EHA-SWG

Pilot Preceptorship on AML Diagnostics

Summary

October 1–3, 2025 | Perugia, Italy

eha

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Introduction

The EHA preceptorship on acute myeloid leukemia (AML), hosted by leading experts in hematology **Prof Maria Paola Martelli** and **Dr Roberta La Starza** from the Laboratory of Integrated Blood Disease Diagnostics, Hematology and Bone Marrow Transplantation Unit at the Center for Hemato-Oncology Research (CREO) in Perugia, Italy, offered participants an intensive, hands-on overview of AML diagnostics. Combining expertise in clinical hematology, laboratory diagnostics, and bone marrow transplantation, CREO – jointly operated by Azienda Ospedaliera di Perugia and the University of Perugia – provided an ideal environment for international training and collaboration.

The program followed the full AML diagnostic workflow, from clinical and hematological assessment to cytomorphology, flow cytometry, cytogenetics, and molecular testing. Attendees developed practical skills in data interpretation and integration for accurate diagnosis, classification, and risk stratification.

CREO functions as a regional and national referral center for hematologic diseases, including rare disorders such as Fanconi anemia, familial AML, telomeropathies, and acute leukemias of ambiguous lineage (ALAL). Laboratory sessions were conducted in state-of-the-art facilities, including analytical flow cytometers (FACSCalibur, FACSCanto II, LSRFortessa) and a FACSAria cell sorter, as well as dedicated areas for immunohistochemistry, cytogenetics, and genomic analysis.

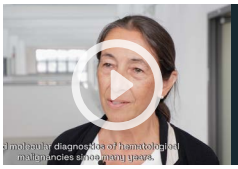
The preceptorship fostered interdisciplinary collaboration between clinicians, laboratory scientists, and researchers, reflecting CREO's mission to advance diagnostic excellence and global hematology education.

This report provides an overview of the two-and-a-half-day event. The opening section presents summaries of three lectures delivered on the first day, with the full recordings available to the public on the EHA platform. Webcasts and presentations from the remaining sessions are accessible to registered participants.

Prof Maria Paola Martelli
Hematology Clinical Immunology, CREO, UNIPG, Hematology and TMO Unit, AOPG

Dr Roberta La Starza
Hematology Clinical Immunology, CREO, UNIPG, Hematology and TMO Unit, AOPG

AML: Diagnosis and genomic-based classification (WHO and ICC) – MP Martelli



Dr Roberta La Starza
Diagnostic Classification of AML

AML is a genetically heterogenous hematologic malignancy primarily affecting older adults, with incidence peaking at age 70. Approximately 40% of younger patients achieve long-term remission, but outcomes remain poor in the elderly. In 2021, AML accounted for 1.68% of all cancer deaths in the EU, with 23,705 reported cases.[1,2]

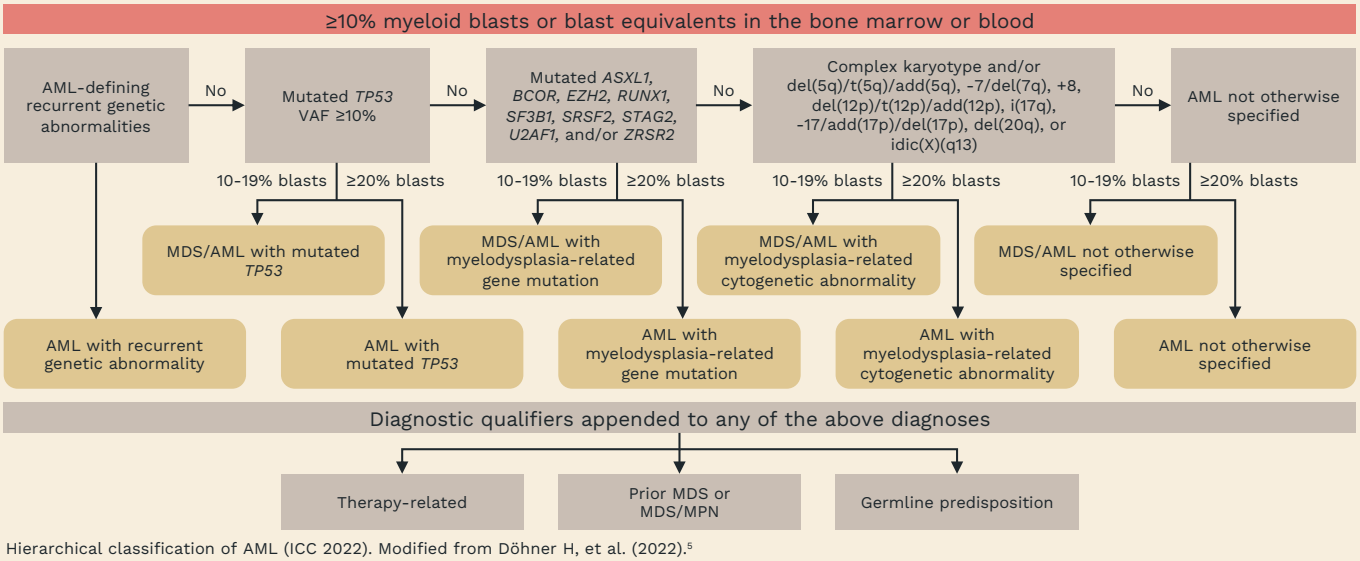
Historically based on morphology and cytochemistry, AML classification now centers on cytogenic and molecular abnormalities determining disease biology, therapy response, and prognosis. Classic karyotype alterations such as t(8;21), inv(16), and t(15;17) confer favorable risk, while complex or monosomal karyotypes indicate adverse outcomes. In 2022, two major classification systems were published: the WHO 5th Edition (WHO-HAEM5), establishing genetic abnormalities as primary diagnostic criteria, and the International Consensus Classification (ICC), which similarly emphasizes genomically defined entities but differs mainly in defining myelodysplasia (MDS)-related AML.[3,4] Revisions over time reflect expanding disease insights, driven by next-generation sequencing (NGS), which has transformed AML into a molecularly stratified malignancy. Complementing these, the European LeukemiaNet (ELN) prognostic risk models (2010–2024) categorize AML as favorable, intermediate, or adverse based on genetics.[5,6] While WHO and ICC define diagnostic entities, ELN recommendations guide risk-adapted therapy.

