Pediatric Acute Myeloid Leukemia

Diagnosis, classification & molecular work-up

F.Malek M.D SBMU MCH

Learning objectives

ORIENTATION

1. **Recognize** the clinical and laboratory patterns that should trigger an AML work-up
2. **Differentiate** myeloblasts from lymphoblasts using morphology, cytochemistry, and flow
3. **Classify** AML using FAB as historical shorthand and WHO-HAEM5 / ICC as the modern framework
4. **Select** the minimum cytogenetic and molecular studies needed at diagnosis
5. **Identify** CNS, metabolic, coagulopathic, and germline-predisposition issues that alter immediate management

How to use the uploaded textbook pages

FRAMING

Classic framework + modern update

What remains highly useful

Clinical presentation
Morphology and cytochemistry
FAB vocabulary
Classic cytogenetic associations

What must be updated

WHO 2008 categories
Fixed 20% blast rule
“AML-NOS” hierarchy
Older gene names and risk assumptions

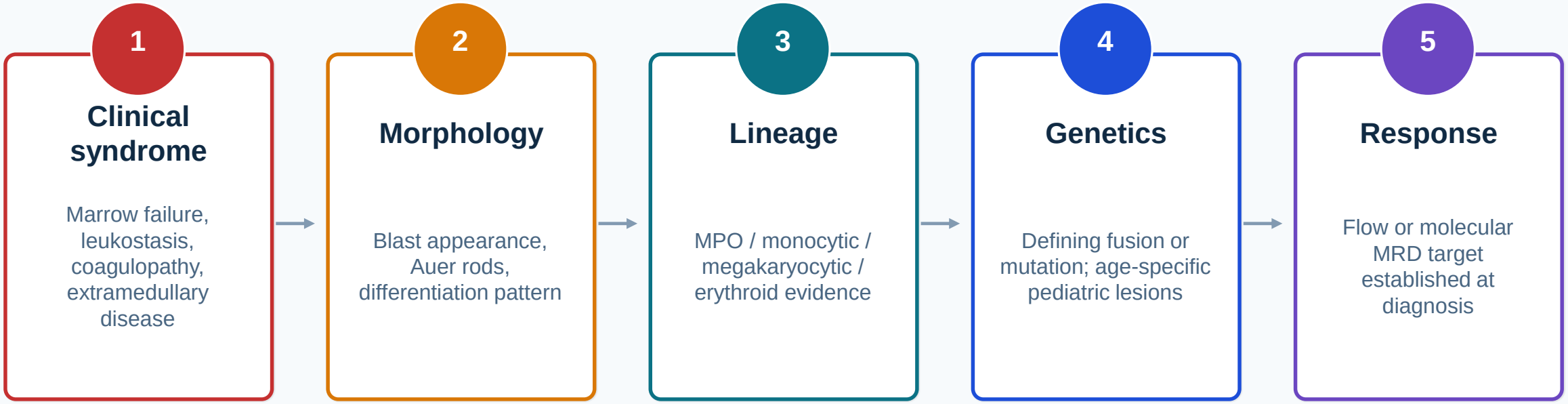
Teaching principle

Use morphology to recognize the disease, immunophenotype to assign lineage, and genetics to define the entity.

Uploaded source: Lanzkowsky’s Manual of Pediatric Hematology and Oncology, Chapter 19, pp. 391–396. Current update: WHO-HAEM5 (2022) and ICC (2022).

AML is a family of biologically distinct diseases

CONCEPT



A complete diagnosis should answer: “What is it, what defines it, and how will we measure it?”

Conceptual synthesis based on the uploaded chapter and WHO-HAEM5 / ICC 2022.

Inherited predisposition to pediatric AML

PREDISPOSITION

Chromosome / DNA repair	Down syndrome; Fanconi anemia; Bloom syndrome; ataxia-telangiectasia; Li-Fraumeni syndrome
Bone-marrow failure	Severe congenital neutropenia; dyskeratosis congenita; Shwachman–Diamond syndrome
Platelet / hematopoietic genes	RUNX1, ETV6, ANKRD26, CEBPA, GATA2 and other germline predisposition genes
RAS / developmental syndromes	NF1 and RAS-pathway disorders; SAMD9/SAMD9L syndromes

The source text correctly highlights syndromes

Modern practice extends this to gene-defined predisposition categories and donor-selection implications.

