities are sometimes found. At present chronic myelomonocytic leukaemia (CMML) and atypical chronic myeloid leukaemia (aCML) fall into this group. Although MDS has been placed in the second group, there is only a single cytogenetically defined entity and otherwise its definition remains largely clinicopathological and morphological; it has long been suspected that specific genetic abnormalities should be identifiable in subgroups of MDS but these have been slow to reveal themselves.

Myeloid neoplasms have been classified by various expert groups under the aegis of the World Health Organization (WHO) as shown, in simplified form, in *Table 1.1* (overleaf).

Oncogenic mechanisms

Oncogenic mechanisms differ between the chronic MPN and AML. The essential difference between the genetic events in the two groups of disorders is that in MPN they result in an expanded clone of proliferating cells able to

differentiate into end cells of one or more myeloid lineages, whereas in AML cells continue to proliferate but are mainly unable to differentiate to end cells.

Mutations in myeloid malignancies include novel fusion genes and mutated genes. Fusion genes can result from a translocation, inversion, insertion or cryptic deletion. Mutated genes may harbour a point mutation, a partial duplication or a small insertion or deletion that alters the reading frame. Genes can be triplicated as the result of trisomy. Genes can be amplified (multiple copies) in double minute chromosomes or in homogeneously staining regions within chromosomes. There can also be epigenetic effects, such as an altered methylation status that alters gene expression. All these changes are related to the formation or activation of oncogenes. In addition, deletion or inactivation of tumour suppressor genes can contribute to oncogenesis.

In MPN there is often a mutation in a gene encoding a protein on a signalling pathway between the surface membrane and the nucleus; often this protein is a tyrosine kinase that becomes constitutively activated as a result of the mutation. The neoplastic cells are thus able to proliferate and differentiate without being dependent on growth factors. Examples of such constitutively activated tyrosine kinases include the product of the *BCR-ABL1* fusion gene in CML, and the product of a mutated *JAK2* gene (*JAK2* V617F) in almost all cases of polycythaemia vera and in some cases of essential thrombocythaemia, primary myelofibrosis and refractory anaemia with ring sideroblasts and thrombocytosis (RARS-T).

In AML there appears to be a need for at least two mutations to convey the leukaemic phenotype to the neoplastic cells and in some types of AML there are multiple mutations. Particularly in AML with multilineage dysplasia, secondary AML, therapy-related AML and AML in the elderly there are likely to have been multiple mutational events (which can include those leading to loss of activity of tumour suppressor genes). The first genetic subtypes of AML recognized were those associated with recurrent cytogenetic abnormalities that gave rise to fusion genes. Specifically these were: $t(15;17)(q22;q12)$ associated with a *PML-RARA* fusion gene; $t(8;21)(q22;q22)$ associated with *RUNX1-CBFA2T1*; and either $inv(16)(p13q22)$ or $t(16;16)(p13;q22)$ associated with *CBFB-MYH11*. Each of these subtypes was found to have characteristic cytological features. More recently, genetic subtypes of AML have been recognized, mainly among patients with normal cytogenetic analysis, that are characterized by gene mutation without

4 Molecular basis and classification of myeloid neoplasms

Table 1.1 An overview of the classification of myeloid neoplasms

<i>Category</i>	<i>Important subcategories</i>
Acute myeloid leukaemia (AML)	Therapy-related myeloid neoplasms AML with recurrent cytogenetic/genetic abnormalities AML with myelodysplasia-related changes AML not otherwise categorized
The myelodysplastic syndromes (MDS)	Refractory cytopenia, including refractory anaemia, with unilineage dysplasia Refractory anaemia with ring sideroblasts Refractory cytopenia with multilineage dysplasia (with or without ring sideroblasts) Refractory anaemia with excess blasts 5q- syndrome Myelodysplastic syndrome, unclassifiable Childhood myelodysplastic syndrome
Myeloproliferative neoplasm (MPN)	Chronic myelogenous leukaemia (with <i>BCR-ABL1</i> fusion gene) Chronic neutrophilic leukaemia (occasionally associated with <i>JAK2</i> V617F mutation) Chronic eosinophilic leukaemias and other chronic myeloid leukaemias (including those associated with rearrangement of the <i>PDGFRA</i> , <i>PDGFRB</i> and <i>FGFR1</i> genes)* Polycythaemia vera (usually has <i>JAK2</i> V617F mutation) Essential thrombocythaemia (often has <i>JAK2</i> V617F mutation) Myelofibrosis (often has <i>JAK2</i> V617F mutation) Mast cell disease Cutaneous mastocytosis including urticaria pigmentosa Systemic mastocytosis (usually associated with <i>KIT</i> ^{D816V} mutation) Mast cell leukaemia
The myelodysplastic/myeloproliferative neoplasms (MDS/MPN)	Chronic myelomonocytic leukaemia Atypical chronic myeloid leukaemia Juvenile myelomonocytic leukaemia (often associated with either <i>PTPN11</i> or <i>NF1</i> or <i>RAS</i> mutation)