WHO-HAEM5 classifies AML into four categories: AML with defining genetic abnormalities, AML defined by differentiation, AML, myelodysplasia-related (AML-MR), and AML, post-cytotoxic therapy.

ICC 2022, using a genetics-first approach, designates MDS-related AML by characteristic mutations (e.g., *ASXL1*, *BCOR*, and *EZH2*) or cytogenetic abnormalities (e.g., complex karyotype, del(5q), -7), defining these as two distinct entities. It also recognizes mutated *TP53* as a separate defining genetic lesion. In contrast, WHO-HAEM5 does not recognize AML with mutated *TP53* as a distinct entity. The ICC AML classification follows a hierarchical structure: AML with recurrent genetic abnormalities; AML with mutated *TP53* (VAF ≥10%) if recurrent abnormalities are absent; AML with MDS-related mutations; AML with MDS-related cytogenetic abnormalities; and AML, not otherwise specified. This framework underpins the 2022 ELN risk model.

Recent data show therapy-related or secondary AML can mirror *de novo* cases. For example, therapy-related AML (t-AML) with *NPM1* mutation resembles *de novo NPM1*-mutated AML in phenotype and overall survival (OS), suggesting prior cytotoxic exposure may be incidental. Accordingly, ICC no longer defines t-AML as a distinct entity but considers prior cytotoxic therapy or antecedent MDS/myeloproliferative neoplasms (MPN) as diagnostic qualifiers.

Another key diagnostic divergence between WHO-HAEM5 and ICC is the blast threshold. ICC defines AML at ≥10% blasts if a defining genetic abnormality is present, otherwise ≥20%. WHO-HAEM5 generally requires ≥20% blasts, though certain disease-defining lesions have no minimum blast requirement. Clinically, blast percentage differences underlie diagnostic discordance.



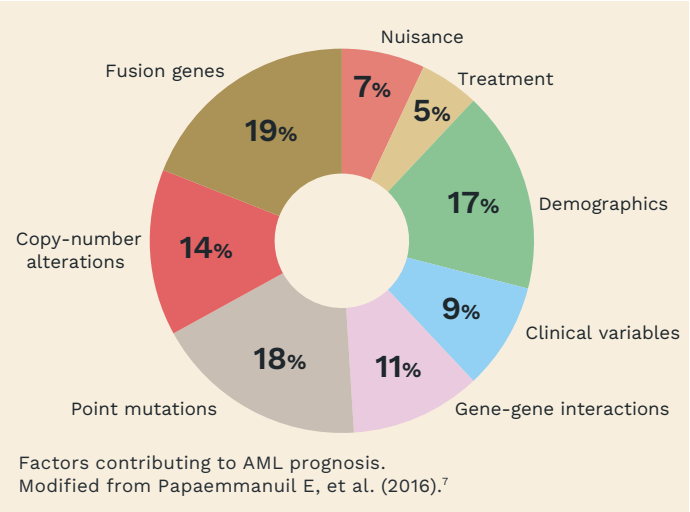
Hierarchical classification of AML (ICC 2022). Modified from Döhner H, et al. (2022).[5]

Prognostic markers and risk stratification – J Esteve

Prof Maria Paola Martelli
Prognostic Stratification of AML

Accurate prognostic assessment in AML guides treatment intensity, predicts outcomes, and informs follow-up. Prognostic markers estimate overall outcome (survival, relapse risk), while predictive markers reflect likelihood of response or resistance to therapy. Prognostic evaluation serves three purposes: (1) outcome prediction via models such as ELN 2022/2024 or emerging artificial intelligence (AI) algorithms; (2) response prediction identifying biomarkers of sensitivity to intensive chemotherapy (IC), low-intensity therapy (LIT), or allogeneic transplantation (alloHCT); and (3) treatment adaptation, where measurable residual disease (MRD) dynamics enable pre-emptive modification.