Why it matters

Conditioning intensity, surveillance of family members, organ toxicity, and related-donor safety may change.

Do not use blood for confirmation

When a germline variant is suspected, confirm in non-hematopoietic tissue—commonly cultured skin fibroblasts.

Sources: uploaded Table 19.2; WHO-HAEM5 (Khoury et al., Leukemia 2022); ICC (Arber et al., Blood 2022); NCI PDQ.

Acquired and treatment-related predisposition

PREDISPOSITION

Pre-existing marrow disorders

- Myelodysplastic syndrome
- Aplastic anemia
- Myeloproliferative neoplasm
- Acquired amegakaryocytic thrombocytopenia
- Paroxysmal nocturnal hemoglobinuria

Prior cytotoxic exposure

- Alkylating agents
- Topoisomerase-II inhibitors / epipodophyllotoxins
- Ionizing radiation

Latency, cytogenetic pattern, and prior therapy should be recorded as diagnostic qualifiers.

Modern classification point

WHO-HAEM5 and ICC prioritize the genetically defined AML entity first. “Post-cytotoxic therapy” or “therapy-related” is then added as a qualifier rather than replacing the biologic diagnosis.

Sources: uploaded Table 19.2; WHO-HAEM5 and ICC 2022.

When should a fellow suspect germline predisposition?

PREDISPOSITION

Family pattern	MDS/AML, thrombocytopenia, marrow failure, or early-onset cancers across generations
Personal phenotype	Long-standing cytopenias, abnormal platelets, immunodeficiency, lymphedema, deafness, pulmonary fibrosis, café-au-lait lesions
Age / genotype mismatch	Very young age, monosomy 7, GATA2/SAMD9/SAMD9L-type presentation, or unexpectedly persistent “somatic” variant
Transplant implications	Related donor under consideration or donor with unexplained cytopenia / similar phenotype

Clinical rule: germline evaluation should begin before selecting a related stem-cell donor.

Sources: WHO-HAEM5 / ICC 2022; NCI PDQ.

Clinical presentation: five domains

CLINICAL

Marrow failure

Pallor, fatigue, infections, bruising, mucosal bleeding

Proliferative burden

Leukocytosis, leukostasis, respiratory or neurologic compromise

Coagulopathy

Especially APL: DIC, hypofibrinogenemia, severe bleeding

Extramedullary disease

Skin, gingiva, orbit, bone, nodes, myeloid sarcoma

CNS involvement

Headache, cranial neuropathy, or asymptomatic CSF disease

Uploaded chapter: broad presentation from fever/anemia/thrombocytopenia to life-threatening coagulopathy and extramedullary disease.