* In the WHO 2008 classification, myeloid and lymphoid neoplasms associated with rearrangement of neoplasms *PDGFRA*, *PDGFRB* and *FGFR1* are assigned to a separate category.

chromosomal rearrangement. Specifically these are associated with mutations in either *NPM1* [2] or *CEBPA* [3]. Neither is associated with distinctive cytological features. It has been postulated that for any case of AML

there is a need for two different types of mutation, one designated type I to indicate a mutation that conveys a proliferation or survival advantage to the cells and another, designated type II, which interferes with differentiation [4].

Table 1.2 Type I and type II mutations that can interact in the pathogenesis of AML

<i>Class II mutation (interferes with differentiation)</i>	<i>Class I mutation (conveys proliferation or survival advantage) (reported incidence in subtype shown in brackets)</i>
<i>RUNX1-CBFA2T</i> , usually resulting from t(8;21)(q22;q22)	<i>KIT</i> mutation (12–47% of cases) <i>NRAS</i> (c 10%) <i>FLT3-ITD</i> (c 4%)
<i>CBFB-MYH11</i> , usually resulting from inv(16)(p13q22) or t(16;16)(p13;q22)	<i>NRAS</i> (c 30–40%) <i>FLT3-ITD</i> (c 7%) <i>KIT</i> mutation (22–47% of cases)
<i>PML-RARA</i> , usually resulting from t(15;17)(q22;q12)	<i>FLT3-ITD</i> (c 30%) <i>NRAS</i> (c 2%)
<i>CEBPA</i> mutated	<i>FLT3-ITD</i>
<i>NPM1</i> mutated	<i>FLT3-ITD</i>
ITD, internal tandem duplication	

Type I and type II mutations are associated with each other in a non-random manner. It is the type II mutation that can be related to the clinical and haematological phenotype of the disease but the type I mutation is also likely to be essential for leukaemogenesis and often affects prognosis (Table 1.2).

In MDS, multiple genetic events occur, which can include changes in oncogenes and tumour suppressor genes. These processes are generally poorly understood. The net result is continuing cell proliferation but with ineffective haemopoiesis, i.e. with an increased rate of apoptotic death of haemopoietic cells in the bone marrow and a resultant failure of production of adequate numbers of end cells. The only subtype of MDS so far linked to a specific cytogenetic abnormality is the 5q- syndrome, in which there is an interstitial deletion of part of the long arm of chromosome 5; several candidate genes that are often deleted have been identified of which *RPS14* appears the most likely to be relevant [5]. A deletion of the tumour suppressor gene *TP53* at 17p13.1 occurs in some patients with MDS.

Aetiology

The aetiology of most instances of myeloid neoplasms is unknown. AML, MDS and MDS/MPN can result from exposure to radiation, anticancer chemotherapy and chemical carcinogens such as benzene. Cigarette smoking also increases the incidence of AML. CML can follow exposure to irradiation or topoisomerase-II-interactive drugs. Genetic predisposition also has an aetiological role. Down's syndrome predisposes to transient leukaemia in the neonatal period and to acute megakaryoblastic leukaemia in infants. Inherited defects in proto-oncogenes can predispose to leukaemia, e.g. germline mutation in *RUNX1* and in *CEBPA* predispose to AML. Germline mutation of *NF1* in neurofibromatosis type 1 and of *PTPN11* in Noonan syndrome predispose to JMML. Inherited defects in tumour suppressor genes likewise predispose to various types of leukaemia. Germline mutation of *TP53* in the Li Fraumeni syndrome, of *RBI* in familial retinoblastoma families and of *WT1* in familial Wilms' tumour families predispose to AML.

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