AML prognosis is multifactorial, driven by disease biology (genomics, cytogenetics) and host/treatment factors (age, comorbidity, performance, therapy). Genomic features explain roughly two-thirds of prognostic variance, with the remainder influenced by clinical parameters. Finally, the nature of prognosis is dynamic, evolving over the disease course: demographic and clinical factors dominate at diagnosis, whereas molecular and MRD-based markers predominate during remission and follow-up.



Factors contributing to AML prognosis.
Modified from Papaemmanuil E, et al. (2016).[7]

The ELN classification remains the cornerstone of genomic risk stratification in AML, evolving with accumulating molecular and clinical data. The 2022 model and 2024 update contextualize prognosis by treatment intensity, distinguishing IC- from LIT-treated patients and emphasizing therapy-dependent risk.[5,6] Genetic lesions such as *TP53*, *NPM1*, or *FLT3*-ITD carry distinct prognostic implications under IC vs. hypomethylating agent (HMA)+venetoclax (VEN) regimens. In VEN+azacitidine (AZA)–treated cohorts, a three-tier molecular signature defines response: *TP53*-WT/*FLT3*-ITD–negative/*KRAS*/*NRAS*-WT patients show the highest benefit, *TP53*-WT with *FLT3*-ITD or *KRAS/NRAS* mutations intermediate benefit, and *TP53*-mutated AML the poorest OS. ELN 2024 further identifies *IDH1/2*, *NPM1*, and *DDX41* as favorable, while *KRAS* and *PTPN11* confer new adverse risk. These refinements underscore the treatment-dependent nature of AML prognosis.

FLT3 mutations remain among the most clinically relevant alterations. The RATIFY trial demonstrated that adding midostaurin to IC improved OS in *FLT3*-mutated AML, with greatest benefit in ITD mutations limited to the juxtamembrane domain.[8] Similarly, the QuANTUM-First trial showed that quizartinib combined with frontline chemotherapy in *FLT3*-mutated AML improved OS vs. chemotherapy alone, with additional benefit when followed by alloHCT in first remission.[9]

FLT3-ITD prognosis depends on mutation subtype, allelic ratio, and insertion site; co-mutations refine risk: *NPM1* co-mutation mitigates adverse impact, whereas the *DNMT3A/NPM1/FLT3*-ITD "evil triplet" predicts poor MRD clearance and early relapse. Low-allelic-ratio *FLT3*-ITD/*NPM1* AML may not benefit from alloHCT, yet FLT3 inhibitor exposure and optimal transplant timing can modify risk.

Relapse remains the main cause of treatment failure in AML, with prognostic drivers varying by disease stage. At diagnosis, demographics and leukemia presentation dominate; in post-remission, genetic features determine relapse risk. Non-relapse mortality is largely influenced by demographic factors, while MRD status and treatment intensity drive post-remission survival.

A survival analysis of 3,653 AML patients revealed stage-specific patterns across genomic subtypes: acute promyelocytic leukemia (APL) carries high early mortality from disseminated intravascular coagulation (DIC) but excellent cure rates; inv(16) AML remains salvageable even after relapse; *TP53*-mutated or inv(3) AML have dismal survival due to resistance and relapse. Prognostic modeling must therefore account for stage, treatment, genetic risk, and relapse kinetics.

MRD is a continuous evaluator that integrates disease biology and treatment response, providing prognostic information at specific timepoints: diagnosis, post-induction, end of treatment, and during follow-up. MRD negativity is influenced by *DNMT3A* mutation, *FLT3*-ITD co-mutation, non-canonical *NPM1* mutations, and *WT1* expression. However, persistent MRD does not always indicate active leukemia. In some patients (30%), low-level MRD spontaneously clears, while others (12%) may remain molecularly positive without relapse. Conversely, relapse can occur despite prior MRD negativity,

as observed in cases of *NPM1*-mutated AML with MRD negativity 3 months prior, emphasizing the need for careful longitudinal interpretation. MRD status also guides therapeutic interventions. Protocols such as those by Dr Esteve's group use MRD dynamics to tailor treatment with VEN+AZA, adjusting therapy based on response or persistence. Similarly, the CETLAM-12 study demonstrated that early MRD-directed intervention in ELN 2017 favorable-risk *NPM1*-mutated AML improves outcomes.[10] Moreover, before alloHCT, pre-transplant MRD burden and *FLT3*-ITD status strongly predict post-transplant survival: generally, MRD negativity confers low risk, while high MRD burden or *FLT3*-ITD positivity denotes relapse risk. Therapeutic adaptation, including myeloblative conditioning (MAC)-alloHCT in MRD-positive patients (BMT CTN 0901 randomized trial) can mitigate this.[11] In contrast, reduced intensity conditioning (alloRIC) is associated with poorer survival in MRD-positive or *FLT3*-ITD AML prior to transplant. In addition, as shown in the MORPHO trial, maintenance with gilteritinib after alloHCT provides additional benefit in MRD-positive patients.[12] MRD thus serves as a dynamic prognostic corrector and guide for personalized AML management.