Extramedullary disease and CNS assessment

CLINICAL

10–20%

Historical estimate of extramedullary disease in pediatric AML

Skin / subcutis

Leukemia cutis

Orbit / bone

Myeloid sarcoma

Gingiva

Monocytic differentiation

CNS / CSF

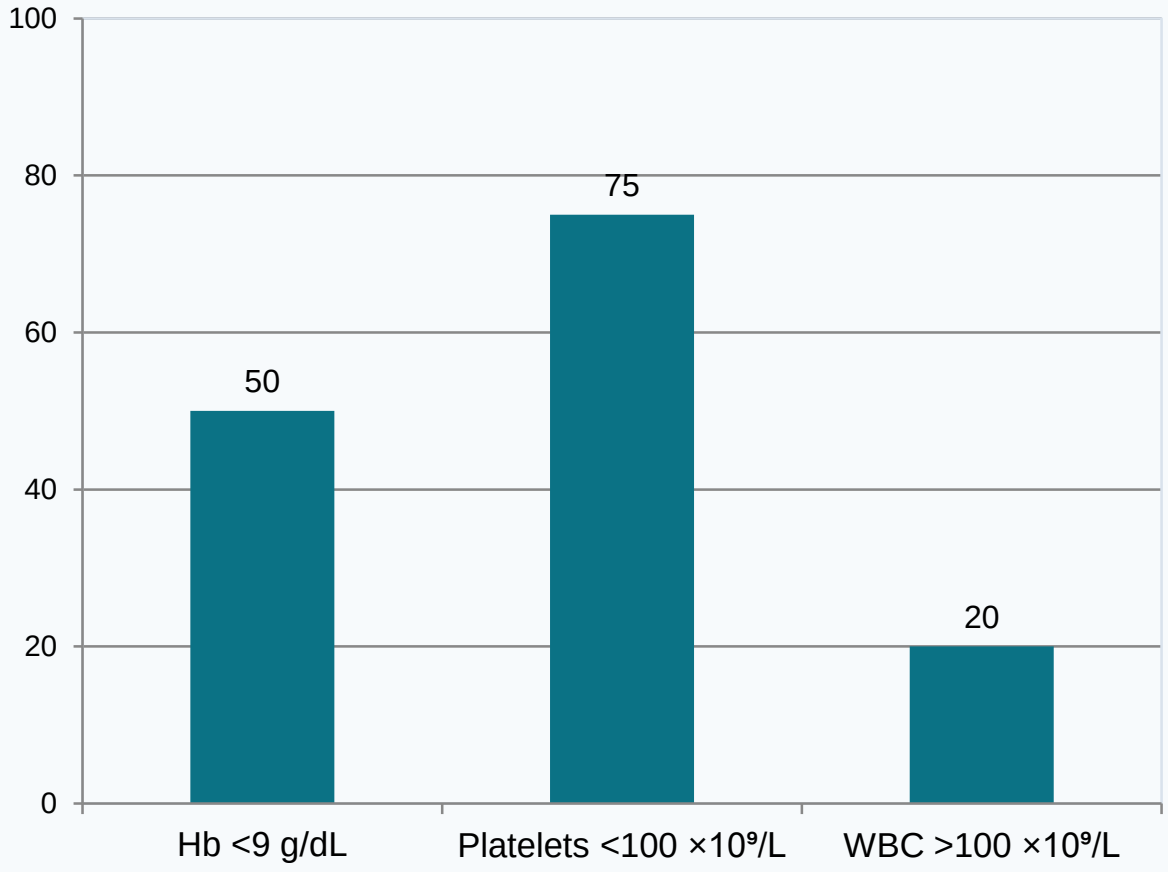
May be asymptomatic

Lumbar puncture timing is protocol-specific; stabilize coagulopathy and avoid a traumatic procedure when unsafe.

Uploaded source: EMD ≈10–20%; CNS disease reported in ~5–10% historically. Current management follows protocol and clinical stability.

Initial laboratory pattern—and what is immediately dangerous

CLINICAL



Hyperleukocytosis / leukostasis

Clinical symptoms matter more than the number alone. Evaluate respiratory, neurologic, renal, and ocular compromise.

Tumor lysis risk

Send potassium, phosphate, calcium, uric acid, creatinine, LDH; start protocol-appropriate prophylaxis and monitoring.