Traditional prognostic models in AML are limited by their reliance on linear associations and a finite number of genetic variables. Machine learning (ML) and AI-based models use large, non–hypothesis-driven datasets to capture complex, non-linear relationships between genomic, clinical, and therapeutic features. These approaches can integrate high-dimensional genomic and clinical datasets, often achieving superior predictive accuracy (e.g., C-index, AUROC) compared with traditional models. Challenges include interpretability, overfitting in small cohorts, and limited clinical adoption. Emerging applications, such as personalized AML risk calculators and molecular decision-support tools, promise to complement existing prognostic frameworks.

The backbone of AML-oriented therapy and new tailored compounds – C Papayannidis

Prof Maria Paola Martelli
Genetic-Targeted Treatment in AML

AML therapy requires alignment of treatment with patient fitness. Standard IC (e.g., 3+7) confers higher response but also higher toxicity, particularly in older or comorbid patients. Fitness assessment, integrating physical, comorbid, cognitive, and biological domains, remains the initial step guiding selection between IC and LIT. Increasingly, fitness is interpreted in the genomic context, since biological stratification is mandatory in the era of targeted agents. Minor treatment delays to obtain key molecular results (*FLT3*, *NPM1*, *IDH1/2*) are acceptable in most patients, except in cases of APL, severe hyperleukocytosis, or critical presentation.

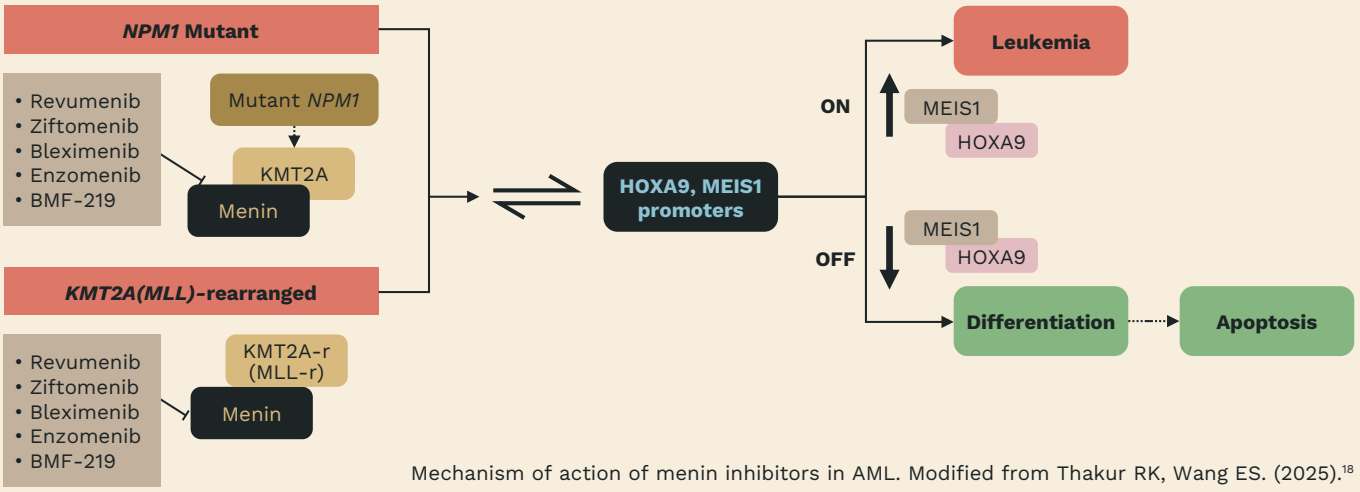
Therapeutic approaches in patients with ND AML unfit for IC

For newly diagnosed (ND) AML unfit for IC, HMA+VEN constitutes standard of care. AZA+VEN significantly improves response and OS vs. AZA alone, though long-term cure rates remain below 20%. Clinical trial participation is recommended when feasible.

Targeted therapy options are emerging for molecularly defined subgroups. Currently, ivosidenib (IVO) is the only approved targeted agent in Europe for *IDH1*-mutated AML ineligible for IC. In the AGILE Phase 3 trial, IVO+AZA achieved a median OS of 24 months vs. 7.9 months with placebo (PBO)+AZA, and a complete remission (CR) rate of 47% vs. 11%.[13] When compared across trials, IVO+AZA (AGILE) exhibited comparable rate of response but superior OS to AZA+VEN (VIALE-A) (29.3 vs. 10.2).[14] The combination is well tolerated, with manageable risks of QT prolongation and differentiation syndrome (rate of incidence ≈10–15%). Co-mutations in *DNMT3A*, *RUNX1*, *SRSF2*, and RTK pathway genes predict enhanced sensitivity, supporting the move toward genomic-guided therapy selection in this patient population. In *IDH1*-mutated AML, combining IVO with AZA and VEN improves outcomes. Early data from pooled analyses of triplet regimens (AZA+VEN+IVO or decitabine [DEC]+VEN+IDHi [enasidenib or IVO]) demonstrated composite CR rates (CRc) of 92–98%, 2-year OS up to 84%, and minimal early mortality. Nevertheless, efficacy appears limited in t-AML.[15]

Menin inhibitors represent a major step toward targeted epigenetic therapy for *NPM1*-mutated and *KMT2A*-rearranged (*KMT2A*r) AML – two subtypes dependent on the menin–KMT2A interaction for leukemic gene expression. Menin inhibitors, such as bleximenib, disrupt the menin–KMT2A interaction that drives leukemogenesis in *NPM1*-mutated and *KMT2A*r AML, thereby suppressing *HOXA9/MEIS1* expression, restoring myeloid differentiation, and inducing maturation and apoptosis of leukemic blasts.

Clinically, AZA+VEN+bleximenib triplet regimen has shown promising early results in ND IC-ineligible and relapsed/refractory (R/R) AML with *NPM1* mutation or *KMT2A*r, achieving overall response rates (ORR) of 94% in ND *NPM1*-mutated and 75% in ND *KMT2A*r patients (n=40, early data).[16] The caMeLot-2 Phase 3 trial aims to confirm the early efficacy and safety signals observed in Phase 1/2 studies. Despite the major progress achieved with HMA+VEN combinations, several molecu-



Mechanism of action of menin inhibitors in AML. Modified from Thakur RK, Wang ES. (2025).[18]

lar subgroups of AML remain without effective targeted options. For *FLT3*-ITD–mutated AML, outcomes with AZA+VEN are suboptimal. However, the triplet combination of HMA+VEN+gilteritinib in ND *FLT3*-mutated AML has shown a CR rate of 90%, with 87% MRD negativity, 71% 2-year OS, and minimal early mortality.[17] The ongoing VICEROY Phase 2 trial (USA) is now testing this regimen prospectively.

TP53-mutated AML is a profound unmet clinical need in all patients regardless of age and fitness. While VEN+AZA induces transient responses, remissions are short-lived, and no targeted therapies are currently approved. The anti-CD47 antibody magrolimab failed two Phase 3 trials (ENHANCE-2 and ENHANCE-3).[19,20] Despite meaningful progress, durable disease control remains elusive, particularly for *TP53*-mutated and *FLT3*-ITD subsets.

The ASCERTAIN-V study evaluates an all-oral regimen of DEC+cedazuridine (DEC-C)+VEN in ND AML as a convenience-oriented alternative.[21] Early data show CRc ≈63%, 9-month remission durability >75%, and MRD negativity in ~55% of patients. Patient numbers are limited and follow-up short, but ASCERTAIN-V illustrates the feasibility of effective oral regimens improving convenience and quality of life in older or frail patients.

Relapsing after HMA+VEN remains a major unmet need and mandates re-biopsy with molecular testing to detect clonal evolution (e.g., *DNMT3A*, *FLT3*-ITD, and *TP53*). Current salvage options are limited. Gilteritinib is approved in R/R *FLT3*-mutated AML after HMA+VEN failure but produces modest activity (30% response rate; median OS 4.4 months). When *NPM1* mutation or *KMT2A* rearrangement is present and not previously targeted, menin inhibitors (ziftomenib, bleximenib, revumenib, or enzomenib) yield 30–40% single-agent responses, with differentiation syndrome and QT prolongation as primary toxicities. Combination regimens (AZA+VEN+menin inhibitor) improve depth of response, though hematologic recovery may be delayed; early cytopenias should not prompt immediate discontinuation.

Mechanisms of resistance to menin inhibitors include acquired *MEN1* mutations that perturb the menin binding pocket and epigenetic adaptation with altered *HOXA/MEIS* expression. Primary resistance mechanisms remain incompletely defined. Rational combination strategies that include potentially synergistic compounds (menin+VEN/HMA/FLT3i) and prospective molecularly stratified trials represent the next frontier to overcome resistance.

Therapy for ND AML patients fit for IC

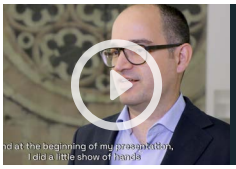
For fit ND AML, treatment selection is guided by molecular subtype and disease biology. In core-binding factor (CBF) AML, standard 3+7 induction with gentuzumab ozogamicin (GO) remains optimal, supported by robust data in this favorable-risk population. In *FLT3*-mutated AML, the addition of FLT3 inhibitors (midostaurin or quizartinib) to 3+7 improves outcomes, as shown in RATIFY and QuANTUM-First trials.[8,9] Ongoing studies (PrECOG 0905 and PASHA) are aiming to refine inhibitor selection. For t-AML, AML with antecedent hematologic disease (AHD), or AML-MR, CPX-351 (daunorubicin+cytarabine) achieves superior survival to standard IC, particularly in MDS-related genotypes; however, no benefit is seen in *TP53*-mutated disease. Recognizing that standard 3+7 regimens are insufficient for many high-risk patients, new strategies are being explored. VEN combined with fludarabine, cytarabine, and idarubicin (GIMEMA AML1718) achieved high CRc (84%) and MRD negativity (~70%) with encouraging survival.[22] Menin inhibitors (e.g., ziftomenib, bleximenib) are being evaluated in *NPM1*-mutated or *KMT2A*r AML, in triplet combinations with standard IC. Finally, trials investigating de-intensified induction (e.g., VEN+AZA before alloHSCT) aim to balance efficacy with reduced toxicity. In addition to menin, FLT3, and IDH inhibitors, novel targeted approaches are emerging in the fields of immunotherapy and antibody–drug conjugates (ADCs), particularly CD123-targeted agents such as pivekimab.