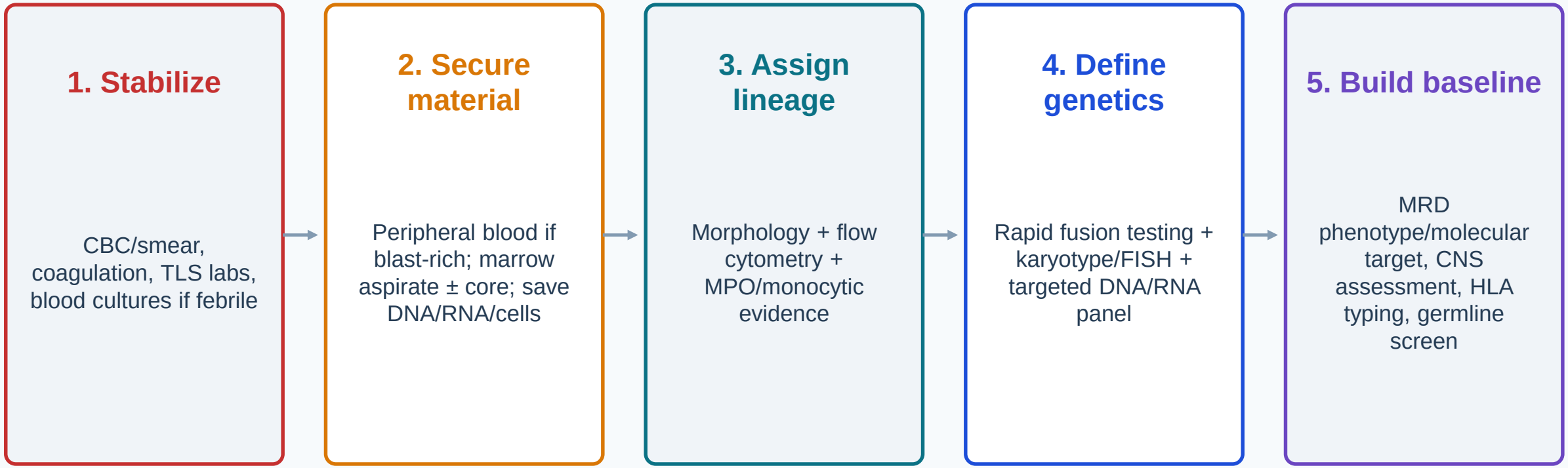
Coagulation emergency

PT, aPTT, fibrinogen, D-dimer—especially when APL is possible. Treat suspicion urgently while confirming PML::RARA.

Historical frequencies from the uploaded textbook page; individual cohorts vary.

Diagnostic workflow for suspected pediatric AML

DIAGNOSIS



Do not consume all diagnostic tissue before cytogenetic, RNA-fusion, and MRD studies are secured.

Diagnostic synthesis based on WHO-HAEM5, ICC, and NCI PDQ.

Morphology: lymphoblast versus myeloblast

DIAGNOSIS

Feature	Lymphoblast	Myeloblast
Typical size	10–20 µm	14–20 µm
Chromatin	Smooth, homogeneous	Loose / spongy meshwork
Nucleoli	0–2, often indistinct	2–5, often distinct
Nuclear contour	Usually smooth	May be irregular
N:C ratio	High	Lower
Cytoplasm	Thin blue rim	More abundant, blue-gray
Granules / Auer rods	Absent	May be present

Morphology is a hypothesis

Auer rods strongly support myeloid differentiation, but their absence does not exclude AML.

Lineage requires integration

Use flow cytometry, cytochemistry, and genetics—especially in minimally differentiated or mixed-phenotype cases.

Table reconstructed from the uploaded textbook page 392.

Cytochemistry: still useful, but no longer sufficient

DIAGNOSIS

Myeloperoxidase (MPO)

Strong evidence of myeloid lineage; may be negative in AML with minimal differentiation or megakaryoblastic/erythroid AML

Sudan black B

Parallels myeloid granule content; historical adjunct where flow is limited

Nonspecific esterase

Supports monocytic differentiation; interpret with immunophenotype and morphology

PAS / other stains

Selected differential diagnoses; largely secondary to contemporary flow and genetics

Current practice: cytochemistry supports lineage; flow and molecular genetics establish the entity.

Uploaded source mentions histochemistry as part of detailed classification.

Flow cytometry: lineage, maturation, and an MRD fingerprint

DIAGNOSIS

Lineage / stage	Key markers (examples)	Teaching caveat
Immaturity	CD34, CD117, HLA-DR, TdT	Variable; APL often lacks CD34/HLA-DR
Myeloid	MPO, CD13, CD33, CD117, CD15	MPO is the strongest lineage marker
Monocytic	CD64, CD14, CD11c, lysozyme, NSE	Require a coherent monocytic pattern
Megakaryocytic	CD41, CD42b, CD61	Exclude platelet adherence artifact
Erythroid	Glycophorin A, hemoglobin, CD71	Modern entities differ from historic FAB M6

Record the diagnostic leukemia-associated immunophenotype before therapy for flow-MRD comparison.