AML: The diagnostic journey

Dr Calogero Vetro
Cytomorphology in AML Diagnostics

Prof Cristina Mecucci
Cytogenetics in AML Diagnostics

Prof Francesco Buccisano
Flow Cytometry in AML Diagnostics



Prof Klaus Metzeler
NGS in AML Diagnostics

The modern diagnosis and classification of AML require integration of cytomorphology, flow cytometry, cytogenetics, and molecular genetics, each contributing complementary insights into disease biology. Minimizing turnaround time (TAT) across diagnostic modalities is critical given AML's clinical urgency, and emerging technologies such as optical genome mapping (OGM) and whole genome sequencing (WGS) promise to refine genomic resolution. Moreover, multiparameter flow cytometry and molecular techniques (e.g., RT-qPCR, ultra-sensitive NGS) enable highly sensitive detection of MRD in the post-remission phase to refine transplant decisions and prompt a pre-emptive treatment of relapse. The following tables summarize the comprehensive discussions delivered by Dr C Vetro, Prof F Buccisano, Prof C Mecucci, and Prof K Metzeler.

Cytomorphology

Cytomorphology remains the first-line diagnostic tool in AML, offering rapid visual assessment of blasts and dysplasia and forming the foundation of integrated diagnostics.

Principle Microscopic evaluation of Wright–Giemsa–stained blood/marrow smears to assess blast morphology, maturation, and dysplasia. Cytochemical stains (MPO, NSE, Prussian blue) aid lineage assessment.	**Sample** Peripheral blood smear and bone marrow aspirate/biopsy.	**Diagnostic role** Confirms suspicion of AML (≥20% blasts, except in genetically defined entities). Provides first-line clues on lineage (myeloid vs. lymphoid) and dysplastic changes suggestive of antecedent MDS.
Key features **Myeloid:** Auer rods, azurophilic granules, MPO+. **Monocytic:** Folded nuclei, NSE+. **Megakaryoblastic:** Small blasts, cytoplasmic blebs. **Lymphoid:** High nucleus-to-cytoplasm (N:C) ratio.	**Strengths/Limitations** Rapid, cost-effective, widely available, allows morphological context/Expertise-dependent, limited sensitivity in low-blast or mixed-phenotype cases, cannot identify genetic or immunophenotypic aberrations, though it may provide a clue based on distinctive morphological patterns.	**TAT/Sensitivity** Hours to one day/Detects blast excess reliably when ≥5% of cells are abnormal.
Application beyond diagnosis Monitoring marrow recovery, blast clearance, and morphologic relapse.	**Sources of discrepancy** Observer variability, hemodilution, poor sample preservation, and overlap between regenerating myeloblasts and leukemic blasts.	**Integration/Role of AI and ML** Guides flow cytometry, cytogenetics/FISH, and molecular testing/AI and ML will support automated blast recognition and dysplasia quantification.

Flow cytometry

Flow cytometry refines lineage assignment at diagnosis and detects aberrant antigen expression and MRD, offering high sensitivity and speed for diagnosis and post-treatment monitoring.

Principle
Fluorescently labeled antibodies detect surface/cytoplasmic antigens, defining lineage, maturation, and aberrant antigen expression.

Sample
Fresh bone marrow aspirate or peripheral blood (EDTA or heparin).

Diagnostic role
Assigns lineage, identifies mixed phenotype acute leukemia (MPAL) and acute undifferentiated leukemia (AUL), detects aberrant antigens, and distinguishes leukemic blasts from regenerating precursors.

Key antigens/Panels
Myeloid: CD13, CD33, CD117, cytMPO, CD64, CD34. **B-lymphoid:** CD19, CD79a, cytCD22, TdT. **T-lymphoid:** CD3 (surface and cytoplasmic), CD2, CD5, CD7, TdT. **Stem/progenitor:** CD34, CD38, HLA-DR, CD123. **Aberrant:** CD7, CD56, CD19, CD2.

Diagnostic nuances
AUL: Lacks lineage-defining markers but may express stem/progenitor markers. **ETP-ALL:** cytCD3+, with stem/progenitor features. **MPAL T/myeloid:** Co-expression of cMPO/CD13/CD33 with cytCD3/CD7. **AML-M0:** Minimal differentiation; stem/progenitor markers; weak or absent myeloid markers; MPO−.

Strengths/Limitations
Gold standard technique for lineage assignment. Quantitative, sensitive, able to detect abnormal immunophenotypes/ Requires viable cells, antigen expression varies with therapy, operator dependent.

TAT/Sensitivity
1–2 hours for diagnostic panels/MRD sensitivity up to 10^{-4}.

Application beyond diagnosis
MRD monitoring; remission confirmation; detection of immunophenotypic shifts at relapse.

Sources of discrepancy
Antigenic modulation, inter-laboratory variability, and phenotypic overlap with regenerating cells.