Current lineage concepts: WHO-HAEM5 / NCI PDQ; classic marker associations also reflected in uploaded Table 19.9.

Acute leukemia of ambiguous lineage: current lineage criteria

DIAGNOSIS

Myeloid lineage

MPO expression
OR
Monocytic differentiation with ≥2 of: NSE, CD11c, CD14, CD64, lysozyme

T lineage

Cytoplasmic or surface CD3 with lineage-appropriate intensity

CD2, CD5, or CD7 alone are not lineage-defining.

B lineage

Strong CD19 + ≥1 strong B marker
OR
Weak CD19 + ≥2 strong markers among CD10, CD22, CD79a

Current criteria: WHO-HAEM5 (2022), summarized in NCI PDQ. The uploaded page shows the older 2008 criteria.

Blast threshold: the key historical-to-modern change

CLASSIFICATION

Framework	General rule	Important exceptions / nuance
WHO 2008 (source)	≥20% blasts	APL, RUNX1::RUNX1T1, and CBFB::MYH11 could be AML regardless of blast percentage
WHO-HAEM5 (2022)	No fixed threshold for most AML-defining genetic abnormalities	Requires increased blasts and clinicopathologic correlation; BCR::ABL1 and CEBPA-mutated AML retain ≥20%
ICC 2022	Usually ≥10% for recurrent genetic abnormalities	≥20% remains for BCR::ABL1 and non-genetically-defined categories; 10–19% may be MDS/AML in other settings

Clinical implication: do not delay a genetically defined AML diagnosis merely because the blast count is below 20%.

Sources: uploaded page 392; WHO-HAEM5 (Khoury et al., 2022); ICC (Arber et al., 2022).

FAB classification: historical morphologic shorthand

CLASSIFICATION

M0	Minimal differentiation
M1	Without maturation
M2	With maturation
M3	Acute promyelocytic leukemia
M3v	Microgranular APL
M4	Myelomonocytic
M4Eo	Myelomonocytic with eosinophilia
M5	Monocytic
M6	Erythroid (historical category)
M7	Megakaryoblastic

Use FAB to describe differentiation—not as the final contemporary disease label or risk group.

Reconstructed from uploaded Table 19.6.

WHO 2008 framework shown in the source

CLASSIFICATION

AML with recurrent genetic abnormalities

t(8;21), inv(16), PML::RARA, KMT2A, DEK::NUP214, MECOM, RBM15::MRTFA; provisional NPM1 and CEBPA

AML with myelodysplasia-related changes

History, cytogenetics, and morphology were combined

Therapy-related myeloid neoplasms

A separate disease category

AML, not otherwise specified

Subdivided by differentiation / FAB-like morphology

Other related entities

Myeloid sarcoma; myeloid proliferations related to Down syndrome; BPDCN

This framework is historically important but should not be copied unchanged into a current diagnostic report.

Uploaded Table 19.5 (adapted from WHO 2008).

Modern AML architecture: WHO-HAEM5 and ICC

CLASSIFICATION

WHO-HAEM5

1. AML with defining genetic abnormalities

2. AML defined by differentiation

Qualifiers: post-cytotoxic therapy, germline predisposition, antecedent myeloid disease

ICC 2022

Hierarchical approach:

- Recurrent genetic abnormalities
- TP53-mutated AML
- MDS-related gene mutations
- MDS-related cytogenetic abnormalities
- AML, NOS

Both systems prioritize genetics; differences should be stated explicitly in reports and teaching.

WHO-HAEM5: Khoury et al., Leukemia 2022. ICC: Arber et al., Blood 2022.