Integration/AI
Complements cytomorphology; guides molecular testing/ML supports automated gating, cluster analysis, and rare population detection.

Cytogenetics

Conventional and molecular cytogenetics continue to underpin AML risk stratification by revealing prognostically relevant chromosomal abnormalities, despite advances in genomic technologies.

Principle
G-banding karyotyping and FISH detect structural/numerical chromosomal abnormalities. Karyotyping visualizes metaphase chromosomes, while FISH uses fluorescent probes to target specific loci.

Sample
Bone marrow aspirate; peripheral blood if blasts are abundant.

Diagnostic role/Key features
Defines AML subtypes with recurrent translocations (e.g., t(8;21), inv(16), t(15;17), *KMT2A* rearrangements); informs risk stratification, including ELN. **Favorable:** t(8;21), inv(16), t(15;17). **Adverse:** Complex karyotype, −5/del(5q), −7/del(7q), 11q23/ *KMT2A* rearrangements.

Strengths/Limitations
Genome-wide detection of large structural variants and numerical changes/Low resolution (~5–10 Mb), requires cell culture and dividing cells, <5% failure rate, FISH is locus/gene-specific.

TAT/Sensitivity
2–14 days for karyotype; 24–48h for FISH/ Clonality is defined by two metaphases with identical structural changes or three with monosomies. FISH requires ~1–5% abnormal cells.

Application beyond diagnosis
Monitoring clonal evolution, detecting cytogenetic relapse, complementing molecular assays.

Sources of discrepancy
Culture failure, poor sample/metaphase quality, subclonal lesions below detection threshold, and interpretive subjectivity in complex karyotypes.

Emerging adjuncts/AI
OGM detects numerical and structural variation with high genomic resolution/ WGS captures sequence-level variants. AI-assisted tools can automate karyotyping and flag atypical karyotypes for expert review.

NGS

NGS enables detailed genomic profiling essential for precision medicine, while emerging tools like OGM and WGS are advancing toward comprehensive, genome-wide AML characterization.

Principle
Parallel sequencing of targeted panels, exomes, or genomes to detect mutations, insertions/deletions, fusions, and copy number changes. RNA-seq complements DNA testing for gene expression and fusion detection.

Sample
Bone marrow aspirate (preferred) or peripheral blood with sufficient blasts. DNA/RNA extracted for library preparation.

Diagnostic role
Identifies driver mutations defining AML subtypes (e.g., *NPM1*, *CEBPA*, *RUNX1*, *TP53*, *FLT3*), informs ELN 2022 and ELN 2024 risk categories, guides use of targeted therapies, and detects co-mutations influencing prognosis.

Strengths/Limitations
High sensitivity, reveals clonal architecture, enables MRD monitoring via VAF, identifies cryptic mutations below cytogenetic resolution/Bioinformatics-intensive, limited detection of balanced translocations without RNA-seq, expensive, long TAT depending on laboratory and assay.

TAT/Sensitivity
Typically 7–14 days (depends on sequencing depth and pipeline)/VAF detection 1–2%; ultra-deep sequencing 0.1% for MRD.

Integration/Emerging technologies and AI
Complements cytogenetics and flow cytometry/WGS, WTS, and OGM enable genome-wide profiling in a single workflow. AI and ML assist in variant annotation, fusion detection, and pathogenicity prediction, prioritizing clinically relevant mutations for reporting.

Discrepancies
Inter-laboratory differences largely arise from pre-analytical factors and post-analytical variables, rather than core sequencing performance. Detection of CHIP mutations unrelated to active leukemia further complicates analysis. Standardized quality metrics, reference materials, and harmonized pipelines minimize discrepancies. Cross-referencing population (e.g., gnomAD) and cancer (e.g., COSMIC) databases and expert manual review are mandatory.

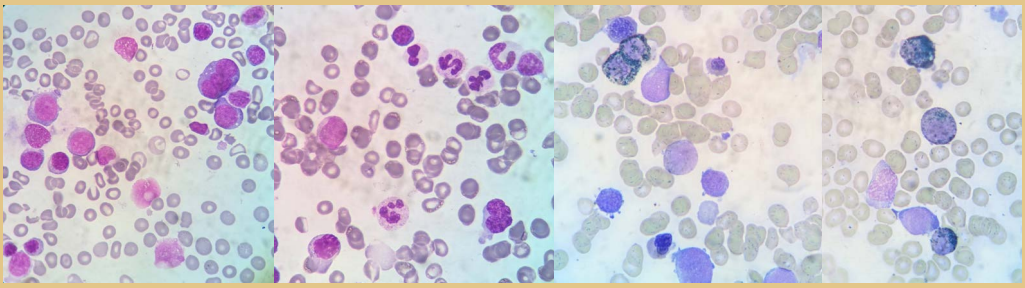
AML diagnosis and classification in practice: Challenging cases

The following section highlights two illustrative clinical cases presented by preceptorship participants, showcasing the practical application of evolving AML management strategies and the challenges integrating molecular and clinical data into real-world therapeutic decisions.