AML with defining genetic abnormalities

GENETICS

Entity / alteration	Typical teaching association
PML::RARA	APL; coagulopathy emergency
RUNX1::RUNX1T1	Classic t(8;21); often maturation / myeloid sarcoma
CBFB::MYH11	inv(16)/t(16;16); abnormal eosinophils
KMT2A rearrangement	Infancy; monocytic phenotype; many partners
DEK::NUP214	t(6;9); often FLT3-ITD
MECOM rearrangement	inv(3)/t(3;3); aggressive biology
RBM15::MRTFA	Infant acute megakaryoblastic leukemia
NUP98 rearrangement	Pediatric-enriched structural lesions; partner matters
NPM1 mutation / CEBPA mutation	More common in older children/adolescents; molecularly defined entities

WHO-HAEM5 / ICC 2022; pediatric context informed by NCI PDQ and Bolouri et al., Nat Med 2018.

Core-binding-factor AML

RUNX1::RUNX1T1

Cytogenetics: t(8;21)
Morphology: often AML with maturation
Clues: large blasts, salmon-pink granules, possible myeloid sarcoma
MRD: fusion transcript can be tracked

CBFB::MYH11

Cytogenetics: inv(16) or t(16;16)
Morphology: myelomonocytic with abnormal eosinophils
Clues: historical FAB M4Eo
MRD: fusion transcript can be tracked

Do not equate “favorable genetics” with low-intensity follow-up: MRD and cooperating lesions still matter.

WHO-HAEM5 / ICC; uploaded textbook Tables 19.5 and 19.10.

Acute promyelocytic leukemia: diagnose and act simultaneously

GENETICS

Suspected APL + bleeding/coagulopathy = medical emergency. Start protocol-directed ATRA while urgently confirming PML::RARA.

Morphology

Hypergranular promyelocytes, bundles of Auer rods; microgranular variant may mimic monocytic AML

Flow

Often MPO bright, CD33 bright, HLA-DR negative, CD34 often negative—patterns are not absolute

Laboratory

Fibrinogen, PT/aPTT, D-dimer, platelets; repeat frequently during stabilization

Molecular

Rapid RT-PCR/FISH for PML::RARA; preserve quantitative baseline for molecular monitoring

WHO-HAEM5 / ICC; emergency management principle consistent with pediatric APL standards.

Pediatric-enriched genetic entities and patterns

GENETICS

KMT2A rearrangements	Prominent in infants; often monocytic; fusion partner affects biology
NUP98 rearrangements	Pediatric-enriched; NUP98::NSD1 and NUP98::KDM5A are important examples
RBM15::MRTFA	Infant AMKL; historical t(1;22)
CBFA2T3::GLIS2	Non–Down syndrome AMKL; cryptic fusion, often adverse
GATA1-mutated ML-DS	Distinct Down-syndrome-associated disease; often megakaryoblastic phenotype

Pediatric AML cannot be fully characterized by an adult-only mutation panel.

Pediatric genomic context: Bolouri et al., Nature Medicine 2018; NCI PDQ; WHO-HAEM5.

NPM1, CEBPA, FLT3 and KIT: classification versus modifiers

GENETICS

Alteration	Role in current diagnosis	Fellow teaching point
NPM1 mutation	Defines an AML entity in WHO/ICC	Uncommon in young children; more frequent with increasing age
CEBPA mutation	Defines an AML entity; current definitions emphasize bZIP in-frame / appropriate mutation pattern	WHO retains a 20% blast requirement
FLT3-ITD / TKD	Usually a cooperating lesion, not the main entity name	Allelic burden, co-mutations, and protocol matter
KIT mutation	Modifier in CBF AML	Does not replace the fusion-defined diagnosis

Write the defining entity first; list cooperating mutations separately for risk, treatment, and MRD planning.

WHO-HAEM5 / ICC 2022; pediatric context: NCI PDQ.

AML, myelodysplasia-related—and therapy-related qualifiers

CLASSIFICATION

What changed from older WHO

Morphologic multilineage dysplasia alone no longer defines AML-MR. Current systems rely on a prior myeloid neoplasm and/or specified cytogenetic or molecular abnormalities.

How to report context

Name the AML entity, then add qualifiers such as:

- post-cytotoxic therapy
- germline predisposition
- antecedent MDS/MDS-MPN

This avoids losing the core biology.

WHO-HAEM5 and ICC 2022.

Immunophenotypic patterns by differentiation

DIAGNOSIS

Pattern	Common marker combination	Caution
Myeloblastic / maturation	CD13, CD33, CD117, MPO ± CD34/HLA-DR	Aberrant lymphoid markers may occur
APL	MPO bright, CD33 bright, HLA-DR−, CD34 often −	Microgranular APL can be misleading
Myelomonocytic / monocytic	CD64, CD14, CD11c, lysozyme ± CD36	MPO may be variable
Erythroid	Glycophorin A, CD71, hemoglobin	Apply current erythroid criteria
Megakaryoblastic	CD41, CD42b, CD61	Exclude platelet adherence; assess fibrosis
Minimal differentiation	Immaturity markers with weak/absent MPO	Genetics and broad flow panel are critical

Modernized interpretation of marker associations from uploaded Table 19.9.

Cytogenetic and molecular test menu at diagnosis

WORK-UP

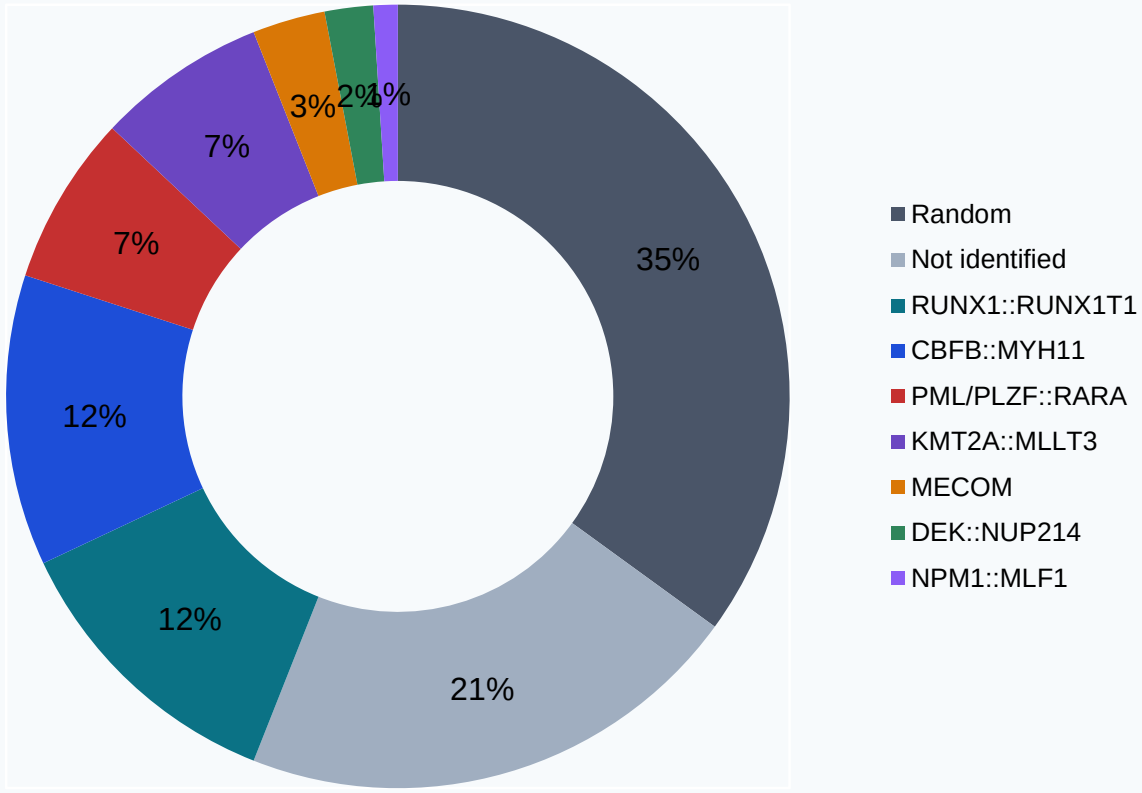
Immediate / same day	PML::RARA when suspected; rapid CBF fusion assessment when available
Core cytogenetics	Conventional karyotype + targeted FISH to detect balanced and unbalanced abnormalities
DNA testing	FLT3, NPM1, CEBPA, KIT and broad myeloid panel according to local platform
RNA fusion testing	KMT2A, NUP98, MECOM, RBM15::MRTFA, CBFA2T3::GLIS2 and other cryptic fusions
MRD preparation	Baseline flow phenotype; quantitative fusion/mutation assay when validated

A DNA-only hotspot panel can miss clinically important pediatric fusion-driven AML.