CASE 1 – Presented by Cristina Notario Mc Donnell,
Hospital Universitario Nuestra Señora de Candelaria (Tenerife, Spain)

A 74-year-old man presented to the Emergency Room complaining of weight loss and anorexia.
Blood counts: Hb: 85 g/L; Platelets: 47 × 10^9/L; WBC: 6.2 × 10^9/L

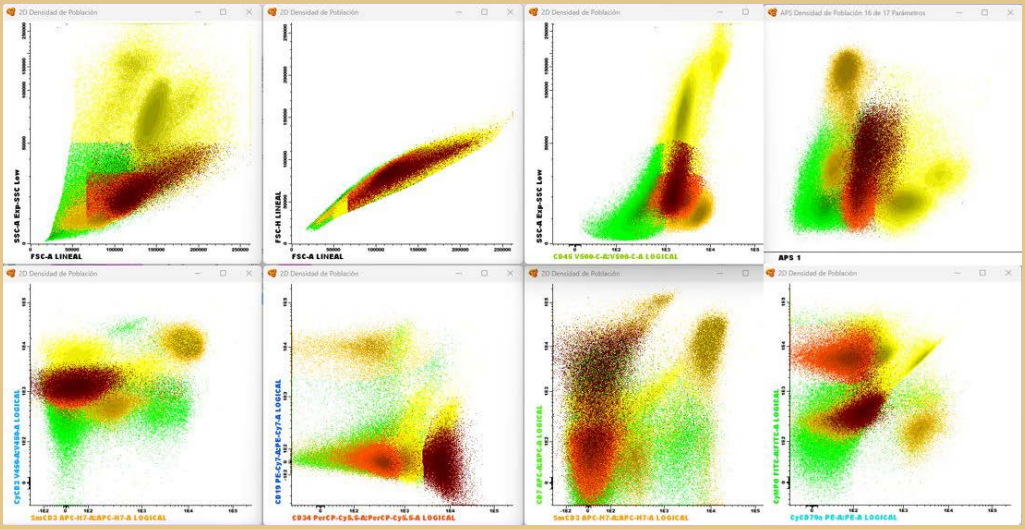
Bone marrow aspiration



- Cellularity: normal
- Myelopoiesis: 19%
- Lymphocytes: 6%
- Erythropoiesis: 45%
- Blasts: 30%

MPO: positive >3% blasts

Flow cytometry: ALOT (Acute Leukemia Orientation Tube) panel (2 populations)



- Population 1: MPO+, CD34-
- Population 2: MPO-, CD7-/+, CD34+

Karyotype: 45, XY, 5q-. NGS: *TP53* mutated (VAF 52.18)

QUESTION: Given the phenotypic and genomics data, what is the diagnosis of this patient according to the ICC 2022 and WHO 2022 classifications? (select all that apply)

A AML with mutated *TP53*

B AML, myelodysplasia-related (AML-MR)

C Pure erythroid leukemia (PEL)

D Acute erythroid leukemia (AEL)

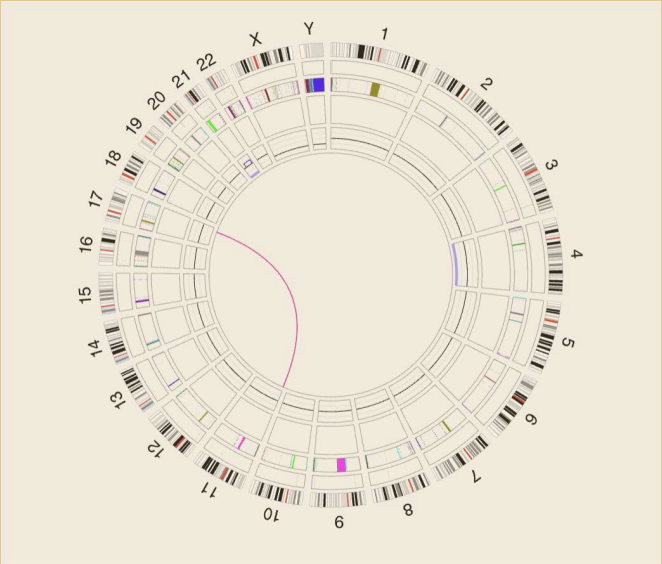
Answer

CASE 2 – Presented by Guillermo Ramil, Hospital Sant Pau (Barcelona, Spain)

A 12-year old male patient presenting with asthenia, pallor, and petechiae receives a diagnosis of AML with minimal differentiation (M0), based on bone marrow aspiration (95% blast infiltration with minimal residual hematopoiesis) and flow cytometry analysis (blasts with CD45dim, MPO-(5%), CD7++, CD34+, CD3-, CD117+, CD13-, CD33+).

Conventional karyotype analysis was unsuccessful due to poor quality metaphases. However, a t(11;17) was detected using OGM.

Further molecular analysis detected a *NUP98::BPTF* fusion, along with pathogenic variants in *TP53* and *RUNX1*.



QUESTION: What is the proper diagnosis of this patient, according to the ELN 2022 recommendations?

A AML with mutated *TP53*

B AML with myelodysplasia-related gene mutations

C AML with *NUP98* rearranged with other partners

D AML not otherwise specified

Answer

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