WHO-HAEM5; ICC; pediatric genomic landscape (Bolouri et al., 2018).

Historical cytogenetic distribution in the uploaded text

HISTORICAL DATA



Why this figure is useful

It shows the historical transition from “random / unidentified” karyotypes toward recurrent fusion-defined AML.

Why it must not be used as current prevalence

The data are old, gene names have changed, and modern RNA sequencing identifies additional pediatric structural lesions.

Teaching use

A historical context slide—not a contemporary epidemiology slide.

Recreated from uploaded Figure 19.1 (historical pediatric AML translocation distribution).

Baseline assessment before protocol therapy

WORK-UP

Disease burden

CBC/differential, marrow, organomegaly, EMD imaging as indicated

Metabolic

Electrolytes, creatinine, uric acid, phosphate, calcium, LDH

Hemostasis

PT, aPTT, fibrinogen, D-dimer; repeat urgently if APL suspected

Cardiac

ECG and echocardiography before anthracycline exposure

Infection

Cultures if febrile; baseline viral serology per institutional protocol

CNS

Protocol-defined LP/CSF evaluation when clinically safe

Add HLA typing, fertility counseling, and germline evaluation according to age, protocol, and clinical context.

Uploaded textbook pages 392 and 391; current practice is protocol-specific.

Fellow exercise: build the complete diagnostic label

CASE DISCUSSION

Case 1	**8-year-old; 12% marrow blasts; Auer rods; t(8;21) positive**	What is the diagnosis under WHO-HAEM5 and ICC? What MRD target should be stored?
Case 2	**18-month-old with Down syndrome; AMKL phenotype; GATA1 mutation**	How does this differ from sporadic pediatric AMKL?
Case 3	**10-year-old; 15% blasts; KMT2A rearrangement; monocytic phenotype**	Does the blast count prevent an AML diagnosis? Which classification are you using?

Suggested discussion time: 2 minutes per case, then reveal the classification logic.

Take-home messages

SUMMARY

1	**Treat the emergency before the taxonomy**	APL coagulopathy, leukostasis, tumor lysis, and sepsis cannot wait.
2	**Morphology starts the diagnosis; genetics finishes it**	FAB language is descriptive, not the final modern entity.
3	**The 20% rule is no longer universal**	Genetically defined AML may be diagnosed below 20% blasts.
4	**Pediatric AML is fusion-rich**	Use karyotype plus broad RNA-fusion testing—not a DNA-only hotspot panel.
5	**Build MRD and germline plans at diagnosis**	Secure the diagnostic phenotype, molecular target, tissue, and donor-safety assessment early.

The best diagnostic report is clinically actionable, genetically precise, and MRD-ready.

Selected references

REFERENCES

1. Uploaded textbook pages: Lanzkowsky’s Manual of Pediatric Hematology and Oncology. Chapter 19, pp. 391–396.

2. Khoury JD, Solary E, Abla O, et al. The 5th edition of the WHO Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36:1703–1719. doi:10.1038/s41375-022-01613-1.

3. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias. Blood. 2022;140:1200–1228. doi:10.1182/blood.2022015850.

4. PDQ Pediatric Treatment Editorial Board. Childhood Acute Myeloid Leukemia Treatment (PDQ®), Health Professional Version. National Cancer Institute.

5. Bolouri H, Farrar JE, Triche T Jr, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. Nat Med. 2018;24:103–112. doi:10.1038/nm.4439.

Use the current institutional / cooperative-group protocol for treatment and surveillance decisions.